ABSTRACTS

of M.Sc. and Ph.D. Dissertations on Spice Crops (2006 - 2022)

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M.Sc. ABSTRACTS

BLACK PEPPER

1. **Morpho-molecular characterization, pathogenicity and fungicidal sensitivity of *Fusarium* species infecting black pepper (*Piper nigrum* L.)**

Ms. B. Alsha/Mahatma Gandhi University/2022/Guide: Dr. C.N. Biju

Fusarium species were isolated from infected plant parts showing yellowing symptoms and was characterized based on the morphological characteristics and molecular tools. Based on the macro-morphological features like colony morphology and growth rate, and on microscopic observations like structure of conidia, the isolates were primarily identified as *Fusarium* species. The isolates attained a colony diameter of 90 mm within 8-9 days after inoculation. Pathogenic assay was conducted on black pepper variety Subhakara and under *in vitro* conditions flaccidity, epinasty and later complete death of the plant was observed. The pathogen was re-isolated from the infected plant parts and resembled the original cultures thereby proving Koch's postulates. DNA isolation and PCR were carried out for ITS, β-tubulin gene, and elongation factor and expected amplicons were observed. For evaluation of fungicidal sensitivity, contact, systemic, and combination group of fungicides under *in vitro* conditions were used. Among the fourteen fungicides Bordeaux mixture and propineb completely inhibited the mycelial growth. The effect of different pH and temperature on mycelial growth was also studied under *in vitro* and was concluded that 30 is the optimum temperature and pH of 6 and 8, the optimum pH for maximum growth of *Fusarium*.

2. **Amplification and cloning of RxLR effector genes from *Phytophthora* species infecting black pepper**

Ms. E.M. Haritha/Bharathiar University/2022/Guide: Dr. A. Jeevalatha

Isolated *Phytophthora* from infected black pepper leaf and identified using morphological characters of colony growth and sporangia and further confirmation was carried out by analysing the ITS region. Then, RxLR effector genes (RxLR 11 & RxLR 29) were amplified from isolates, *P. capsici* (05-06) and *P. tropicalis* (98-93), cloned in a T/A cloning vector and sent for sequencing. The zoospores were used to inoculate the black pepper plants and the samples were collected at different time intervals. These samples will be used in qPCR analysis to check the expression of these RxLR effector genes during *Phytophthora* infection in black pepper.

3. **Studying the water stress alleviating effect of melatonin in susceptible variety and expression of stress related candidate gene in contrasting arieties of black pepper (*Piper nigrum* L.)**

Ms. Renuka Suresh/Bharathidasan University/2022/
Guide:Dr. M. Alagupalamuthirsolai

The results revealed that exogenous melatonin could alleviate the oxidative damage caused by water stress. Moreover, exogenous melatonin improved water stress tolerance by enhancing the antioxidant enzyme activity and reducing ROS production, as well as compatible osmolytes like proline and reducing chlorophyll degradation which leads to maintaining net photosynthetic activity. The data presented in this study demonstrated that melatonin at 100 µM concentrations significantly alleviated the adverse effects of water deficit stress on the black pepper plants compared to lower concentrations. However, further studies are necessary to elucidate the interrelation between melatonin and the signaling molecules in response to water stress, as well as the role of melatonin in the expression of genes related to water stress resistance. BAHD (N-acyltransferases) expression was higher in drought tolerant variety as well as it correlated with piperine content of the varieties. This indicates that BAHD could be a candidate gene of the pathways related to drought responses and piperine biosynthesis. Further studies are necessary on functional validation of the gene.

4. **Studies on leaf blight of black pepper (*Piper nigrum* L.) caused by *Colletotrichum* spp.**

Ms. K. K. Amitha/Kerala University of Fisheries and Ocean Studies/2021/
Guide: Dr. C.N. Biju

Two symptomatic variants (SV) namely, SV A and SV B were morphologically characterized and variability observed with respect to colony, conidia lcharacters and its dimensions. Pathogenicity studies indicated that, two isolates could infect black pepper variety Sreekara by inducing brown necrotic lesions with yellow halo. *In vitro* studies showed that, two isolates could produce microsclerotia-like structures. PCR conditions for ITS, β tubulin gene regions were standardized leading to the generation of expected amplicons. The isolates were analyzed based on the similarity with the bestaligned sequence of the BLAST search. The molecular study confirmed the association of *Colletotrichum* with leaf blight of black pepper. However, further analysis needs to becarried out to confirm the species associated with black pepper leaf blight. The studies on biochemical alterations induced by two SVs (representing IISR Thevam and Pournami), indicated that *Colletotrichum* infection

alter the physiological conditions of black pepper by increasing the activity of enzymes such as peroxidase, catalase, polyphenol oxidase and also the phenolic content.

5. Studies on the effect of mycorrhizal colonization on biochemical and molecular defence responses to *Phytophthora capsici* in black pepper

Ms. R. Radhika/Central University of Tamil Nadu/2021/Guide: Dr. C. Sarathambal

In this study, we investigated the effect of mycorrhizal colonization on biochemical, molecular defense responses, and root exudates composition in the *Phytophthora capsici* -black pepper host-pathosystem. Four biochemical parameters such as total phenols, orthodihydroxy (OD) phenols, peroxidase activity, and lignin were studied to delineate the effect of mycorrhizal colonization upon pathogen inoculation. Total phenol, OD phenol, and lignin content increased consistently upon challenge inoculation with *P. capsici* as well as in AM alone inoculated plants in both leaves as well as roots. Similar peroxidase activity was noticed in AM alone and challenged inoculated plants. The qPCR analysis showed that AM pre-inoculation led to up regulation of pathogenesis related genes *viz*., cAPX (PR7), Osmotin (PR5) and β-1,3-glucanase (PR2), phenylalanine ammonia-lyase (PAL) and NPR 1 in black pepper leaves and roots upon the inoculation of *P. capsici* inoculation. Sole inoculation of *P. capsici* also influenced the copies of most genes studied.

6. Biochemical characterization of black pepper (*Piper nigrum* L.) and vanilla (*Vanilla planifolia*) genotypes for its quality parameters

Ms. Dayana Paul/Mahatma Gandhi University/2020/Guide: Ms. R. Sivaranjani

The present work was undertaken to analyze the quality parameters of genotypes of black pepper and vanilla. In 43, black pepper germplasm, essential oil, oleoresin, piperine, starch, sugar, total phenolic content were estimated. The results showed that genotypes Acc. 1207, Acc. 2445, Acc. 5755, Acc. 6648, Acc. 5757, Acc. 1090, OPKM, HP 728 with more than 6 % piperine content and all these genotypes showed more than 10% oleoresin content. The genotypes OPKM and Acc. 6648 had given more than 2% essential oil content. There found a strong negative correlation between starch content and important quality parameters like piperine, oleoresin and essential oil. In five *Vanilla planifolia* genotypes and one each of *Vanilla aphylla* and *Vanilla pilifera* genotypes, total phenolics, total flavanoids, total soluble sugar, percentage of vanillin, vanillic acid, 4-hydroxybenzaldehyde and 4-hydroxybenzoic acid were estimated. The results showed that one *V. planifolia* genotypes showed higher percentage of flavor compounds. The flavor profile of *V. aphylla* and *V. pilifera* was

found to be different and main flavor constituent vanillin was absent in these species. The study concludes that the genotypes present in India have wide variability for both primary and secondary metabolites. The results of the present study could be utilized in the selection of genotypes for crop improvement programs.

7. Identification and characterization of *Fusarium* species associated with collar rot induced yellowing in black pepper (*Piper nigrum* L.)

Ms. R. Anusree/Periyar University/2020/Guide: Dr. C.N. Biju

In black pepper, collar rot associated yellowing was noticed in surveyed locations of Kerala and Tamil Nadu. Further, the symptomatology was recorded in three locations *viz*., Kothamangalam and Peruvannamuzhi (Kerala) and Gudalur (Tamil Nadu) and four isolates were collected. The symptoms of yellowing initiated on the vines during summer and pronounced during February to March which included general decline of plant health, flaccidity, epinasty and yellowing. In the later stages, the entire vine exhibited yellowing leading to wilting. The symptoms manifested on collar region included; formation of necrotic regions which later extended both upwards and downwards and disintegration of affected tissues. Initial observations on the infected tissues revealed the association of *Fusarium* species based on the production of characteristic fusiform conidia upon incubation under high humidity. The isolates were morphologically characterized based on colony, conidial and chlamydospore features. DNA isolation and PCR conditions were standardized for ITS region and based on the molecular analysis the pathogen was identified as *Fusarium solani*. The Koch's postulates were proved based on two approaches *viz*., inoculation with and without injuring the roots. The symptoms were manifested as complete wilting of the plants within 20 to 25 days post inoculation (injured) whereas without injury, the inoculated plants exhibited symptoms like flaccidity and epinasty 55 to 65 days after inoculation. All the isolates were compatible with each other as revealed through vegetative compatibility experiments. Most isolates produced conidia (manifested as yellowish or orange conidiation) on bark of black pepper when exposed to 12 hours photoperiod under *in vitro* condition. Based on growth inhibition, sporulation and conidial density with two isolates (Peruvannamuzhi and Gudalur 2), Bordeaux mixture (1%), copper oxychloride (0.2%) and carbendazim (0.1%) were found superior under *in vitro* condition. Among the bioagents evaluated under *in vitro* conditions, *Trichoderma harzianum* and Act 5 were found effective compared to other bioagents. In the present study a new culture medium was developed for the specific isolation of *Fusarium solani.*

8. Effect of arbuscular mycorrhizae fungi on antioxidant properties of black pepper cuttings

Ms. Anamika Baburaj/Bharathiar University/2020/Guide: Dr. C. Sarathambal

Effect of arbuscular mycorrhizal fungi inoculation on root colonization, growth, and biochemical parameters of black pepper cuttings were evaluated under poly house conditions. Black pepper seedlings were grown in the presence and absence of AM combinations for 150 days in under poly house. AM inoculated treatment had overall significantly maximum mycorrhizal root colonization (95%) and spore numbers (312/50 g of sample). AM inoculation significantly enhanced nutrient uptake and positively correlated to root colonization. The effect of AM fungi was more prominent in root biomass than above ground biomass. AM inoculation has not shown any influence in the stimulation POD activity in root and leaves. It was observed that the activities of poly phenol oxidase, and β gluconase shows an increase in the inoculated AM fungi pots experiment as compared to uninoculated AM fungi pot. It was also observed that decrease in the activity of malondialdehyde (MDA) in inoculated AM fungi pot compare to uninoculated AM fungi pot. Based on these results, we found that AM inoculation at earlier stage of plant development can enhance symbiosis, and increased plant growth in the nursery which may improve the performance after planting in the field.

9. Intrinsic quality evaluation and biochemical characterization of selected black pepper (*Piper nigrum* L.) germplasm

Ms. Fathimath Shamna/Kerala University of Fisheries and Ocean Studies/2019/ Guide: Ms. R. Sivaranjani

Twenty five cultivars and collections of black pepper were selected for the intrinsic quality analysis. The range of bulk density among cultivars is 338 g/L to 617g/L. Among them, Acc. 7220 showed highest bulk density of 617 g/L followed by chumala with bulk density of 593 g/L. The other cultivars with higher bulk density are HP 105 (581 g/L), Acc. 1041(574 g/L), Narayakodi (566 g/L) and Kaniakadan (564 g/L). The cultivars with low bulk densityare Kalluvally (338 g/L), Balancota (376 g/L) and Acc. 856 (373 g/L). All other cultivars showed moderate bulk density. High bulk density cultivars usually fetch premium prices to farmers. The study concluded that the effect of climate and agronomic practices influences bulk density of acultivar. The range of essential oil content in cultivars analyzed was found to be 2.6 % to 5.4 %.The highest essential oil content was found in P-24 with 5.4 % and Kalluvally (5.3 %). Neelamundi and Jeerakamunda recorded the lowest essential oil content of 2.6 %. The highest

oleoresin content was observed in Kalluvally with 12.7% followed by P-24 (11.6%) and Acc.856 (11.3 %). The lowest oleoresin content was obtained in the cultivars Acc. 1071(6.0 %) followed by HP-105 (6.6 %) and Chumala (6.7 %). The results showed that Cul. 5128 has the highest volatile oil content of 5.06% (v/w),which was significantly higher than all other varieties followed by Acc. 2426 (Kottanadan) and it was lowest in Panniyur-3. The oleoresin content was the highest (9.4% w/w) in Panniyur-l and minimum (7.6% w/w) in Panniyur-2. However, differences among the varieties were not significant. The pungency in black pepper is due to the presence of alkaloid, piperine. The most cost-effective one is using spectrophotometer. In our studies, the analysis of piperine using spectrophotometer has given piperine content in the range of 2.5 % (Narayakodi) to 6.1 % (KS-27). The other cultivars which has given highest piperine are Acc.856 (6 %), Kalluvally (5.9%), KS- 14 (5.7 %) and Jeerakamunda (5.7 %). The lowest piperine were observed in chumala (2.9 %), Acc.1071(3.1 %) and Acc.7220 (3.2 %). The range of piperine obtained in 1.0 % to 4. 0%. The highest piperine content was observed in KS-27 (4 %), Panniyur – 4 (3.9 %), Acc.856 (3.8 %) and Jeerakamunda (3.7 %). The lowest piperine content was observed in Narayakodi (1.0 %) followed by Acc. 1071 (1.5%) and Acc.7220 (1.6 %). The average percentage variation between HPLC method and spectrophotometer method was found to be 56.5% which means that the piperine value obtained in spectrophotometer method would be 56.5% more than HPLC method. The lowest piperine content obtained in HPLC method is due to higher sensitivity of HPLC towards differentiation of different isomers of piperine and related piperamides which is not possible to differentiate in spectrophotometer-based methods. The sensitivity of the detection could be further improved with the use of LCMS/MS which is the most sensitive instrument

10. Analysis of antioxidant and antidiabetic properties of allspice (*Pimenta dioica*)
Ms. Nimisha Abraham/Mahatma Gandhi University/2019/
Guide: Ms. R. Sivaranjani

We analysed the antioxidant and antidiabetic properties of allspice using the crude extract of hexane, chloroform, methanol and water. Total phenol and flavonoid contents were also analysed. Hexane extract (222.4 mg/g) of allspice showed highest phenolic content compared to other extracts. In total flavonoid content analysis, we found that water extract of Allspice showed highest flavonoid content 47.5 mg/g. Antioxidant potential of allspice extracts were analysed by 2 methods. In DPPH (α, α-diphenyl-β-picrylhydrazyl) radical inhibition assay, methanol extract showed the

highest antioxidant property with very low IC50 value of 20.8 µg/mL, followed by water and chloroform, 85.15 µg/mL and 114.81 µg/mL respectively. Hexane extract showed the lowest activity (125.85 µg/mL). In ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) inhibition assay the chloroform extract showed the higher activity (149.1µg/mL) followed by water extract (158.1 µg/mL). Methanol and hexane extracts showed almost similar activity, 166.03 µg/mL and 166.99 µg/mL respectively. *In vitro* antidiabetic activities of the extracts were analysed by 2 methods. In α- glucosidase inhibitory assay hexane extract showed highest antidiabetic activity with low IC50 value of 69.5 µg/mL. In glucose uptake potential of allspice extracts using yeast suspension. Hexane extracthas given the highest antidiabetic activity with low glucose concentration of 157.0µg/ mL. Based on *in vitro* results, chloroform extract was selected for purification of compounds using column chromatography. We collected 510 fractions. Among them, two fractions arepartially purified and α-glucosidase inhibitory assay was conducted and these fractions showed good antidiabetic activity than standard acarbose. Fraction 3 have highest antidiabetic activity than fraction 1 (IC50 values of 1304.1 µg/mL and 1242.7 µg/mL respectively). In conclusion, the allspice extracts showed good antioxidant and antidiabetic activity.

11. Influence of nutrient management systems on microbial diversity and soil fertility of black pepper (*Piper nigrum* L.)

Ms. Archana K. Kumar/Mahatma Gandhi University/2019/

Guide: Dr. C.K. Thankamani

The experiment was carried out by adopting three factor factorial design in RBD. Three nutrient management systems, formed one factor. Two improved varieties formed second factor. Two soil depths constituted third factor. Total 12 treatment combinations were replicated thrice in RBD. The enzyme activities such as acid phosphatase, alkaline phosphatase, dehydrogenase were noticed maximum in organic management at 0-15 cm depth in the varieties IISR Thevam followed by Panniyur 5.Organic Nutrient Managaement (ONM) improve the soil fertility by increasing the availability of pH, NPK, Secondary and Mn contents significantly over Intergrated Nutrient Management (INM) and Chemical Nutrient Management (CNM). ONM improved the soil OC content. Highest activities of microbes observed in ONM depicted by higher enzymes activities. Higher soil respiration and dehydrogenase activities in ONM, showing the substrate. Stability for growth of microbes under ONM

as compared to INM > CNM. Top soil (0-15 cm) showed higher microbial than subsoil (15-30 cm) as it is high in OC, pH and other fertility aspects. ONM is the best system for sustainable soil fertility and health management in black pepper. The study revealed that organic nutrient management greatly influenced soil fertiltiy, microbial diversity and enzyme activities in the varieties of black pepper namely IISR Thevam and Panniyur 5 at a soil depth of 0-15 cm.

12. Growth perfomance of rooted black pepper cuttings with different potting mixtures

Ms. P. Saranya/Mahatma Gandhi University/2019/Guide: Dr. C.K.Thankamani

Growth performance of rooted black pepper cuttings with different potting mixtures revealed that medium containing soil, enriched compost with rock phosphate (CERP) and coir block (2:1:1) ratio was found to be efficient for enhancing the growth of black pepper cuttings in serpentine method followed by the potting mixture containing soil, enriched compost with rock phosphate and granite powder (2:1:0.5). The medium can be used efficiently in organic cultivation considering the physiochemical parameters such as OC, potassium, calcium, bacterial counts, P solubilising activity, enhanced growth performance of rooted black pepper cuttings. From the results, it may be concluded that preparation of potting mixture using dairy waste (grass, and paddy straw) and CERP and coir block as ingredients with soil is eco-friendly for organic cultivation.

13. Biochemical and anatomical studies of abscission zone in black pepper (*Piper nigrum* L.)

Ms. M. J. Ashly/Mahatma Gandhi University/2019/Guide: Dr. K. Anees

The current study has designed to throw some light into the biochemical aspects of spike and leaf shedding as a prologue to develop a chemically induced harvesting method. The current study attempted to characterize the abscission process of black pepper using physical, biochemical and histological analysis of the abscission zone (AZ) of both leaf and spike. As the ripening progress the detachment force reduces significantly and it was found to vary among different cultivars. Protein content increased gradually as the ripening of leaf, spike and berry progressed. There was a perfect correlation between the protein content of spike abscission zone and berry abscission zone indicating the effect of protein translocation to berry during maturity. Reducing sugar content increased gradually during maturation process in spike, leaf and berry. The effect of sugar translocation to berry during maturity was clearly visible

as indicated by increased sugar content. The involvement of reactive oxygen species (ROS) is more in case of leaf for its abscission than in case of spike indicating differential involvement of reactive oxygen species in the abscission of both leaf and spike. SDS PAGE of mature and immature spike AZ protein revealed four specific proteins in mature spike abscission protein out of which a low molecular weight protein (~15kDA) was found to be prominently expressed in mature spike abscission zone. Phloroglucinol staining of AZs revealed clear lignifications of the AZ in berry and spike as the maturity of the berry increases. While in case of leaf AZ significant difference in lignifications was not visible during maturation process. Ruthenium red staining of AZs showed no significant variation for mucilage substances in case of berry and spike. While leaf showed an accumulating pattern for mucilage substances in AZ during full maturity which showed disappearing upon ripening. Ruthenium red-Toluidine blue staining gave a clear AZ demarcation of leaf AZ due to pectin degradation. The current study thus has been successful in establishing the fact that there is a mechanistic difference between the abscission of the leaf and spike in case of black pepper. This difference in biochemical mechanism can be harnessed for developing a tool for induced harvesting in black pepper. For this, further studies to study the proteins and metabolites involved in the process needs to be characterized.

14. Exploring source of *Phytophthora* resistance among black pepper (*Piper nigrum* L.) accessions using biochemical defence molecules

Ms. Aswathy R Nair/Mahatma Gandhi University/2019/

Guide: Dr. R. Suseela Bhai

Forty-five germplasm accessions were screened using aerial inoculation to studythe symptom development on stem and leaf and also root screening adopting hydroponicsystem. The defence response of black pepper accessions was evaluated using two tropical isolates of black pepper *Phytophthora viz*., *P. capsici* and *P. tropicalis* was studied. Role of cell wall reinforcement and cell membrane integrity (important structural barriers to pathogen ingress were studied using parameters such as conductivity, total phenol, polyphenol and orthodihydroxy phenol. The overall result showed that the accessions show differential reaction towards different isolates and also showed varying degrees of resistance / susceptibility. However, when the conductance is low, there is less membrane leakage indicating cell membrane integrity with reduced total phenol content. So among the 45 accessions only six accessions were found to be showing stem lesion in the range of 0-5 mm size (highly resistant /resistant group) with

Total phenol<5 mg/100 mg of the tissue, <10 mg poly phenol and OD phenol with conductivity <300 mg. These accessions *viz*. 5764, 6787, 7243, 7319, 7218 and 7344 rated as resistant.

15. Antifungal activity of novel indole derivatives and bacterial isolates against fungal pathogens of spices

Ms. Titty Thomas/Mahatma Gandhi University/2019/Guide: Dr. R. Praveena

The study was conducted to understand the mitigation of moisture stress by different *Trichoderma* isolates*viz*., *T. harzianum* (MTCC 5179), *T. lixii* (KA 15), *T. asperellum* (TN 3), *T. harzianum* (KL 3), *T. erinaceum* (APT1) and *T. atroviride* (APT2) in black pepper plants. The estimation of biochemical parameters *viz*., proline, protein, phenol, lipid peroxidation, chlorophyll a and b and relative water content showed that the inoculation of *Trichoderma* alleviated the stress induced due to moisture stress. Under moisture stress, the proline content of plants increased, whereas in plants inoculated with *Trichoderma* showed an additional increase in proline that provided an extra protection to the plants under drought. The black pepper plants inoculated with the isolate *T. atroviride* (APT2) showed the highest protein content which projected out its ability to provide drought tolerance to the plants, while in un inoculated plants, there was a significant reduction in protein content. The biochemical parameters, leaf proline content, lipid peroxidation increased in response to moisture stress but these parameters decreased in plants inoculated with *Trichoderma* isolates *viz*., *T. harzianum* (MTCC 5179) and *T. atroviride* (APT2). The plants inoculated with the isolates *T. harzianum* (MTCC 5179) and *T. atroviride* (APT2) showed a lower rate of reduction in RWC, even at 25 per cent moisture stress. The higher values of RWC provided by these two isolates were considered as an index of stress tolerance provided to the black pepper plants. The total phenol content in control plants increased under moisture stress. But in the case of those plants inoculated with *Trichoderma* isolates showed a lower phenol content when compared with that of control plants. The plants inoculated with the isolates MTCC 5179 and APT2 showed the presence of constant phenol content indicating the reduction in moisture stress. From the observations on different growth parameters of the black pepper plants inoculated with the different isolates of *Trichoderma*, it was found that the isolates *T. harzianum* -MTCC 5179 and *T. atroviride* -APT2 have the potential to induce tolerance in plants towards drought stress, by its involvement in root and shoot growth promotion, and in addition to triggering the protective mechanisms which avert the oxidative damage. Drought affects physiological and biochemical

processes in plants, thus resulting in altered growth and development. Our study demonstrated that *Trichoderma* could play a role in alleviating drought effect, by improved production of proline concentration in plant tissues and the synthesis of growth hormones. *Trichoderma* also bring growth regulation by protecting membranes from Reactive oxygen species (ROS) and enhance root system accessing to more nutrients. Our experiment showed that drought affects black pepper growth by altering the synthesis of chlorophyll pigments and several other biochemical parameters. It can be concluded from the present study, that few *Trichoderma* isolates are promising in mitigating the negative effects of drought stress by the influencing the plant secondary metabolites.

16. Studies on collar rot associated yellowing of black pepper (*Piper nigrum* L.)

Ms. Reeba Sasikumar/Mahatma Gandhi University/2019/Guide: Dr.C.N. Biju

The *Fusarium* isolate was morphologically characterized and the *in planta* pathogenicity studies indicated that, the isolate could infect Panniyur 1 (susceptible host) by inducing prominent wilting symptoms. The isolate produced chlamydospores under *in vitro* conditions indicating its survival potential. PCR condition targeting ITS region was standardized leading to the generation of expected amplicon. Molecular analysis tentatively placed the pathogenic isolate in the species *Fusarium solani*. However, extensive studies including surveys in different black pepper growing tracts, symptomatology, cultural as well as molecular studies of the isolates and molecular studies employing MLST are highly imperative to prove the association of the pathogen with yellowing of black pepper. Among the fungicides evaluated, all the fungicides except metalaxyl, propiconazole and hexaconazole completely inhibited mycelial growth of the pathogen under *in vitro* conditions

17. Expression of key genes involved in lysine biosynthesis in black pepper (*Piper nigrum* L.)

Ms. C. Aiswarya/Kerala University of Fisheries and Ocean Studies/2019/
Guide: Dr. K. Johnson George

The important key enzymes involved in lysine biosynthesis, aspartate kinase (AK) and dihydrodipicolinate synthase (DHPS) were identified from transcriptome data of black pepper using bioinformatics tools. These genes were selected for the gene expression analysis based on semi quantitative PCR in black pepper leaf and berry. The given transcriptome sequence of aspartate kinase is closely related to *Macleaya cordata* (OVA09663.1) and *Cinnamomum micranthum* (RWR86934.1) and

dihydrodipicolinate synthase transcriptome sequence is closely related to *Populus trichocarpa* (XP_002301301.1) and *Theobroma cacao* (EOX96183.1). The relative expression study on the lysine biosynthesis genes (AK and DHPS) based on the amplified sequences from RNA indicated that, the expressions of both genes are higher in berry when compared to the black pepper leaves.

18. Expression studies on alpha-guaiene synthase and alpha guaiene-2-oxidase in black pepper

Ms. K. Sowparnika/Cochin University of Science and Technology/2019/
Guide: Dr. K. Johnson George

The genes responsible for the biosynthesis of rotundone *viz*., alpha guaiene synthase and alpha guaiene-2-oxidase were identified from the transcriptome of black pepper variety, "IISR Thevam" using bioinformatics tools. Expression studies conducted using semi-quantitative RT-PCR revealed higher expression of these genes in the berries compared to leaves. More accurate quantification and comparative gene expression analysis should be possible using qRT-PCR. *In vitro* enzyme assays of these genes are desirable for functional validation of these genes.

19. Identification of gene specific markers in black pepper (*Piper nigrum* L.)

Ms. C. V. Archana/Cochin University of Science and Technology/2019/
Guide: Dr. P. Umadevi

In the study, identification of PCR based markers from functional genes in black pepper for *Phytophthora* resistance was tested. Three primers from three different genes were taken. The amplified product size for all the primers were of more than the expected product size. Pep 8 and 9 amplified single size fragments whereas pep 2 amplified 2 fragments. The amplified product of pep 8 was above 1kb. The amplified product of pep 9 was below 500 bp. The amplified productof pep 2 was above 700 bp. The genotypes *viz*., P24-O4 and vadakkan did not show any amplification for pep 9. Genotypes Hybrid HP 780, P24-O4, Panniyur 5, Chumala, Vadakkan, Agali, Pournami and PLD-2 did not show amplification for pep 8 at the set parameters. The genotypes chumala and vadakkan did not show amplification for pep 2. The absence of amplification suggests the sequence divergence. The sequence divergence and the presence of introns might have hindered the amplification in certain genotypes. The sequence variation obtained from the sequencing of amplified product will provide further information on the suitability of the selected primers in developing allele specific PCR.

20. Identification of gene specific markers in black pepper (*Piper nigrum* L.)

Ms. K. Sai Sneha/Bharathiar University/Coimbatore/2019/Guide: Dr. P. Umadevi

Primer set 1 (Pep 5) was able to discriminate the genotype Narayakodi from all the other genotypes. Narayakodi showed 2 bands: bright band of size between 300 and 400 bp and; light band just above 500 bp. This is the first of its kind finding in black pepper. In amplification with the *Phytophthora* tolerant and susceptible genotypes, Shakthi showed differential abundance with bright band (400 bp) and the lighter band (just below 300 bp) when compared to Sreekara (bright band- 300 bp and light band-400 bp) and Subhakara (bright band-300 bp and light band-400 bp). For primer set 2 (Pep 10), no differential pattern was observed in any genotype. Among the two primers, Pep 5 was taken for further analysis by sequencing for which the bioinformatics analysis is yet to be done.

21. Defense response of black pepper cultivars to tropical species of *Phytophthora*

Ms. Riya Alex/Mahatma Gandhi University/2018/Guide: Dr. R. Suseela Bhai

The aim of the research work was to study the defense response (lignin and peroxidase) of black pepper varieties to *Phytophthora* infection. Five isolates of *Phytophthora* coming under two groups *viz*. *P. capsici* and *P. tropicalis* were used in the study. Morphologically, the five isolates showed three different colony morphologies like chrysanthemum, stellate and floral cottony pattern. While growth rates of the isolates showed no significant difference and all the five isolates were heterothallic. The symptomatology was studied by direct inoculation of the isolates on the leaf, stem and root of four black pepper varieties namely 04-P24, IISR Shakthi, Sreekara and Panniyur-1. Of these four varieties, 04-P24 showed no symptoms of infection in any of the plant parts except a few specks on leaf and stem with one isolate and was proved as resistant by earlier studies. Other isolates on other varieties showed dark brown advancing lesions with fimbriate margin. However, the lesions produced by *P.capsici* are comparatively larger when compared to *P. tropicalis*. The study on biochemical response such as lignin and peroxidase activity showed 04-P24 as having highest activity of peroxidase and lignin content. Thus, it was proved that increased levels of lignin and peroxidase can contribute to development of resistance to *Phytophthora* infection leading to foot rot incidence. Since peroxidase is a precursor for lignin biosynthesis, the study proposed the use of peroxidase activity of plants as a marker for screening plants for resistance to *Phytophthora* infections. Four accessions and seven released varieties were screened using peroxidase activity keeping the *Phytophthora* root resistant line 04-P24 as a reference standard and found that accessions 7731 and

7583 have comparatively higher peroxidase activity when compared to other accessions and released varieties.

22. Morphological and chemical analysis of black pepper (*P. nigrum*)

Mr. P. Muhammed Farshad/Sri Dev Suman Utharakhand University/2018/
Guide: Dr. K. Anees

The present study attempted to characterise eight different black pepper varieties/cultivars with respect to its morphology and physic-chemical properties. The degree of crinkling due to drying was maximum in Panniyur-1 and Girimunda while Sreekara and Shubhakara showed least degree of crinkling. Maximum shrinkage ratio was observed for Sreekara (0.69) and the least was for Girimunda (0.40) followed by Panniyur-1 (0.42). Overall bulk density of selected black pepper berries was in the range of 428.67to 568.67 g/L and was highest in Panniyur-1 and lowest in Panchami. The Malabar excel showed the highest L* value, ie., it is more light-coloured powder than any other selected samples and Panchami have the lowest L*value and hence it is darker coloured. The total carbohydrate content (43.09 to 46.59%) was found to be highest in Girimunda whereas lowest in Thevam. The total protein content was ranging from 3.06 to 5.65% and was highest in Girimunda and lowest in Malabar excel. The total fat content estimated as per section, ranged from 5.29 to 7.34%. The highest fat content was recorded in Panchami and the lowest was in Subhakara. The crude fibre content estimated, varied from 10.65 to 11.55% and it was highest in Panchami and lowest in Subhakara. The essential oil content, estimated by hydro distillation method, explained on section, is varied between 2.29 to 4.95%. The highest essential oil content was recorded in Malabar excel and lowest was in Panniyur-1. The piperine content of selected black pepper cultivars is showing variability. The percentage of piperine is ranged from 3.8 to 6.55%. Maximum piperine content was observed in Kottanadan and minimum piperine content observed in Panniyur-1. Oleoresin, the concentrated form of spice, was estimated by cold percolation technique and the oleoresin content of selected black pepper varieties ranges from 7% (Thevam) to 11.86% (Shubhakara). The antioxidant property, which was measured in term of DPPH radical scavenging activity and expressed as IC50 (µg/mL), of selected black pepper varieties noticed from 22.82 to 58.43 µg/mL. The variety Sreekara shows higher anti-oxidant property.

23. Gene expression analysis of β-caryophyllene synthase gene in black pepper (*Piper nigrum* L.)

Ms. E. K. Resmi/Kannur University/2018/Guide: Dr. K. Johnson George

The study was undertaken to understand the expression of β-caryophyllene synthase gene in different cultivars of black pepper. For this, leaf and berry samples of major black pepper cultivars from IISR Experimental Farm Peruvannamuzhi, Calicut, were collected and studied by using real time PCR. The result of this study shows high variations in the expression of this gene in different cultivars. These variations are may be due to the different cultivars, altitude and location.

24. Characterization of *Colletotrichum* spp. associated with leaf blight of black pepper (*Piper nigrum* L.)

Ms.V. P. Bhavya/Mahatma Gandhi University/2018/Guide: Dr. C.N. Biju

Sixteen isolates representing Kerala and Karnataka were morphologically characterized and considerable variability could be observed with respect to colony, conidial as well as appressorial characters and its dimensions. *In planta* pathogenicity studies indicated that, three isolates could infect Panniyur1 (susceptible host) by inducing prominent yellow halo. While, *in vitro* studies showed that, infection could occur within 72 hours after inoculation with the formation of acervulus initials. Vegetative compatibility studies among selected isolates representing Kerala and Karnataka indicated that, isolates 2 and 4 (representing Kerala) were more compatible with other isolates indicating it's a sexual out crossing potential. *In vitro* survivability studies showed that, all the isolates were capable of producing microsclerotia indicating its survival potential in dormant forms. PCR conditions for ITS (ITS 1 & 2), Actin and CHS regions were standardized leading to the generation of expected amplicons. However, sequencing need to be carried out further to delineate the species associated with black pepper leaf blight.

25. Detection and differentiation of *Phytophthora* species through cytochrome oxidase (Cox) gene analysis and PCR assay

Ms. M. Sruthi/Mahatma Gandhi University/2018/Guide: Dr. A. Jeevalatha

In this study, black pepper cultivar Sreekara produced typical symptoms of leaf blight after inoculation with *Phytophthora* isolate 98-02 and the pathogen was re-isolated from the inoculated plants to prove Koch's postulate. The cox II, intergenic region and cox I gene (~ 2.2. kb) of *Phytophthora* isolates 05-06 and 97-55 were cloned and sequenced. The nucleotide sequences of Cox region of *Phytophthora* isolates 05-06

and 97-55 were used in BLAST programme in NCBI to search similar sequences. Since only mitochondrial genome sequences of *Phytophthora* species available in NCBI were used in analysis, they shared a maximum of 91.8 to 91.9 % identities with *P. sojae* from USA. Then only the Cox sub unit I of isolates 05-06 and 97-55 were used in analysis. The isolate 05-06 shared a maximum of 99.4 % identity with *P. capsici* isolate 302 from *Capsicum annuum* (Florida, USA) and isolate 97-55 shared 99.2 % identity with *P. tropicalis* voucher CBS43491 from Hawaii, USA. In a phylogenetic analysis, isolate 05-06 was found to cluster with *P. capsici* isolates (AY129166 and KJ631596). Isolate 97-55 was found in a separate sub cluster with *P. tropicalis* isolates (HQ261467 & HQ708417). So it is concluded that the Cox I gene analysis could differentiate *P. capsici* and *P. tropicalis* and according to the results, the isolate 05-06 belongs to *P. capsici* and 97-55 belongs to *P. tropicalis*. Species specific primers based on ITS region were designed and used in PCR standardization. The results indicated that the designed primers were highly specific and the amplifications were observed only in respective *Phytophthora* species except PMPCO-F & COM-R. The primers PCPT-F & COM-R were validated with 46 isolates of *Phytophthora* from black pepper and the assay showed successful amplification of 335 bp band in all the tested isolates except two isolates indicating they are either *P. capsici* or *P. tropicalis*. In this study we could not design primers to differentiate *P. capsici* and *P. tropicalis* based on ITS region as the ITS regions of these two species are more conserved. However, the developed primers can be used in the initial identification of *Phytophthora* species associated with black pepper and further sequencing of ITS or Cox gene is required for confirmation.

26. Analysis of R genes in black pepper

Ms. K.N. Aswathi/Kannur University/2018/Guide: Dr. P. Umadevi

Good quality RNA was extracted from all the varieties under study and cDNA was synthesized. The synthesized cDNA was of good quality which was checked by GAPDH gene. To understand the differential expression of five R genes among varieties in natural condition the real time PCR was attempted. The repeated analysis resulted in the amplification in NTC also. The other strategy to find out the sequence variation in the R genes using sequencing of PCR products was undertaken. The sequencing of the products is under way. The variation expected from the amplified products will yield the difference in the R gene among varieties under natural condition without the infection of *Phytophthora*. The variant base would become possible SNP candidate for screening the black pepper genotypes in future. The *in silico* analysis of other 4 R gene loci from

the transcriptome yielded the defined phylogeny for each R gene locus. The R gene locus 43684, 1179 belonged LRR-3 super family and Pkc-like super family respectively. The R gene loci-15607 and 8252 were found to be of PLN00113 super family.

27. Molecular characterization of mealy bugs (Hemiptera: Seudococcidae) infesting black pepper (*Piper nigrum* L.)

Ms. K. T. Busthana Sidheque/Kannur University/2017/Guide: Dr. T. K. Jacob

In the study, the DNA barcoding method was used in a more comprenensive, way, by testing- its performance on two species of mealy bugs belonging to the same family infesting the same crop collected from two different locations of Kozhikode district in Kerala. The results indicated that the CO I based barcoding was consistent with identification by morphological characters. The study indicates that the mealy bugs can be efficiently identified using the standard barcode region of CO I gene. The technique should be useful in developing quick species-specific control strategies against mealy bugs.

28. Response of black pepper (*Piper nigrum* L.) to drought and exogenous application of $CaCl_2$

Ms. M.V. Fathima Nasreen/University of Calicut/2017/Guide: Ms. P. Umadevi

The study was conducted to identify the cost-effective molecule to mitigate drought and changes in stomatal, physiological parameters such as relative water content, membrane leakage, reactive oxygen species and protein content in black pepper. For this, four varieties of black pepper namely Subhakara, Acc. 1622, Acc. 4226 and Acc.1495 were subjected to drought by water with holding water. The stomatal behaviour was studied using microscope. Here the size of the stomatal pore is small and numbers of stomata closed were higher in drought samples compared to the control samples. The relative water content showed a decreasing trend whereas membrane leakage shows an increasing trend in the drought samples when comparing the control sample. The total protein content in control sample and drought sample (10^{th} day with holding water) was analysed by electrophoresis to achieve the identification of differentially expressed protein. The application of calcium chloride solution showed significant changes in the desiccation-imposed plants. The relative water content was higher whereas membrane leakage is lower when compare to desiccation control sample in $CaCl_2$ sprayed samples. The size of stomatal pore is reduced in $CaCl_2$ sprayed samples when compared to desiccation control sample.

29. DNA barcoding for identification of scale insects (*Diaspididae: Homoptera*) infesting black pepper (*Piper nigrum* L.)

Ms. M. R. Aparna/Kannur University/2017/Guide: Dr. C. M. Senthil Kumar

In the present study, morphological identification of *Protopulvinaria longivalvata, Lepidosaphes piperis* and *Marsipococcus marsupialis* were done. PCR amplification of mt-CO1 gene and the band sizes of the mt-CO1 genes were studied. The PCR amplification of the CO1 gene produced characteristic bands of approximately 600 bp for both the species. Sequencing of CO1 genes of scale insects an approximate 700 bp portion of the mt-CO1 gene region was amplified and sequenced for three scale insects. An NCBI (National Centre for Biotechnology Information) BLAST similarity search was conducted to search for homologous sequences identical to the mt-COI sequence of the sequenced insects. However, none of the sequences had similarity with existing scale insects in the Gen Bank database because sequences of these insects are not available in the database.

30. Molecular characterization of selected cultivars in black pepper

Ms. Farhana Muhammadali/University of Calicut/2017/Guide: Dr. K. V. Saji

In the present study, molecular characterization of 16 selected cultivars of black pepper was undertaken. For conducting the study, DNA of the cultivars was isolated using a modified SDS protocol. Protocol for isolation of DNA from black pepper leaves was optimized, since the existing methods are very expensive and time consuming. Phenol: chloroform: isoamylalcohol is used (25: 24: 1) instead of acid phenol: chloroform for purifying nucleic acids and to eliminate proteins and lipids. Alterations in concentrations of PVP (Poly vinyl pyrrolidone) and SDS (sodium dodecyl sulphate) yielded good quality DNA with 1.5 to 1.8 ranges of absorbance. RNase and Proteinase treatment was included in the procedure for yielding pure DNA without contaminants. Use of β-mercaptoethanol is avoided since it doesn't change the quality of DNA. PCR-IISR analysis was carried out as per procedure optimised in lab. Six IISR primers were used for the genetic analysis of the black pepper cultivars. The percentage polymorphism varied in different primers, three of them showed 100% polymorphism among the six primers, UBC 812 gave higher number of amplified fragments where as UBC 1 gave lowest number of amplified fragments. The six primers produced 49 bands of different sizes with an average of 7.6 polymorphic bands per primer. 45 out of 49 fragments were polymorphic and 4 were monomorphic, with 91.38% of mean percentage of polymorphic bands. Unique markers were identified

based on the presence or absence of bands produced. Six cultivars out of 16 produced unique bands in different cultivars *viz*., IISR-Malabar Excel, Kottanadan, Nedumchola, IISR-Thevam and Karivilanchi, hence can be used to distinguish from other cultivars. The rest of the cultivars did not yield any unique bands may be used as markers for identification of these cultivars.

31. Gene expression study of a thaumatin like protein gene in black pepper (*Piper nigrum* L.) under drought stress

Ms. P. Raveena/Kannur University/2017/Guide: Dr. K. Johnson George

The tolerant black pepper (Acc.4226), responded to drought stress at molecular level and in this study, the TLp gene showed higher level of expression (5.4 fold) under the water stress. Differential expression of TLp gene during drought suggests that higher expression of this gene is beneficial to combat drought at cellular level. The differential expression of this import drought tolerant gene (TLp) in black pepper indicates its further usefulness in developing varieties with improved water stress tolerance.

32. Isolation, identification of entomopathogenic nematodes through molecular characterization and their biocontrol potential against insect pest infesting black pepper (*Piper nigrum* L.)

Mr. A.C. Thasneem/University of Calicut/2015/Guide: Dr. Rashid Pervez

Random survey and 76 soil samples were collected from black pepper rhizosphere from different locations of Kozhikode and Wayanad (Kerala) and Kodagu (Karnataka) districts. EPNs isolated from the soil using the insect baiting technique. All isolated EPNs were cultured as per the procedure described. Soil samples were analyzed for soil type and pH. DNA extracted from the entomopathogenic nematodes by following phenol -chloroform method. The isolated DNA was amplified by using 18S (Forward) and 26S (Reverse) primers. The sequencing was done using purified PCR product. The phylogenetic history was erected using the Neighbour-Joining method.

33. Assessment of single nucleotide polymorphic (SNP) markers in the identification of black pepper cultivars

Ms. V. K. Sobhida/University of Kannur/2015/Guide: Dr. K. Johnson George

The study was undertaken to assess single nucleotide polymorphic (SNP) marker in the identification of black pepper cultivars. We could identify a number of SNP sites based on RAD sequence analysis of *Piper nigrum.* The II locus were selected

based on the analysis and was used for the allele-specific -SNP primer designing. To validate the SNPs that were identified by us, 4 out of these loci were found to be polymorphic in black pepper and 7 polymorphic primers of these locus were used for assessing Single nucleotide polymorphism (SNP) in the identification of black pepper cultivar. Variation in SNP between the 24 selected black pepper cultivars was studied by the simple technique of Allele Specific PCR. SNP profile have been developed for 24 pepper cultivars; such as Panniyur 1, 2, 3, 4, 5, 6, 7 and 8, IISR Thevam, IISR Girimunda, IISR Shakti, IISR Malabar Excel, Sreekara, Subhkara, Panchami, Pournami, PLD2, Arka Coorg excel, Agali pepper, Chumala, Acc819, OPKM, Vadakkan, Narayakkodi. We could optimize PCR profile for selected SNP primers. In this study, we could differentiate 14 out of 24 black pepper cultivars by its unique SNP pattern. The result demonstrated that the allele-specific -SNP markers used in this study are extremely useful for cultivar identification. The same technique may also be successfully used in other plant species to help in cultivar identification at molecular level.

34. Studies on effect of water replacement on the production of white pepper

Ms. Joselin Ann Joy/Karunya University/2015/Guide: Dr. E. Jayashree

The white pepper is the value-added product of black pepper (*Piper nigrum L.*) which is obtained by removing the outskin or the ripe or matured green berries or dried black pepper. In the current study, experiments were conducted to determine the effect of water replacement on the production of white pepper. The treatments considered were 0 percent, 25 percent, 50 percent 75 percent, 100 percent water replacement and nutrient broth inoculated with 24 h *Bacillus licheniformis.* Green pepper and the fermentation medium were maintained at 3:3 ratio. The white pepper obtained was analysed various physical. biochemical and microbial test following stand ard procedures. The results of the study showed that complete conversion of green pepper to white pepper was obtained on the sixth day for 100 percent water replacement. The 100 percent water replaced sample showed highest dry recovery of 22.8%. White pepper obtained by water replacement in 100 percent water replaced sample showed more colour compared to other treatments. The physical properties studied showed that the dry white pepper had an average moisture content of 10.32%, berry size 4.32 mm, sphericity of D.97 and bulk density of 473.23 kg/m^3. Primary metabolites like total carbohydrates, total fats and total proteins present in dry white pepper had an average value of 67.76%. 7.11% and 7.69%. respectively. Essential oil content were 2.41%.

Oleoresin was 15.25% and for piperine it was 5.26%. The volatile components or essential oil distilled from white pepper were analysed in gas chromatography - mass spectrometry and alpha -limonene showed the highest relative peak area or 22.99% followed by sabinene 13.96% and pinene 9.32%. The compound germacrene was found in minimum quantity 0.23%. From the present study, it was concluded that fermentation or green pepper by replacement 01% water by different treatments were efficient for white pepper production. But considering the quality of white pepper obtained it was concluded that the white pepper obtained by 100 percent water replacement treatment provided better results.

35. Detection of mineral oil adulteration in black pepper and the influence of solvent washing on its quality

Ms. P. Aiswarya/Karunya University/2014/Guide: Dr.T. John Zacharia

In the present study, mineral oil adulteration in black pepper was determined as per procedure enumerated by Indian Council of Medical Research (1990) by following thin layer chromatographic technique. Further, attempts for the removing the mineral oil contamination were done by washing the adulterated black pepper using organic solvent hexane at 50 rpm in a mechanical shaker for different time intervals of 5, 10, 15, 20, 25, and 30 min. After washing the black pepper, chromatographic analysis was again carried out to determine the removal of mineral oil. A five minutes wash with organic solvent hexane was found efficient in removing the mineral oil from the surface of adulterated black pepper. Chromatogram obtained by spotting the essential oil and oleoresin extracted from the washed and unwashed black pepper showed bright yellow fluorescence which indicated the possible penetration of the mineral oil into the inner core of black pepper. Quality analysis of unwashed and washed black pepper was done by comparing it with the control samples such as Panniyur-l, Panniyur-3 and Panniyur-5. Physical properties like bulk density and Hunter colour value (L*) was found to increase from an initial value of 456.91 kg/m^3 and 19.59 to a final value of 468.1 kg/m^3 and to 24.79, respectively. Primary metabolites analysed were carbohydrates, fat and proteins. Carbohydrate content decreased from 64.26 to 52.70%, fat decreased from 6.6 to 4.4 % and protein content decreased from 8.28 to 8.13 % after washing for 30 min. Analysis of secondary metabolites indicated that essential oil remained unchanged after washing for 30 min, while the oleoresin and piperine content decreased by 5.7% and 36%, respectively. The analysis of volatile constituents of essential oil obtained from unwashed and washed black pepper by Gas Chromatography-Mass Spectroscopy,

indicated that out of the seven major oil constituents identified, five constituents like a-limonene, caryophyllene, a- phellandrene, carene, a-pinene, p-myrcene, and a-thujene was found to decrease while carene and a-pinene was found to increase after washing for 30 min. From the study it could be concluded that the mineral oil contamination of the black pepper cannot be removed completely even after solvent washing for 30 minand hence mineral oil adulterated black pepper could be unsafe for human consumption

36. Studies on production of white pepper from green pepper using selected bacterial strains

Ms. P.V. Suvina/Mahatma Gandhi University/2014/Guide: Dr. E. Jayashree

In the current study, experiments were conducted to determine the effect of three varying load i.e. 25, 50 and 100 kg of green pepper in five different fermentation medium. The fermentation medium considered were 7 mL of IISR WP38 (*Bacillus subtilis*) in 1/4 strength nutrient broth, IISR WP43 (*Bacillus licheniformis*) in 1/4 strength of nutrient broth, IISR WP38 in water, IISR WP43 in water and a control without inoculation of any bacterial culture. Green pepper and the fermentation medium were maintained at 1:1 ratio. The white pepper obtained was analyzed for various physical, biochemical and microbial test following standard procedures. The results of the study showed that complete conversion of green pepper to white pepper was obtained on the sixth day for control sample, IISR WP43 and IISR WP38 in nutrient broth as fermentation medium for all the quantities of pepper considered. The control sample showed highest dry recovery of 21.20, 22.6 and 21.6% corresponding to 25, 50 and 100 kg of green pepper fermented in water. White pepper obtained in a fermentation medium of IISR WP43 in strength nutrient both as fermentation medium and in water (control sample) showed more colour compared to other treatments. The physical properties studied showed that the dry white pepper had an average moisture content of 9.24%, berry size of 4.26 mm, sphericity of 0.98. Primary metabolites like total carbohydrates, total fats and total proteins present in dry white pepper had an average value of 69.36%,7.38% and 8.68%, respectively. The secondary metabolites of white pepper such as, essential oil, oleoresin, piperine were determined and the mean values for essential oil content were 2.86%, oleoresin was 13.50% and for piperine it was 2.97%. The volatile components of essential oil distilled from white pepper were analysed in gas chromatograph and D- limonene showed the highest relative peak area of 29.79% followed by sabinene 11.37% and p-caryophyllene 11.197%. The compound

gennacrene was found in minimum quantity 0.017%. From the present study, it was concluded that fermentation of green pepper using bacterial cultures like IISR WP38 and IISR WP43 (7 mL) in nutrient broth as fermentation medium along with control (fermentation in water) were efficient treatments for white pepper production. But considering the quality of white pepper obtained it was concluded that the white pepper obtained from control treatment provided better results.

37. Studies on production of white pepper from black pepper using selected bacterial strains

Ms. P. Linsi/Mahatma Gandhi University/2014/Guide: Dr. E. Jayashree

In the current study, white pepper was produced from black pepper by fermenting it in four different fermentation medium i.e1/4; strength nutrient broth with 1.25 mL of *Bacillus licheniformis* (IISR WP43) 1 litre, 1/4; strength nutrient broth with 1.25 mL of *Bacillus subtilis* (IISR W^{p}38) 1 litre, water (unchanged during the experimental period) and water (changed every day till complete decortications was obtained). The experiment was conducted at room temperature until complete decortication of black pepper was obtained. After decortications was completed, fermentation media was removed and the berries were rubbed, washed in tap water and sun dried for 3 days. The result of the study showed that complete conversion of black pepper to white pepper was obtained on the 17th day when the 2 .5 mL bacterial culture of IISR WP43 and IISR WP38 in 1/4; strength of nutrient broth. In the case of control samples like as water (unchanged during the experimental period) and water (changed), the conversion was obtained on the 20th and 92nd day of fermentation, respectively. The highest dry recovery of 68.30% was obtained when black pepper was fermented in water when water was unchanged for the entire fermentation period.

38. Variability in the quality profile of black pepper

Ms. Anjali Aravind/Mahatma Gandhi University/2014/Guide: Dr. T. John Zachariah

In the present study, dried black pepper berries of 17 different accessions (S1 to S 17) from Sirsi area, Karnataka were powdered and subjected to variability inquality profile. High yielding black pepper variety Panniyur- 1 was used as reference sample. Quality in terms of their physical, biochemical and microbial analysis were done. Biochemical analysis includes primary and secondary metabolites. Physical properties studied were moisture content and bulk density. Major primary metabolites studied include total carbohydrate, protein and starch and secondary metabolites include essential oil, oleoresin, piperine and total phenol. Antioxidant activity of black pepper

accessions were also examined in the study. Variation in volatile oil components was done using GC analysis.

39. Expression analysis of glucanase inhibitor (GI) and glycoside hydrolase (GH) genes of *Phytophthora capsici* in challenge inoculated leaves of *Piper colubrinum* Link

Ms. A. Soorya/Kannur University/2014/Guide: Dr. K. Johnson George

Experimental data on *Phytophthora capsici* Glucanase Inhibitor (GI) and Glycoside Hydrolase (GH) gene function play a key role towards better understanding of oomycete- plant interactions which eventually may result in identification of host defense genes for breeding programs. In this study it was found that GI genes showed higher level of expression during the first 2- 4 h after inoculation. Differential expression of GI genes during infection suggests that more expression of this gene is required early during infection as its function is to counteract host defense through targeting host hydrolytic enzymes. Glycoside Hydrolase (GH) is mainly involved in the degradation of host cell wall for pathogen invasion and it showed highest level of expression at 16 hpi.

40. Effect of varying temperature on stored black pepper powder (*Piper nigrum)*

Ms. Sumi Sara Paul/Karunya University/2014/Guide**:** Dr. T. John Zachariah

Storage of black pepper varities Panniyur -1and Panniyur-5 were studied for a period of 12 weeks under varying temperatures of 30, 40, 50˚C. Pulverized black pepper powder (25 0 mesh size) was packaged in three layered metalized polyester covers (12,1 polyester + 12,1 metalized polyester + 80,1 LDPE). The varieties of black pepper powder stored at different temperatures and for different storage period were studied for the variation in physical properties, biological properties like the primary metabolites and secondary metabolites and microbial analysis was done at the beginning and end of storage period. Physical properties studied are moisture content, water activity and colour. Major primary metabolites studied were total carbohydrate, fat, protein and starch. Major secondary metabolites studied are essential oil, oleoresin, piperine and total phenol content. The antioxidant activity, the variation in volatile constituents of essential oil and total plate count were also determined during the storage period. For both varieties of black pepper powder, during storage at ambient condition for 12 weeks the physical properties like moisture content and water activity increased but during storage at 30, 40, and 50^0C, the moisture content and water activity decreased. The colour has a significant effect on black pepper powder during the storage. The

biochemical properties studied include primary and secondary metabolites. Initially the black pepper powder had primary metabolites like carbohydrate, protein, fat and starch content of 38.42, 6.02, 7.52 and 35.62%, respectively more in variety Panniyur -1, towards the end of storage period, there was no considerable change. The secondary metabolites of stored black pepper powder such as essential oil, oleoresin and piperine content were determined. There was no significant change in essential oil content at ambient temperature. The initial oleoresin content of black pepper powder was 8.09 and 7.55 for variety Panniyur -1 and Panniyur-5 respectively. At the end of 12^{th} week of storage, the oleoresin content is decreased with increase in temperature. The piperine content of stored black pepper powder decreased with increase in temperature and storage period. It was reduced by 21.62% and 22.03% for ambient and 50°C. respectively volatile variety Panniyur -1 towards the end of storage. The total phenol content and antioxidant activity of the powder increased with increase in temperature but it is decreased during storage at ambient temperature. Volatile constituents of essential oil was determined us in Gas Chromatography. The constituents like pinine, camphene, sabinene, β pinine, mycrene, aphellandrene, limonene, β caryophyllene reduced during the storage period at different temperatures while the two constituents elementes and germacrene was found to increase with increase in temperature. On microbial analys is, for variety Panniyur -1, it was found that increase in temperature decreased microbial load. For variety Panniyur -1 the total plate count of stored black pepper powder decreased from $3.0x\ 10^4$ cfu/mL to $1.0x\ 10^4$ cfu/mL at 5°C towards the end of storage period. Similar trend of decrease in total plate count during storage with increase in temperature was observed for variety Panniyur -1. From the current study, it was found that temperature had a significant effect on storage of black pepper powder. Ambient temperature was found better for storage period of black pepper powder up to 12 weeks since it retains essential oil with a minimum reduction in oleoresin and piperine content.

41. Studies on conversion of green pepper to white pepper using different bacterial cultures

Ms. Minu Poulose/Karunya University/2014/Guide: Dr. E. Jayashree

In the current study, experiments were conducted for optimization of temperature for pectinase enzyme production by four different bacterial isolates (IISRWP 33, IISRWP 34, IISRWP 38 and IISRWP 43) in a production medium and to identify the most important bacterial organism for production of white pepper from

green pepper. For temperature optimization studies to determine the pectinase enzyme production, 1 mL of each bacterial isolate was added to 50 mL of the modified enzyme assay medium and incubated at different temperatures *viz*., 30, 40 and 50°C for a period up to 96 h. The results of the study indicated that IISRWP 34 and IISRWP 38 had maximum enzyme activity of 197.46 µl /mL and 208.2 µl /mL, respectively after 24 h of incubation when the temperature was 40°C. While IISRWP 33 showed highest activity of 107.63 µl/mL at 24 h. The lowest enzyme activity was obtained when the temperature was 50°C. For the second experiment, freshly harvested green pepper (variety Panniyur -1) was washed and 5 kg each of fresh pepper was transferred in to buckets which contained 5 l of 1/4 strength nutrient broth. The inoculum were prepared using different bacterial strains and 1.25 mL of 24 hold cultures of each organism were added to the nutrient broth and incubated at room temperature until complete decortication was obtained. After incubation, the fermenting broth was removed and the berries were rubbed and washed in tap water to remove degraded pericarp and bacteria and sun dried for 3 days. Control was prepared by adding 5 litres of distilled water to 5 kg of green pepper. The results of the study showed that complete conversion of green pepper to white pepper was obtained on the sixth day when a bacterial culture of IISRWP 34 and IISRWP 38 was used. Control also showed maximum conversion within 6 days. The creamy white pepper obtained after manual washing of green pepper was sun dried for three days and the qualities of white pepper in terms of its physical and biochemical properties were evaluated. The highest dry recovery of 19.82 % was obtained for control sample. The physical properties studied showed that the dry white pepper had an average moisture content of 10.27 %, berry size of 4.30 mm, sphericity of 0.95 and bulk density of 521.67 kg/m^3.The maximum colour value was obtained for control sample with hunter colour values of L* 52.41, a* of 5.82 and b* of 2 1.44. Primary metabolites like carbohydrate, fat, protein and starch present in dry white pepper were 77.11, 8.44, 8.59 and 66.17% respectively. The secondary metabolites of white pepper such as, essential oil, oleoresin and piperine were determined and the mean values for essential oil content was 3.59%, oleoresin was 1.43% and for piperine content it was 2.78%. Volatile constituents of essential oil are determined and eleven important compounds were analysed. The important constituents are limonene of mean value of 24.32%, element with mean value of 3.56%, pinene with 5.42% mean value and p-caryophyllene with mean value of 25.5%. The surface microbial load varied from 2.8 x 10^1 to 3.04x 10^2 cfu/mL. From the present study it was concluded that the bacterial cultures IISR WP 34, IISRWP 38 along with control proved efficient in convert green pepper to white pepper.

42. Antioxidant potential of black pepper and cinnamon and their synergistic effect
Ms. A. Shahida/University of Calicut/2013/Guide: Dr. N.K. Leela

Selected promising accessions of black pepper and a market sample of cinnamon were evaluated for their quality attributes and antioxidant activity. Essential oil content and oleoresin content were analyzed by ASTA methods. The chemical constituents of the oil were determined by GC- MS analysis. Sabinene, Ct- pinene, pinene, caryophyllene were the major constituents in black pepper oil. T-cinnamaldehyde was the chief constituent of cinnamon oil. The chief components of oleoresin of black pepper and cinnamon were piperine and cinnamaldehyde. Essential oil, oleoresin and soxhlet extract of black pepper and cinnamon were tested for their antioxidant potential by DPPH and phosphomolybdenum assays. The antioxidant activity of essential oil of black pepper was higher than that of cinnamon. Methanol extracts of cinnamon and black pepper showed higher antioxidant potential compared to the corresponding hexane extracts. This indicates that the constituents contributing antioxidant property are polar in nature. The antioxidant potential of oleoresin of black pepper was higher than that of cinnamon. But the antioxidant potential of cinnamaldehyde was higher than that of piperine. Piperine showed very low antioxidant potential by DPPH method, compared to the standard BHT. When a combination of piperine and cinnamldehyde was used, the antioxidant potential increased marginally. The study also indicates the presence of more potent constituents other than piperine in black pepper. Further studies are warranted to draw final conclusions on this matter.

43. Functional annotation of whole genome sequence of *Phytophthora* isolate infesting black pepper and *in silico* protein-protein interaction studies
Mr. Faisal Moossa Athikkavil/Bharathiar University/2012/
Guide: Dr. Santhosh J. Eapen

This study was conducted with the aim of annotating the proteins present in the whole genome sequence of an oomycete *Phytophthora* isolate (IISR) from black pepper and to show the mode of interaction of some plant defence proteins against some of these *Phytophthora* proteins annotated. Since this study generated a large amount of data on proteins, it also helped in creating a database with the proteins annotation result. Since this pathogen is highly destructive to many important plants another study was conducted to understand the host plant's defence mechanism against the infective proteins present in *Phytophthora*. The proteins selected for defence mechanism in plants were osmotin (PR- 5 family) and pectin methyl esterase inhibitor and the proteins selected in *Phytophthora* isolate annotated were nitrogen activated protein kinase and

pectin methyl esterase. Osmotin showed activity towards the nitrogen activated protein kinase of pathogen and pectin methylesterase inhibitor showed activity against pectin methylesterase (cell wall breaking protein) of *Phytophthora*. Apart from this we could also list out some important proteins of *Phytophthora* isolate (IISR) which were required for its pathogenic, biological and metabolic activities.

44. *In silico* docking studies to identify potential inhibitory secondary metabolites from *Pseudomonas putida* against *Phytophthora capsici*

Mr. U. S. Vishnu/Bharathiar University/2012/Guide: Dr. Santhosh J. Eapen

The present study was initiated with the intent of searching for secondary metabolites from a gram negative bacterium, *Pseudomonas putida* having activity against *Phytophthora capsici*. The oomycete focused in this study, *P.capsici*, a major soil borne pathogen that cause great loss in agriculture field. Glucanase Inhibitor Protein from *P.capsici* was exploited as target as this protein serve a major role in pathogen's defence mechanism. In the present study 141 secondary metabolites from *Pseudomonas putida* predicted and reported to have potential antifungal, antiprotozoal activity have been docked with GIP of *P. capsici*. The binding energy scores have been found to be in the ascending order 3-demethylubiquinol-8 <2-methoxy-6-(all-lrans-octaprenyl) phenol < 2- octaprenylphenol <plastoquinol < 6-methoxy-3-methyl-2-all-trans-octaprenyl- I,4- benzoquinol <plastoquinone <hydroxy-IJ-zeacarotene <canavaninosuccinate. From the docked results it was clear that, of the 141 secondary metabolites chosen as ligands, most of them docked well and showed good interaction with the target protein GIP. These highly docked compounds with higher interaction will be a potential inhibitor for GIP and can be a good bacterial compound that can be deployed for control of *P. capsici*.

45. Studies on the effects of plant growth promoting rhizobacteria on bio-chemical and microbial properties of soil under black pepper (*Piper nigrum* L.)

Ms. Reshma Balakrishnan/Amrita University/2011/Guide: Dr. R. Dinesh

A green house study conducted on black pepper (*Piper nigrum* L.) with the treatments comprised of shortlisted PGPR isolates (BRB-3, -13, -23) and different levels of NPK fertilizers (0, 75% and 100%) applied alone or in combinations. The objectives of the study were to determine the effects of PGPR on physicochemical characteristics of soils and to determine their effects on biochemical microbial parameters reflecting soil quality under black pepper (IISR Thevam). The soil physico-chemical characteristics studied were soil pH, soil organic C, mineral N, Bray P,

Exchangeable- K, -Ca & -Mg and the biochemical microbial parameters analyzed were dissolved organic -C (DOC) & -N (DON), microbial biomass -C, -N & -P, soil respiration (SR), N mineralization, metabolic quotient activities of enzymes like dehydrogenase (DH), urease (UR), acid phosphatase, beta-glucosidase (BG) and arylsulphatase (AS). The results on physico-chemical properties of soils revealed that PGPR or NPK had little effects on soil pH, while mineral N, Bray P, exchangeable -K, -Ca & -Mg and SOC accumulated at significantly greater levels in treatments with combined application of PGPR and NPK fertilizers. Dissolved organic-C (DOC) was also positively influenced by the combined application of NPK and PGPR.

46. Development of six linked SCAR marker in *Piper betle* L.

Mr. Anto Paul/Bharathiar University/2009/Guide: Dr. T.E. Sheeja

The molecular markers have been used to identify the sex of the dioecious *Piper betle* in the study. Of the 101 RAPD primers used for the study, three random decamer primers exhibited sexual polymorphism. The OPEO I and OPB20 primers gave a female-specific 403 bp and 468 bp markers respectively and OPE11 gave a male-specific 600bp marker. Since the RAPD technique is having the drawback of poor reproducibility, SCAR markers have been developed to improve the efficiency of the marker in the sex determination. Hence, by the advent of the molecular markers the difficulties in the sex identification of the plantlet in the earlier stage by the use of conventional methods have been solved. Through the above work, it was clear that the technique of RAPD along with the development of SCAR marker is a very powerful tool in the identification of sex in the dioecious plants such as *Piper betle*. The SCAR marker developed from the RAPD markers are more reliable because they can detect only a single locus and their amplification is less sensitive to the reaction conditions. SCAR markers also have the capability to become co-dominant markers which are much effective in the marker-assisted selection.

47. Plant regeneration, genetic transformation and molecular characterization of *Piper betle* L.

Ms. Amritha Vijayan/Vellore Institute of Technology University/2009/
Guide: Dr. K. Nirmal Babu

A simple and efficient protocol for micro propagation and plant regeneration was standardized for *Piper betle* which can be used for production of disase free planting materials. The plant regeneration system can be used for increasing the spectrum of variation through somaclonal variation and genetic manipulation in this

rarely seed setting vegetatively propagated crop for crop improvement. A protocol was partially standardized for *Agrobacterium* mediated genetic trans formation of *Piper betle* for the first time. This needs more time and efforts for completion. Molecular markers can effectively be used for diversity analysis and varietal characterization of *Piper betle* germplasm. With more efforts elven markers associated with sex can be identified for quick selection of productive males among seedling progenies

48. Survey of weed flora in black pepper plantation

Ms. A.V. Sajini/University of Calicut/2009/Guide: Dr. K. Kandiannan

The study was mainly aimed at identify the weed flora present in different pepper fields, identify the most predominant weed species, calculate the density and dominance, and analyse the nutrient (NPK) content of weed species. Weed survey was done by using quadrate and various types of weed species were collected from different pepper fields for identification. Name of the weed species, family, common name and their important features were recorded and presented. There were 51 weed species belonging 47 genera and 31 families were identified, 3 were monocotyledonous, 47 dicotyledonous and 1 terrestrial fern. Dicot species were more common than monocot species. Among the dicot weed species, most predominant weed species were *Ageratum conyzoides* L., *Peperomia pellucida* (Linn.) HB&K, *Spermacoce latifolia* Aiton., *Mimosa diplolricha* L. Monocot weeds were less in number and only 3 monocots were identified. The monocot weed species were *Commelina diffusa* Burm., *Kyllinga monocephala* Rottb. and *Cynodon dactylon* (L.) Pers. *Pteris quadriuarila* Retz. was the terrestrial fern present in the pepper field. *Ageratum conyzoides* L., *Peperomia pellucida* (Linn.) HB&K., *Spermacoce lalifolia* Aubl. and *Oldenlandia auricularia* (L.) F. Muell. were themost densely populated weeds of pepper crop with average density of 8.04, 7.98, 4.34 and 3.72, respectively. The mean data showed that *Ageratum conyzoides* L., *Cynodon dactylon* (L.) Pers. and *Spermacoce latifolia* Aubl. were the most frequently occurring weeds in pepper crop having average frequency of 62%, 62% and 52%, respectively. The maximum nitrogen content was noted in *Eupatorium odoratum* L. (3.85%), followed by *Justicia adhatoda* L. (3.84%), *Mimosa diplotricha* L. (3.42%) and *Centrosema molle* Mart. Ex Benth. (3.37%). The phosphorous (P) and potassium (K) content of weed species ranged from 0.02% to 0.33% and 1.3% to 3.02%.

49. Comparison of leaf dimensions and major nutrient contents in orthotropic and plagiotropic leaves of black pepper (*Piper nigrum* L.)

Ms. V. P. Suhitha/University of Calicut/2009/Guide: Dr. K. Kandiannan

Leaf samples from 30 black pepper varieties were collected from Experimental Farm, Peruvannamuzhi used for the study. The mean of the leaf length was ranged from 8.2cm (Vellamunda) to 18.3 cm (Cheppukulam munda) with the SD of 2.0 and CV of 15.4% in the plagiotrpic leaves collected from bush pepper varieties. Similarly, mean of the leaf length orthotropic leaves was ranged from 8.3 cm (Nedumchola) to 16.4 cm in (Vattamundi), with the SD of 2.0 and CV of 15.3%.The mean leaf width of plagiotropic leaves were ranged from 3.4 cm (Vellamunda) to 9.9 cm (HPI411) with the SD of 2.3 and CV 15.7%.The mean leaf width of orthotropic leaves were ranged from 5.2 cm (Kathirinmel kathir) to 11.8 cm (Panniyur-1) with the SD of 1.6 and CV 20.3%.The leaf area of plagiotropic leaves ranged from 29.8 cm^2(HP 728) to 114.3 cm (Cheppukulam munda) with the SD of 21.7 and CV of 33.3%). The leaf area of orthotropic ranged from 32.1 cm^2 (Kathirinmel kathir) to 123.5 cm^2 (Panniyur 1) with the SD of 23.7 and CV of 31.9%. The mean leaf factor for plagiotropic leaves ranged from 1.4 (Aimpiriyan) to 2.6 (Otaplackal II) with the SD of 0.3 and CV of 17.6% whereas, in orthotropic leaves it ranged from 1.28 (Panniyur 1) to 2. 11 (O.P Karimunda). In plagiotropic leaves of the selected varieties the nitrogen (N) content varied from 2.23% to 2.87%. The maximum N content seen in Neelamundi and Nedumchola. The minimum level present in Cheppukulam munda. Phosphorous content varied from 0.17% to 0.39%. The P content was maximum in Kalluvally and minimum in Panniyur 1 and HP 728. The value of Potassium varied from 0.75% to 3.16% with a maximum in Narayakodi and minimum in Neelamundi. The relationships between leaf width and leaf length both in plagitropic and orthotropic leaves indicated positive. The magnitude of relationship was high (R^2=0.595) in orthtropic leaves compared to plagiotropic leaves (R^2=0.5109). The relationships between leaf area and leaf length, width and leaf factor was worked out and it is indicated that length and width of orthtropic leaf has high magnitude of relation with leaf area than plagitropic leaf. It is also observed that leaf width has better relation with leaf area (R^2=0.8699 in plagitropic leaf and R^2=0.9249 in orthotropic leaf) than length (R^2=0.791 in plagitropic leaf and R^2=0.8131 in orthotropic leaf) in both types of leaves.

50. Type of planting material and growth medium on performance of bush pepper (*Piper nigrum* L.)

Ms. K. Prathyusha/University of Calicut/2009/Guide: Dr. C.K. Thankamani

External application of growth regulators and other treatment might be forcing root initiation, which is not always repeatable. Profuse rooting in lateral cuttings of both Panniyur1 and Panniyur 2 with orthotropic segment was reported, which requires destructive sampling and may be resulted in reduction in yield in the field. Availability of planting materials limit the extension of planted area of bush pepper. Pruning of laterals may be practiced in potted bush pepper to maintain the optimum canopy for photosynthesis and or better appearance. Suitability of pruned branches for propagation was tested to reduce the scarcity of laterals for bush pepper propagation. Preliminary study indicated that laterals of Panniyur 1 collected from black pepper vines grown on standard and dipping in Jiwamrita/dipping in tender coconut water/Indole Butyric Acid were better in terms of growth performance compared to plants grown in pots. However, validity of the present finding may be tested by conducting repeated experiments.

51. Compatibility of endophytic and rhizospheric bacterial biocontrol agents with agrochemicals in black pepper system

Ms. Sasmita Patnaik/Vellore Institute of Technology University/2009/
Guide: Dr. R. Suseela Bhai

When the endophytes and the rhizosphere bacteria were inoculated in media incorporated with different plant protection agrochemicals in recommended dosages, there is distinguishable difference in growth when compared to growth in media without the agro chemicals. Broth assay of these organisms with the plant protection chemicals showed a more realistic data when compared to plate assay. Here the sensitivity was studied in broth culture by measuring the Optical density of the broth culture and also by estimating the colony forming units (CFU). All the organisms showed comparative reduction in CFU when compared to untreated control showing the sensitivity of the organisms towards the chemicals. The study on the survival of the introduced antagonistic bacteria in the soil in the absence or the chemicals showed that endophytes as well as rhizobacteria can colonize the soil and can proliferate at a taster rate. The evaluation of the chemicals *in vivo* showed an entirely diffrent trend root colonization of the endophyte, with different agrochemicals also showed distinguishable variation in colonization. In general, it is found that BP17, BP 25 and IISR 853 are highly sensitive where as TC10, BP 35 and IISR 6 are less sensitive to most of the chemicals. Rhizobacteria IISR 6 and IISR 853 showed high sensitivity towards copper oxychloride and carbendazim - mancozeb where it is less sensitive to other chemicals.

52. Survival of *Phytophthora capsici* and *Radopholus similis* in black pepper soil under integrated disease management system

Ms. Edna Mary Varghese/Vellore Institute of Technology University/2009/
Guide: Dr. R. Suseela Bhai

In this work, six bacterial biocontrol agents *viz*., BP35, BP25, BPt7, TC10 (endophytic) IISR 6 and IISR 853 (rhizospheric) were integrated with agrochemicals *viz*. Metalaxyl-mancozeb (fungicide) and Phorate (nematicide) in six treatments. A treatment with both the agrochemicals (without antagonists) and a control with no treatments were maintained. A field trial laid out in IISR experimental farm Peruvannamuzhy, Calicut, Kerala during July 2008 containing the above treatments was the base of the work. A duplication of the experiment was carried out in greenhouse conditions (challenge inoculated) to check the *Phytophthora* disease incidence. It was found that the treatments containing TC 10+Metalaxyl-mancozeb and BP25+phorate is effective in reducing *R. similis.* Population in field conditions and treatments containing BP 25 + phorate and BP35+phorate is effective in reducing the *Phytophthora* foot rot disease incidence in greenhouse conditions. The work suggests that the integration of TC 10 and BP35 along with chemicals may be a suitable combination in IDM tor combating both *P. capsici* and nematode population in black pepper system.

53. Detection of *Piper* yellow mottle virus in *Piper* germplasm accessions by PCR

Ms. K. P. Shameeba/Calicut University/2008/Guide: Dr. A. Ishwara Bhat

The study focused on the detection of PYMoV in *Piper* germplasm accessions. PCR was done for the detection of PYMoV. PCR was successful in amplifying the PYMoV and a product of expected size was observed in the infected sample. The PCR based indexing developed can be used in nursery certification programme to identify PYMoV free plants. The method would also be usefull for detection of the virus in planting materials. Screening of germplasm to identity resistance source, detection in potential weed host and vectors and epidemological studies. Indexing by PCR successfuly detected PYMoV in infected plants showing no visible symptoms. Of the 265 symptom Less plants, 53 plants showed positive reaction to PYMoV. This clearly indicates that PCR method can be used to identify PYMoV free plants.

54. Performance of rooted cuttings of black pepper (*Piper nigrum* L.) varieties raised from nodal positions

Ms. S. Anchu/University of Calicut/2008/Guide: Dr. C.K. Thankamani

The present study was conducted to compare performance of three improved black pepper varieties Girimunda, Panniyur 5 andMalabar excel raised from nodal

positions from bamboo splits. The experiment was carried out in two factor randomized block design. Four improved varieties *viz*., Girimunda, Malabar Excel and Panniyur- 5 formed one factor. Ten nodal segments (positions) replications were there. Internodal length of black pepper varieties was higher in 9th node that was on par with 8th, and 10th nodes and the variety Girimunda recorded maximum internodal length. Maximum Leaf area was recorded by Panniyur- 5 that was on par with Malabar excel whereas number of root production was high in Malabar excel followed by Panniyur-5 Among the varieties maximum biomass was recorded by Malabar excel that was on par with Panniyur-5. No specific pattern was observed with regard to carbohydrate content invarious nodal positions. However, with regard to stem and root carbohydrate content, Malabar excel was on par with Panniyur-5, whereas highest leaf carbohydrate was recorded by Girimunda. Results revealed that most of the biometric characters such as height of rooted cuttings, number of leaves, number of roots, and biomass were not significant at 90 DAP. Maximum length of roots was recorded by 9thnode position that was onpar with l0th and 1st node and the variety Malabar excel had maximum length of roots at 90 DAP. Among the varieties, Panniyur- 5 had higher leaf area, and number of' roots. Significantly higher biomass was recorded by Panniyur- 5 that were onpar with Malabar excel. No specific pattern with regard to carbohydrate, starch and phenol content was observed in plants raised from various nodal segments. However, variety Malabar excel had higher carbohydrate content in stem, and root whereas maximum. Starch content in stem recorded by Panniyur- 5. Variety Girimunda had maximum stem and leaf phenol content whereas variety Panniyur- 5 had higher phenol content in roots. Nitrate reductase (NR) activity was higher in 1st node that was on par with 5th node. Among the variety Girimunda had maximum NR activity followed by Panniyur- 5. Regarding NPK concentration no specific trend was observed in plants raised from varius nodal segments. However, variety Malabar excel had higher N&P concentration whereas K concentration was higher in Panniyur- 5. Higher P & K uptake was observed in 9th nodes that were on par with 8thand Ist node, and the variety Panniyur- 5 had maximum P&K uptake. Higher Ca & Mg uptake was observed in 9th node that was on par with 8th and the variety Malabar excel had maximum Ca uptake whereas Panniyur- 5 recorded higher Mg uptake followed by Girimunda.

55. Changes in phenylpropanoid pathway enzymes in response to infestation of black pepper seedlings with *Radopholus similis*

Ms. K. P. Dhanya/Bharathidasan University/2008/Guide: Dr. Shamina Azeez

In the present study, phenylalanine ammonia-lyase (PAL) activity increased in inoculated/wounded leaves within one week to agreater extent than in 48 hrs. PAL activity increased in the roots by almost the same level within 48 hrs and one week after inoculation. The root samples on wounding showed a similar trend of increase. PAL activity increased to a greater extent in the inoculated root when compared to inoculated leaf. No significant change in Butadiynyl (C4H) activity was observed between the control and treated samples of black pepper roots and leaves. The root and leaf cinnamic acid 4-hydroxylase (C4H) activities were comparable. Catechol-O-Methy ltransferase (COMT) activity increased only in leaf samples 48 hours after inoculation. COMT enzyme activity was not detectable in leaves 48 hours after wounding, however, one week after wounding the activity increased. COMT activity decreased in the root samples in 48 hours after inoculation and further decreased by one week after inoculation over control. COMT activity in control roots was over twice that of the leaf activity.

56. Genetic transformation of black pepperusing *Piper* yellow mottle virus (PYMoV) sequence

Ms. N. P. Shaini/Bharathidasan University/2008/Guide: Dr. A. Ishwara Bhat

The present study was aimed for the *Agrobacterium* mediated transformation of black pepper explants using a portion of *Piper* yellow mottle virus (PYMoV) ORF - III region as transgene. The 409 bp ORF-III region of PYMoVS construct was prepared in binary vector pBI121. The presence of 409 bp insert of ORF-III of PYMoV in pBI121 - PYMoVS in transferred *E. coli. Agrobacterium* was confirmed by PCR and restriction analysis. Nodal explants of *in vitro* and green house shown plants of black pepper were used for establishment of mother culture. A total 402 nodal explants were directly placed in WPM. A high percent of survival was seen with *in vitro* explants (65%) compared to explants collected from green house (20%). Explants (leaves, internodes, petioles) were treated with the *Agrobacterium* harboring pBI121- PYMoVS. Totally 1112 explants from *in vitro* and 955 green house (both tender and mature, explants) were used for transformation. Explants were first transferred to the petridishes containing the SH medium and incubated at 28°C for two days. After two days of incubation explants were washed with sterile distilled water containing cephotaxime and streptomycin to kill *Agrobacterium* and other contaminating bacteria in the medium and explants were

placed in SH medium. In the case of *in vitro* explants 225 (46%) of leaf, 24 (8.6%) of internode, and 66 (18.5%) of petiole explants survived till 15 days. Majority of green house explants were lost due to fungal, endogenous bacterial contamination and browning due to high phenolic production in explants. Explants survived for 15 days were transferred to both MS and B5 regeneration medium containing different phytohormones. A total of 225 leaf, 24 internode and 66 petiole explants from *in vitro* were transferred to different MS and B5 regeneration medium. Different types of calli formed in different media combinations during 45 days. A hard green calli was found in 4 combinations ie MS_1, MS_2 Ms_4 and MS_5. Three media combinations gave white powdery callus that is MS_3, MS_6, MS_9 and green callus in three combinations. BAP (1.0, 3.0, 5.0,10.0 mg/L) alone or in combination with NAA (0. 25 and 1.0 mg/L), IAA (1.0 mg/L) and TDZ (0.1 mg/L) were used. NAA (0.25 mg/L) in combination with Kinetin (1.0 and 2. 0 mg/L) was used in a separate combination. A protocol was developed for isolation of total DNA from black pepper and peR for the detection of PYMoV. This method can be used to screen the transgenic plants for the presence of PYMoV ORF-III insert.

57. Major chemical constituents present in the volatile oil of leaf and berry of black pepper (*Piper nigrum*) cultivars- a comparative study

Mr. Safeer Abdul Lathief/Mahatma Gandhi University/2008/
Guide: Dr. T. John Zachariah

In the present study leaf and berry samples of black pepper cultivars grown at IISR experimental farm Calicut and PRS Panniyur were compared for the primary metabolites and secondary metabolites. Major primary metabolites studied are total starch, total free amino acids, total carbohydrate, total phenols and protein. Major secondary metabolites studied are oil, oleoresin, piperine and leaf oil constituents. The study revealed many facts. Total phenol, total amino acids and leaf protein are in very low concentration in the leaves compared to berries at both locations. Between locations cultivars grown at IISR farm Calicut showed more concentration compared to PRS Panniyur. Piperine content of leaf was very low compared to berries.

58. *Agrobacterium* mediated transformation of black pepper using *Piper* yellow mottle virus (PYMoV) sequence

Mr. Niranjan Ganga Hegde/Periyar University/2008/Guide: Dr. A. Ishwara Bhat

The present study was aimed for the *Agrobacterium* mediated transformation of black pepper using a *Piper* yellow mottle virus sequence. The presence of 409 bp insert

ORF III in pBII21-PYMoVS construct of *Piper* yellow mottle virus was confirmed by PCR and restriction analysis. The construct of *Piper* yellow mottle virus pBII21-PYMoVS was successfully transferred to the *Agrobacterium tumefaciens* EHAI05. Well-isolated transformed colonies were found in the YEB selection medium containing kanamycin and rifampicin after the mobilization of pBI 121 -PYMo VS into *Agrobacterium tumefaciens* EHA I05 by triparental mating. The presence of insert ORF III portion in pB121-PYMoVS was confirmed by the PCR analysis and restriction analysis.

59. Effect of pH and moisture on the survival of *Trichoderma harzianum* and *Phytophthora capsici* with reference to black pepper (*Piper nigrum* L.)

Ms. Sithara Raj/Bharathiyar University/2008/Guide: Dr. R. Suseela Bhai

The present study was aimed to understand the ecological factors responsible for the survival of *Trichoderma* and its proliferation in the soil so as to use them effectively for the biological control of foot rot disease of black pepper caused by *P. capsici*. In this respect it is essential to understand the ecology of the pathogen as well as the host under similar conditions. Data on abiotic factors that influence the target biocontrol organisms is a prime requisite for large scale field adoption. Black pepper is favouring acidic soil (pH 5.0- 6.5) with adequate organic matter content for its healthy growth. The seraphic factors vary in different agro climatic conditions which will have profound impact on the soil microflora which in turn affect the growth of the plant. An unbalaned environment will lead to unhealthy soil conditions which will adversely affect the growth of the plant. Most diseases develop under these unbalanced conditions. Ecological fitness of biocontrol agents with back ground knowledge on the pathogen biology and crop cultivation would help in standardization of strategies for greater adoption and functioning of biocontrol agents for disease management. Environmental parameters that control the survival and activity of introduced biocontrol agents are less studied. These ecological factors are the basis for predicting the performance and modifying the soil environment for extracting the better activity of biocontrol agents to obtain acceptable level of disease control. So information on the ecological factors that influence the biocontrol agents is essential.

60. Isolation and partial characterization of black pepper pericarp degrading bacteria

Mr. V. Vinod/Acharya Nagarjuna University/2007/Guide: Dr. A. Kumar

Attempts were made to isolate indigenous bacteria from black pepper associated microbial niches. Forty five bacteria representing diverse group of bactcria were isolated from decomposing black pepper pericarp, soil used for decomposing black pepper pericarp, and commercial white pepper. When evaluated for fermentation of black pepper, eight of them showed promise for production of white pepper. Fermentation temperature was optimized to be 35°C for microbial decortication of black pepper. Boiled pepper was found amenable for satisfactory decortication of black pepper when compared to raw pepper. Among the eight bacterial strains *Bacillus* spp. was dominating as identified by phenotypic and biochcmical methods. Rep-PCR and ARDRA based molecular characterization revealed the diversity of the fermenting bacteria. Short listed strains produced extra cellular enzyme such as cellulase, pectinase, amylase and protease, the major enzymes required for the decortication of pericarp. *Bacillus* species produced all the four enzymes. Further investigations are required to draw conclusions about the species level identity of the bacteria, the strains with the highest potential for the eventual inclusion in the starter culture and nature of thc enzyme produced by the efficient strains and their quantity required in the proccss of decortication.

61. Attempt to identify a physiological marker for disease diagnostics in black pepper

Ms. P. Dilu/Bharathiyar University/2007/Guide: Dr. K. S. Krishnamurthy

Present study is an attempt to identity a physiological marker for disease diagnostics in black pepper variations in the genus *Piper nigrum* with regard to their biochemical constituents and enzyme activities. Two pepper species namely, Panniyur 1 and cv Karimunda used in the studies. The contents of total carbohydrates, starch, reducing sugars, proteins, amino acids and phenols were estimated in the leaves of pepper leaves collections maintained at Indian Institute of Spices Research, Kozhikode. Among the two species, total carbohydrates and starch were maximum in the leaves of *Piper nigrum* where as the amino acids, starch, reducing sugars, chlorophyll, nitrate reductase, protein and total carbohydrates was maximum in Panniyur 1 healthy pepper variety the total phenols were maximum in Karimunda variety. The activity of the enzyme peroxidase was highest in the leaves of pepper, which was followed by Karimunda and Panniyur. The activity of polyphenol oxidase and superoxide dismutase was maximum in the leaves of Panniyur 1. The highest polyphenol oxidase activity in

Panniyur was supported by the disease of pepper leaves. Four peroxidase isozymes were observed in the leaves of two peppers pecies studied. Bands corresponding to Em values 0.3, 0.25, and 0.2 were present in Panniyur and Karimunda. Isozyme profile of polyphenol oxidase in the leaves of pepper species indicated 5 molecular forms with Em values 0.05, 0.075, 0.15, 0.20, and 0.60.There was no common band in the polyphenol oxidase isozyme profile of the two pepper species.

62. DNA Profiling of black pepper (*Piper nigrum* L.) cultivars using Inter Simple Sequence Repeat (ISSR) markers

Ms. Sini Raju/Mahatma Gandhi University/2007/Guide: Dr. K. Johnson George

In the present study, 12 varieties and seven cultivars were used. A total of 10 ISSR primers were used to differentiate various cultivars / varieties of black pepper conserved in the IISR germplasm. The ISSR PCR with thier action components 10x PCR (Ix), $MgCl_2$, dNTPs (10 mM), Tag polymerase (2SU), template DNA (30 ng), Primers (10 picomoles) in 15 µl reaction volume resulted in the successful amplification of genomic DNA from different plants. The primers evaluated showed less polymorphism among the various individuals.

63. Molecular analysis of microbial diversity and structure in soils of the rhizosphere of various standard - black pepper systems by RFLP techniques

Ms. M. Chenchitha/Acharya Nagarjuna University/2006/
Guide: Dr. R. Dinesh & Dr. T.E. Sheeja

The present study was conducted to determine the microbial community structure in soils of the rhizosphere of standard - BP systems through DNA finger printing by PCR and RFLP. Relevant physico- chemical and biochemical characteristics of these soils were also studied. For the study, soil samples (0-30 cm) were collected from the rhizosphere of *Erythrina indica* - BP*, Garuga pinnata- BP, Gliricidia sepium* -BP*, Ailanthus malabarica* – BP and Reinforced Cement Concrete (RCC) pole- BP systems. The major focus of the study was to standardize a simple and rapid soil DNA extraction protocol and optimal PCR conditions for detection through RFLP and to detect possible shifts in the rhizosphere microbial community structure vis-a-vis various standard- BP systems by PCR and RFLP. Relevant physico-chemical (pH, organic carbon, available N, Bray P, exchangeable K, DTPA-extractable Zn, -Cu, -Mn and -Fe) and biochemical microbial biomass carbon (CMIC), basal respiration (CO_2 evolution), metabolic quotient (qCO_2) and dehydrogenase activity characterization of these soils also formed a part of the study.

CARDAMOM

1. **Cloning and Partial sequencing of banana mosaic virus associated with clorotic streak disease of cardamom**

Ms. A. Gopika/Kannur University/2017/Guide: Dr. A. Ishwara Bhat

Virus causing chlorotic streak disease of cardamom was partially characterized on the basis of viral genome nucleotide properties. The viral genome was amplified using reverse transcription- polymerase chain reaction, cloned and sequenced. Sequenced region contained 3416 nucleotides potentially coding for 1138 amino acids. Sequence analyses with available three BBrMV infecting isolates from India and other parts of the world showed an identity ranging from 94.8-97.4% and 97.8- 98.9% at nucleotide and amino acid respectively. Analyses with BBrMV isolates from other host revealed the greatest identity with banana isolate and the phylogram clearly indicated that banana isolate from India is more closely related to the present isolate.

2. **Studies on bacterial endosymbionts of cardamom thrips, *Sciothrips cardamomi* (Ramk.) (*Thripidae: Thysanoptera*)**

Ms. V. Rajalakshmi/Amrita University/2011/Guide: Dr. T. K. Jacob

In the present study, attempts were made to characterize and document the endosymbiotic bacteria present in cardamom thrips through culture- dependent methods. The bacteria were identified using biochemical, molecular and Biolog methods. In arthropods, thelytoky is a form of parthenogenesis where unfertilized eggs give rise to females. It is very common in hymenopteran insects. The thelytoky is often associated with the presence of *Wolbachia* bacterium. Parthenogentic thrips or haplodiploid mites are considered more important groups in which to look for parthenogenetic *Wolbachia*. In the present study attempts were made to detect the presence of *Wolbachia* in cardamom thrips, by amplifying *Wolbachia* specific gene. Culture dependent study of the bacterial endosymbionts of cardamom thrips detected three bacterial associates in them. The biochemical, molecular and Biolog methods used to characterize the bacteria identified them as *Enterobacter cloacae*, *Bacillus subtilis* and *Bacillus pumilus*. The endosymbionts remained the same in adults and larvae and thrips collected from two locations. This has become the first report on the detection of *Wolbachia* in cardamom thrips. Uses of conventional techniques that relay

on the culturing of microorganisms on defined media have itsown disadvantages. Documentation of endosymbiotic bacteria and study of the interaction of these bacteria with the host insects could lead to development of innovative strategies for effective management of insect pests of crop plants.

3. Characterization of shoot borer (*Conogethes punctiferalis*) infesting cardamom, ginger and turmeric through RAPD

Ms. K. Divya/Bharathiar University/2010/Guide: Dr. A. Ishwara Bhat

The present study was focused on the characterization of shoot borer infesting cardamom, ginger and turmeric through RAPD. Good quantity DNA was isolated from shoot borer larvae infesting cardamom, ginger, and turmeric using Fast method modified by Beye and Raeder (1993). RAPD technique was successfully employed for characterizing shoot borer infesting cardamom, ginger and turmeric. A total of twenty-seven decamer primers were selected for RAPD analysis. Out of the twenty-seven decamer primers twenty primers produced polymorphic bands and thirteen primers produced monomorphic bands. When the percentage similarity was compared using the formulae of Nei and Li (1979) ginger and turmeric shoot borer was found to be more similar with 54%, while cardamom and ginger shoot borer are 49% similar and cardamom and turmeric shoot borer are less similar with 40%. Characterization of shoot borer infesting cardamom, ginger and turmeric collected from different locations can also be done by using RAPD technique.

4. Chemical composition of cardamom and its antioxidant potential

Ms. Leena Joseph/Bharathiar University/Coimbatore/2009/Guide: Dr. N.K. Leela

In the present study selected promising accessions, hybrids and new collections of cardamom germplasm were evaluated for their quality attributes and the antioxidant activity of few cardamom accessions was determined. The chief quality determinant in cardamom is its essential oil yield and composition. Out of the sixty two accessions evaluated sixteen accessions recorded above 6% oil yield in cardamom capsules. The chemical constituents of the oil were determined by GC-MS analysis.1, 8- cineole and α-terpinyl acetate were the major constituents in the oil. Based on the chemical composition of the essential oil, the accessions were classified into1, 8-cineole-rich and α-terpinyl acetate rich types. In selected accessions oleoresin content and its constituents were determined. The oleoresin was mainly constituted by α terpinyl acetate and α terpineol. The essential oil of cardamom showed promising antioxidant activity by three different assays.

5. **Partial characterization of the virus associated with foorkey disease of large cardamom (*Amomum Subulatum* Roxb.)**

Ms. V. Sangamitra/Acharya Nagaraja University/2008/Guide: Dr. A. Ishwara Bhat

Virus causing foorkey disease of large cardamom in India was partially characterized on the basis of replicase gene nucleotide properties. A protocol for total DNA isolation from large cardamom infected with foorkey was standardized. Replicase gene of the virus was amplified using Polymerase Chain Reaction (PCR), cloned and sequenced. Sequenced region contains 552 nucleotides potentially coding for 184 amino acids. Sequence analysis with BBTV isolates showed an identity ranging from 92-99% and 93-99% at nucleotide and amino acid respectively. Since LC is showing maximum identity with BBTV isolates, it is considered as a strain of BBTV.

GINGER

1. **Effect of arbuscular mycorrhizal inoculum on seedling growth and quality traits of ginger**

Ms.P.K. Adheeba /University of Calicut/2022/Guide: Dr. C. Sarathambal

The present study investigated the effects of different doses of arbuscular mycorrhizal (AM) fungus inoculum and vermicompost on growth, and quality parameters of ginger (*Zingiber officinale* Rosc.) were evaluated. AM colonization of adventitious roots has potential to enhance ginger growth and tillers. In this study, 50 g AM + 50 g vermicompost increased plant height, number of tillers and yield parameters when compere with control. However, higher dry biomass (61 g) has reported with 75 g AM+75 g vermicompost treatment. As expected, the application of arbuscular mycorrhizae (AM) positively affects spore count and mycorrhizal dependency percentage ranging from 58 to 70.5 spores per 50 g substrate and 19-36% respectively. Biochemical analysis of AM inoculated rhizomes showed that improved production of secondary metabolites like phenols, flavonoids, essential oil and crude fibre content. On the whole the study proves that arbuscular mycorrhizae are a suitable bioinoculant in ginger production for increasing growth and quality of ginger.

2. **Molecular studies on *Zingiber officinale***

Ms. P.K. Arshyadevi/Bharathiar University/2022/Guide: Divya P. Syamaladevi

In this study we have conducted molecular investigations on the Xyloglucan transglycosylase (XET) gene family through *in silco* and *in vitro* approaches. First, we performed a complete sequence-based mining in the whole proteome of ginger and the

complete set of homologues were curated using Conserved Domain searches and CDHit Analysis. This study identified 134 members of the XET family proteins in ginger. The phylogenetic relationship between these sequences were analysed using Molecular Evolutionary Genetics Analysis (MEGA) tool. Here, 40 hypothetical unannotated sequences of ginger were classified and annotated to XET family proteins. The phylogenetic analysis identified six major clusters of XETs. These findings can be taken forward to identify the diversity in conservation patterns among these identified clusters of XET.

3. Use of edible coating for enhancing the shelf life of fresh ginger under ambient and cold storage conditions

Ms. R. Abhirami/Kerala University of Fisheries and Ocean Studies/2021/
Guide: Dr. E. Jayashree

The effect of lac-resin based edible coating, sterilization with sodium hypochlorite and storage condition was studied for enhancing the shelf life of fresh ginger. Incidence of disease was maximum of 3.25% in treatment T1A(no coating and no sterilization) under ambient condition and minimum of 0.89% in T12C (15 minutes sterilization and 10% lac resin-based coating) under cold condition after 120 days of storage. The effect of the components used in the treatments were studied for its efficacy in controlling those fungi affecting ginger during storage. Growth inhibition study showed that Sodium hypochlorite (NaOCl) (5%) exhibited 100% inhibition of both *Macrophomina* sp. and *Pythium* sp. while it exhibited only 13.3% inhibition of *Fusarium* sp. Coating formulation (5%) showed 58.82%, 88.63%, 6.6% inhibition of *Macrophomina* sp, *Pythium* sp, and *Fusarium* sp respectively. Coating formulation (10%) showed 100%, 94.31%, 33.3% inhibition of *Macrophomina* sp, *Pythium* sp, and *Fusarium* sp respectively. The antifungal properites assessed by poison food assay in *Macrophomina* sp. revealed 100% inhibition by both NaOCl (5%) and coating formulation (10%) while 59% inhibition by coating formulation (5%). Poison food assay in *Fusarium* sp. revealed 13.3%, 6.6%, 33.3% inhibition at 9th day after inoculation using NaOCl (5%), coating formulation (5%) and coating formulation (10%) respectively. Poison food assay in *Pythium* sp. revealed 100%, 88.6%, 94.3% inhibition at 3rd day after inoculation using NaOCl (5%), coating formulation (5%) and Coating formulation (10%) respectively. Thus the treatment T12C ie., the ginger stored under cold condition, coated with 10% coating fomulation couple with 15 minutes of NaOCl (5%) sterilisation exhibited better storage properties and was able to extend the shelf life by 4 months.

4. Assessment of genetic fidelity of ginger micro rhizome using SSR and ISSR markers

Ms. K. C. Chaithanya/Bharathiar University/2021/Guide: Dr. Sharon Aravind

Molecular techniques play an important role in determining the genetic fidelity within the species. In the present study assessment of genetic fidelity of micro rhizomes of ginger using SSR and ISSR markers, genetic stability of micro rhizomes of ginger with that of mother parent and subsequent generations (V1 and V2) were analyzed. Also, an attempt was made to find the occurrence of any variability in different stages of sub culture. The variety of ginger taken for study was IISR Varada. The samples were field sample (mother plant in the field), four subcultures (1, 2, 7 and 8 stages), micro rhizomes, V1 and V2 generation pro tray plants. The analysis was carried out using 5 SSR primers and 5 ISSR primers. A total of 343 bands were observed in the study. The distinct bands were produced of size ranging from 100-3100 bp. High degree of genetic uniformity was obtained by employing marker analysis. This indicates that same genomic stability is maintained in field plant as well as in *in vitro* micropropagated plants, micro rhizomes and in their next two generations. It was inferred that the sub cultured samples, V1 generation sample, V2 generation sample and leaf samples of micro rhizomes were genotypically similar to that of mother parent (sample taken from the field) and confirmed clonal fidelity. Also, somaclonal variation was absent in different stages of sub culture which indicates homogenesity of regenerants produced by micro propagation techniques. SSR and ISSR primers are informative and feasibly convenient in validating the uniformity assessment.

5. Optimization of duplex RPA assay for simultaneous detection of *Pythium* spp. and *Ralstonia pseudosolanacearum* from ginger rhizomes

Ms. K. C. Punya/Cochin University of Science and Technology/2021/
Guide: Dr. A. Jeevalatha

Recombinase polymerase amplification (RPA) is a rapid, isothermal amplification method with high specificity and sensitivity. Owing to minimal sample-preparation requirements, low operation temperature, and commercial availability of freeze-driedr eagents, this method has been applied outside laboratory settings and in remote areas. In this study, duplex RPA assay was developed for the rapid detection of *Pythium* spp and *Ralstonia pseudosolanacearum* in ginger rhizome for the first time.The duplex RPA assay could simultaneously detect *Pythium* spp and *Ralstonia pseudosolanacearum* and is simple, time saving, sensitive and specific method. Duplex RPA developed in the present study were validated using field samples confirming its

utility in testing the *Pythium* and *Ralstonia pseudosolanacearum* in field samples. This would help in identifying *Pythium* and *Ralstonia pseudosolanacearum* -free rhizome for propagation and further distribution to farmers that would ultimately check the spread of the pathogens.

6. Morphological and molecular characterization of ginger pathogens and development of multiplex PCR assay

Ms. Ashika George/Kerala University of Fisheries and Ocean Studies/2021/
Guide: Dr. A. Jeevalatha

In the present study, fungal and bacterial pathogens from ginger rhizomes collected from different regions in Kozhikode and Wayanad were isolated. The pathogens were identified as *Fusarium oxysporum*, *Pythium myriotylum*, *Sclerotium rolfsii*, *Macrophomin a phaseolina*, *Colletotrichum* spp and *Ralstonia pseudosolanacearum* using morphological characters and by sequencing ITS regions of fungal pathogens and also an upstream gene region of *Ralstonia pseudosolanacearum*. A multiplex PCR assay was developed to detect *Pythium myriotylum, Sclerotium rolfsii, Macrophomina phaseolina* and *Ralstonia pseudosolanacearum*. The developed assay was highly specific and did not show any cross amplification with other pathogens tested. Four fungicides were tested against these pathogens and found that carbendazim + mancozeb or metalaxyl + mancozeb combinations can be used to manage these pathogen infections in ginger.

7. Morphological andmolecular studies on fungi populationin dry ginger (*Zingiber officinale*) and its management using elicitor treatment

Ms. V. Jisni Johnson/Mahatma Gandhi University/2019/Guide: Ms. R. Sivaranjani

In this study we investigated storage diseases causing fungi in dry ginger. Eight isolates were obtained from non treated dry ginger samples. Microscopicaly and cultural characteristics were used to identify that seven *Aspergillus* sp. and one *Fusarium* sp. Further identification done by ITS PCR analysis. ITS regions of fungal ribosomal DNA (rDNA) are highly variable sequences of great importance in distinguishing fungal species. The isolated DNA were amplified using ITS1 and ITS 4 primers. The amplified fragments were run in agarose gel and purified using Gel elution kit. The purified fragments were once again run in agarose gel and were used for sequencing. Another part of the study, to treat the fresh ginger samples with chitosan and salicylic acid to control the opportunistic fungal infections. We have taken IISR Mahima variety of fresh ginger for this experiment. We used three concentrations of chitosan (100 mg/mL, 200 mg/mL, and 300 mg/mL) and salicylic acid (0.1 mM, 0.5

mM, 1mM) in two-time intervals. Treated samples were used for enzyme extract preparation at 0^{th}, 1^{st}, 2^{nd}, 3^{rd} and 4^{th} hour of treatment. The enzymes extracts were used in the assay of some of the antioxidant enzymes like catalase, peroxidase and superoxide dismutase. In general, treated samples showed better peroxidase activity than catalase. In case of SOD assays proved that chitosan and SA treatments increased their activity in various degrees as compared to control group. After the rhizomes are dried, the dry weight of the samples is noted down and dry recovery was calculated. There found no significant variation in dry recovery of treated and control samples. Quality characterization of dry ginger we found that rhizomes of chitosan at 200 mg/mL concentration treated for half an hour and SA at 0.5 mM concentration treated for one hour showed highest total phenolic content (TPC) as compared to control. The same was the case with total flavonoid content. In addition to the aforementioned treatments, SA at 0.1mM concentration treated for half an hour showed highest total flavonoid content. The results shows the treatment influence the enzymatic activity of treated rhizomes compared to control. The increase in their enzymatic activity is the indicator of activation of defense signaling pathways proteins and induction of antioxidant machinery. This study needs further confirmation by population study of treated samples. We check it some treated samples that shows reduce the fungi load. So, the treated samples after drying were kept in storage and it will be monitored for fungal contamination at regular interval.

8. Chemoprofiling of selected ginger genotypes

Ms. Nasrin Nazar/Mahatma Gandhi University/2019/Guide: Dr. N.K. Leela

The current work attempted to study the chemical profiling of Ginger by its essential oil, oleoresin and crude fibre content. The salient findings of the study are: Essential oil content of 122 accessions of ginger, which contained 1.0–3.0% essential oil. Highest oil was obtained from rhizomes of accession Nos.G282 and G396. Oleoresin content of selected ginger accessions varied between 2.85% and 10.1%, with highest in G282 followed by G5 (8.39%). G282 is identified as a promising accession with high essential oil and oleoresin. Crude fibre content of selected ginger accessions varied between 3.19% and 8.6%, with highest in G 848 followed by NGC 31 (8.2%) and G 222 (8.1%). The high fibre accessions identified, (G 848, NGC 31 and G 222) could be used for making dry ginger. G512 with low fibre (3.19%) and low oil and oleoresin could be used for making candy. Thirty nine constituents of essential oil were characterised by GC- MS analysis. The major constituents were zingiberene, Beta

sesquiphellandrene, Ar-curcumene, alpha farnesene, camphene, Beta citral, Alpha citral, Beta bisabolene and linalool. Zingeberene is the major constituent of ginger essential oil which is responsible for the flavour of ginger. Genotype G396 shows high Zingeberene (30.08 %) and β- Sesquiphellandrene content. The pungent principle of ginger was analysed by using High Performance Liquid Chromatography (HPLC). Four constituents of methanol extract, namely,6-gingerol, 6-shogaol, 8- gingerol and 8-shogaol were identified. 6-Gingerol is the major constituent responsible for the pungency of ginger. G282 shows high 6- gingerol content.

9. Mechanism of action of calcium chloride aganist *Ralstonia pseudosolanacearum*, the ginger bacterial wilt pathogen

Ms. Ammu Raj/Mahatma Gandhi University/2019/Guide: Dr. R. Suseela Bhai

In this research, the mechanisms of action of calcium chloride against *R. pseudosolanacearum* were investigated along with its antibacterial activity against *R. pseudosolanacearum.* $CaCl_2$ at a concentration of 3% was highly effective against *R. pseudosolanacearum* and significantly inhibited the growth. The minimum bactericidal concentration and minimum inhibitory concentration values for $CaCl_2$ was found to be 3 %. Further investigation of the mechanism of action of $CaCl_2$ via scanning electron microscopy assays indicated that the destruction of the cell structure and biological assays indicated the inhibition of biofilm formation and swarming motility due to $CaCl_2$. Taken together, these findings suggest that $CaCl_2$ exhibits strong antibacterial activity against *R. pseudosolanacearum* and has the potential to be applied as an effective antibacterial agent for controlling bacterial wilt caused by *R. pseudosolanacearum.*

10. Characterization and evaluation of bacterial isolates for growth promotion and disease suppression in ginger

Ms. K. Srekha/University of Calicut/2019/Guide: Dr. R. Praveena

In the present study on characterization and evaluation of bacterial isolates for growth promotion and disease suppression in ginger, 18 bacterial isolates including seven bacterial isolates from turmeric rhizosphere soils and 11 isolates from turmeric rhizosphere soils were characterized and evaluated. Among the isolates evaluated, based on growth promoting traits like IAA production, ammonia production, and HCN production and mineral solubilization tests the isolates IISRGB7(3) (*Bacillus* sp.) and IISRTB4 (*Bacillus safensis*) were found to be the most promising isolates formineral solubilization and growth promotion. *In vitro* studies on antagonism againstmajor fungal pathogens of spices showed that the isolates IISR GB1 (*Pseudomonas*

aeruginosa), IISR GB2 (*Bacillus cereus*) and IISR GB7 (3) were the most promising isolates. Under greenhouse conditions also IISR GB7 (3) (*Bacillus* sp.) was effective in preventing diseases development by *P. myriotylum*, which indicates that the bacteria can be further, tested under field conditions for its efficacy. The isolate IISR GB7 (3) (*Bacillus* sp.) was also found to be a promising mineral solubilizer also. The identified bacteria holds great promise as a viable alternative to chemical inputs and can be integrated into appropriate nutrient management and disease management schedules for ginger.

11. Performance evaluation of mechanical washer cum peeler and slicer for ginger (*Zingiber officinale*)

Mr. P. M. Murshid/Maharaja Ranjit Singh Punjab Technical University/2019/
Guide: Dr. E. Jayashree

Ginger (Variety IISR-Rejatha) for the experiment was collected from the ICAR-IISR Kozhikode. About 60 kg of ginger was used for the experimental purpose. Washing and peeling of raw material was done by ginger washer cum peeler machine purchased from Pilotsmith India Pvt. Ltd, Thrissur, Kerala and installed at ICAR-IISR Experimental Farm, Peruvannamuzhi. Experiments on mechanical washing of ginger were conducted for three varying loads of 5, 10 and 15 kg for 5 varying washing duration of 2, 3, 4, 5 and 6 min and the washed ginger was evaluated for its washing efficiency and bruise index. Experiments on peeling of ginger were performed using a mechanical peeler (Pilotsmith India Pvt. Ltd) operated using for three varying loads of 5, 10 and 15 kg for 5 varying peeling periods of 2, 4, 6, 8 and 10 min and the peeled ginger was evaluated for its peeling efficiency. The washing of ginger was effective when the inner stainless-steel drum mounted on the central shaft and the outer drum rotated at varying speeds of 151 rpm and 27 rpm in the same direction. Peeling of ginger was found effective when the inner drum mounted on the cental shaft and the outer drums rotated at varying speeds of 151 rpm and 27 rpm in the opposite direction. The washed and peeled ginger was dried in a solar tunnel dryer of size 12.14 m × 4.15 m and height of 2.67 m covered with 200 microns UV- stabilized transparent polyethylene sheet. The loss in mass during drying was recorded and the drying characteristics were calculated. The physical and biochemical properties of the dried ginger were evaluated. The results of the study showed that the maximum washing efficiency was obtained when the washing duration was 6 min for all the three drum loads evaluated and varied from 98.68 per cent for drum load of 5 kg to 98.10 per cent for drum load of 15 kg. Bruise

index was maximum for washing time of 6 min for all the three drum loads evaluated and was found to vary from 13.2 for drum load of 5 kg to 17.50 for drum load of 15 kg. The peeling efficiency was maximum for peeling duration of 10 min for all the three drum loads evaluated and it was found to vary from 58.10 per cent for drum load of 5 kg to 62.60 per cent for drum load of 15 kg. Among the five peeling durations and three drum loads studied, minimum drying time was required when ginger was mechanically peeled for 10 min and when the drum load was 15 kg. The drying characteristics curves indicated that moisture content during drying decreased exponentially with increase in peeling time and the reduction of moisture content was faster in the initial period of drying and decreased towards the final periods. The initial moisture content of the fresh turmeric was found to be 76.5 (325 db) per cent and reduced to 10.00 per cent by solar tunnel drying. The physical and biochemical quality parameters of peeled dry ginger were determined. The physical quality parameters studied were the bulk density, color and moisture content and while the biochemical parameters studied were total carbohydrates, protein, fat, essential oil and oleoresin. From the study, it was concluded that mechanical washing of ginger was effective when the inner and the outer drums rotated in the same direction at variable speed of 151 rpm and 27rpm respectively. The washing efficiency of 98.68 per cent could be obtained when ginger was washed mechanically for duration of 6 min when the drum load was 15 kg and the bruise index observed for the above conditions was 17.50. The capacity of washing was found to be 128 kg/h. The peeling of ginger was found effective when both the inner and the outer drums rotated in the opposite direction 151 rpm and 27 rpm. The maximum peeling efficiency of 62.60 per cent was obtained when fresh ginger was peeled for a duration of 10 min at drum load of 15 kg. The retention of essential oil and oleoresin content in dry ginger for the above condition was 1.67 and 4.13 per cent, respectively.

12. Molecular characterization of exotic ginger genotypes

Ms. Athira Pramod/Cochin University of Science and Technology/2019/
Guide: Dr. D. Prasath

Molecular characterization of exotic and indigenous ginger genotypes were developed using SSR and ISSR markers. Though the indegenous varities are quite distinct from exotic ginger cultivars in term of its rhizome features and quality traits, there are no studies on the comparative molecular profiling of indegenous varities versus the improved varities of exotic ginger genotypes. Among the 40 molecular markers studied, 14 could easily discriminate the genotypes and showed polymorphism.

Cluster analysis of data using UPGMA dendrogram gives an similarity index of the exotic and indigenious genotypes. The grouping of exotic genotype with indegenious ginger genotypes in the dendrogram implies that there is some phylogenetic relation between the exotic and indigenous varieties. This dendrogram also reveals the relationship of the most similar ginger genotypes. Rio-dejenerio and Nadia had shared high similarity among the 16 accessions.

13. Rhizosphere priming effect on nutrient mineralization dynamics of crop residues in ginger

Mr. P. Mohammad Thanveer/Indira Gandhi Krishi Vishwavidyalaya/2018/
Guide: Dr. V. Srinivasan

An incubation study was conducted to study mineralization of major nutrients especially N from commonly used crop residue mulches (*Gliricidia*, *Ailanthus*, Mixed leaves) with and without rhizosphere priming in ginger, to quantify nutrient addition to soil due to crop residues and rhizosphere priming in ginger and to quantify microbial activity due to rhizosphere priming and crop residue addition in ginger. The priming with crop residues and FYM has been influenced the nutrient mineralization dynamics in ginger. The total N mineralized and added to the soil was significantly higher in FYM and mulch treatments over control. The net N released was 948 and 676 mg/kg soil in FYM and ginger treatments, respectively. Of the net total N released into the soil, up to 33.8-70.3% was contributed by NO_3-N. *Gliricidia* addition contributed highest of 70.3% followed by mixed leaves (65.3%) and the lowest was in *Ailanthus* treatment (33.8%). The NH_4- N addition also followed the same trend with *Gliricidia* and mixed contributing 22.8% & 23.5% NH_4-N to the total fraction with the lowest in *Ailanthus* (9.4%). Both the cumulative and net release of P was higher in the treatments with FYM or ginger. Rather than mulches, priming agent FYM has contributed as a major source for P added to the soil. FYM added 187.3 mg net P as compared to 14.4 mg net P to the soil in without FYM which is almost 13.3 times higher. FYM addition was observed to be the major source of K as it contributed 1.78 times K (1481 mg) as compared to treatment without FYM (830 mg), whereas rhizosphere influence seemed to secondary as it contributed to a net release of 100-130 mg K, when compared between ginger and without ginger treatments. The higher activity of MBC in treatments with FYM or ginger indicated that, priming with the organic substrates improved the MBC accumulation. Among the mulches, *Ailanthus* with higher C:N ratio and lignin or polyphenol content supported higher MBC throughout the incubation period. Among

mulches no definite trend was observed in MBN contents and it did not show much correlation with any of the parameters studied. In control, under restricted supply of P, more of available P was used for microbial assimilation there by increasing MBP during initial phase of incubation (till 60 DAT). The priming effect enhanced the activity of enzymes such as dehydrogenase, urease, acid phosphatase, alkaline phosphatase and β-glucosidase. The addition of leaves and FYM significantly increased the DM and the uptake of N, P and K by ginger. The magnitude of increase was 48% for DM production and 88%, 100% and 75% for N, P and K uptake, respectively with FYM as compared to treatments without FYM. Similarly, addition of mulches increased the DM by 91-102% as compared to control and N, P and K uptake by 155-196%, 111-122%, and 141-158%, respectively over control.

14. Development of simple sequence repeats (SSR) and single nucleotide polymorphism (SNP) markers from transcriptome sequences of ginger (*Zingiber officinale*)

Ms. B. Jilna Babu/Mahatma Gandhi University/2018/Guide: Dr. D. Prasath

The main objective of the present study is to dissect large- scale genomic molecular marker resources for ginger. Molecular markers allow the identification and characterization of plant genotypes through direct access to hereditary material. In ginger, molecular markers are commonly used to identify genetic variation and classify there relatedness among varieties, accessions, and species. Consequently, it provides important input in determining resourceful management strategies for ginger improvement programs. High-throughput next-generation sequencing (NGS) technologies are rapidly transforming the fields of ecology, evolution, and genetics by increasing the amount of large-scale genomic and transcriptomic data that is available for non-model plant species. The denovo assembly of ginger transcriptome yielded 79049 contigs and 78987 unigenes with an average length of 912.83 bp and 840.72 bp, respectively. A total of 16790 Simple Sequence Repeats (SSRs) were identified as potential molecular markers in the unigenes. In gene regions, 4597 SSRs were found in coding region of the sequences. In addition, a total of 578634 SNPs were identified in the transcriptome sequences. These novel SSR and SNP markers identified in the present study are useful for analyses of genetic diversity, genetic linkage mapping, and the identification and improvement of varieties of ginger.

15. Physiological and biochemical changes during ginger- *Bipolaris rostrata* interaction

Ms. Divya Suresh/Kannur University/2018/Guide: Dr. R. Praveena

The objective of the present study was to understand the physiological and biochemical changes occurring during Ginger- *Bipolaris rostrata* interaction. The study included two major aspects *viz*., studies on the infection process of *Bipolaris rostrata* in ginger and physiological and biochemical changes occurring during the pathogen – host interaction. In conclusion, the results from the present study demonstrated that during the infection process of *Bipolaris rostrata* on ginger, the leaf physiology was mainly affected due to the damage to epidermal cells especially at the membrane level. Variations in different enzymes gave an insight into the mechanisms by which the plant metabolism was altered. The ginger plants artificially inoculated with the fungus showed overall decrease in photosynthetic rate due to disease progression and showed decreased amount of healthy leaf area. The fungus *Bipolaris* infect not only staple crops like rice and wheat, but it also affects plants like maize, sorghum, barley and ginger. Ginger is a spice of high importance and the present study focused on the identification of changes in biochemical parameters in ginger infected by *Bipolaris*. These biochemical parameters can function as potential molecular markers for the identification of resistant lines in ginger. This study aids in better understanding of the infection process of the pathogen in ginger plants and enables understanding the role of different enzymes in host pathogen interaction. The effect of the fungi on the nutrient and phytochemical composition of the leaves is well understood here. Study of the infection process and the physiological and biochemical changes occurring during the interaction between Ginger- *Bipolaris rostrata* interaction, aidsin understanding the mechanism of its infection and helps us in adopting new techniques to manage the pathogen and there by reduce ginger yield loss due to leaf blight disease.

16. Quality evaluation of ginger squash during storage

Ms. Athira Pavithran/Mahatma Gandhi University/2017/Guide: Dr. E. Jayashree

Although ginger is widely available in different forms, the scope for product diversification is very high in this spice. The raw materials required for the experiment like the fresh ginger was obtained from ICAR-Indian Institute of Spices Research (ICAR-IISR), Experimental farm, Peruvannamuzhi, Kozhikode, Kerala and nutmeg was obtained from IISR farm, Chelavoor. The fresh lemon and sugar were procured from the local market at Kozhikode. Ginger was washed, peeled and cleaned in water to remove surface impurities. After washing, the peel was removed and ginger extract was

prepared. Fresh ginger was cleaned, peeeled and crushed. The crushed ginger was then boiled with water 2000 mL of water. After crushing, the extract was strained through stainless steel sieve to get ginger extract (1690 mL). Ginger extract was mixed with sugar syrup to obtain ginger syrup of 38° Brix. It was then concentrated to 45° Brix by boiling to obtain ginger squash. After cooling, potassium metabisulphite (KMS) at the rate 0.1 per cent was added as preservative and citric acid of 2 gm added to obtain the pH below 4 and stirred thoroughly. The juice was the filled in to the polyethylene terephthalate bottles (PET) and stored under ambient and refrigerated storage condition.

17. Comparison of R-gene expression of ginger (*Zingiber officinale* Rosc.) and mango ginger (*Curcuma amada* Roxb.) following inoculation with *Ralstonia solanacearum*

Ms. T. V. Lini/University of Kannur/2015/Guide: Dr. D. Prasath

Studies on genetic diversity among exotic ginger (*Zingiber officinale* Rosc.) genotypes were conducted at ICAR-Indian Institute of Spices Research, Kozhikode, Kerala during the period of 2019-2020. The morphological and molecular characterization were undertaken among 19 exotic and five indigenous types. The eight shortlisted high yielding exotic types and indigenous genotypes were further profiled for quality parameters. The study helps to identify the morphological, molecular and biochemical diversity among the exotic and indigenous genotypes. Among the genotypes, highest projected yield/ hawas recorded in the accession Acc. 869 (37.40 t ha^{-1}) followed by Acc. 874 (32.40 t ha^{-1}), Acc. 607 (31.20 t ha^{-1}), Acc. 869 (24.40 t ha^{-1}), Acc. 393 (24.35 t ha^{-1}) and Acc. 833 (23.88 t ha^{-1}). Estimates of PCV were relatively higher than genotypic coefficient of variation for all the traits. The yield parameters along with characters such as number of tillers/clumps, height of shoot, plant height, number of leaves on main shoot and leaf petiole length recorded high heritability coupled with high GAM useful for efficient selection. The correlation and path analysis studies revealed that yield per plant had a positive significant association with plant height, number of tillers/clump, shoot height, number of leaves on main shoot and rhizome thickness at both genotypic and phenotypic level. Out of which, plant height followed by rhizome thickness, number of tillers/clumps, dry recovery and number of leaves on main shoot had highest positive direct effect on yield. Hence the selection of genotypes based on these characters with direct and indirect effect on yield were important consideration for ginger crop improvement programme. In contrast to a wide variation among the genotypes with respect to morphometric and yield characters, a narrow genetic diversity was identified by the molecular study based on ISSR and SSR

markers, which indicate a low efficiency of primers to detect polymorphism and it is essential to characterize the genotypes with more number of polymorphic primers to identify the genetic diversity at molecular level. The genotypes were grouped into various clusters irrespective of their geographical origin which may be due to genetic similarity existing among them. Biochemical characterization of shortlisted genotypes revealed that, highest essential oil and oleoresin content was recorded in Rio-de-Janeiro *i.e.* 2.76% and 6.69% respectively. Among the exotic genotypes, the highest essential oil was recorded in Acc. 869 (2.44%), Acc. 393 (2.42%), Acc. 833 and Acc. 873 (2.10%); highest oleoresin content in Acc. 869 (5.88%), Acc. 874 (5.63%), Acc. 873 (5.34%), Acc. 393 (5.28%), and Acc. 833 (5.15%). Among the exotic genotypes less crude fibre content was observed in Acc. 607 (3.5%), Acc. 736 (3.95%), and Acc. 393 (4.25%), whereas highest in Acc. 869 (5.88%). The α - zingiberene was the major compound identified in essential oil and the higher zingiberene content was reported in Acc. 393 (30.49%) followed by Maran (30.32%) and Acc. 869 (28.92%).

18. Gene expression studies of ginger (*Zingiber officinale* Rosc.) and mango ginger (*Curcuma amada* Roxb.) following inoculation with *Ralstonia solanacearum*

Ms. T. K. Nisheeda /University of Calicut/2015/Guide: Dr. D. Prasath

In this study, we selected two candidate genes from the transcriptome of ginger and mango ginger *viz.,* β1,3-glucanase and HMGS and real time PCR analysis was carried out to study its expression pattern in *Z. officinale* and *C. amada* plants challenge inoculated with *R. solanacearum*. The total RNA from ginger and mango ginger rhizomes tissue after inoculation with bacterial wilt pathogen was isolated at different time intervals (0 HAI, 1 HAI, 4 HAI, 8 HAI, 24 HAI, 48 HAI, 72 HAI, 96 HAI, 120 HAI). The RNA was converted to cDNA by RT-PCR and qPCR gene expression analysis was carried out. The *R. solanacearum* infection did influence the glucanase and 3-Hydroxy-3- methylglutaryl-CoA synthase (HMGS) expression in mango ginger and ginger. Expression of glucanase was up regulated and reached a peak at 8 HAI in *C. amada* and maintained higher expression up to 120 HAI, however, in contrast, its activity decreased dramatically in *Z. officinale*. In the case of, HMGS mRNA level in *C. amada* was significantly elevated (greater than four fold of the basal level) within I HAI. Peaked at I HAI and gradually declined after 4, 8 and 16HAI. Interestingly, no difference in the early induction (I HAI) of HMGS in ginger was observed, suggesting that the early signal for systemic activation of this gene was not there when compared to *C. amada*. Although expression pattern of HMGS in *Z. officinale* was modulated upon

pathogen inoculation, transcript levels were approximately 4-fold lower than *C. amada* (1 HAI). Thus, the very early (l HAI) and robust (nearly 4- fold) accumulation of HMGS transcripts indicates a crucial role for this gene in the *R. solanacearum* resistance machinery of *C. amada*. This study identified two potential candidate genes of the basal defense of *C. amada* against *R. solanacearum*.

19. Isolation and characterization of PR5 gene from different species of Zingiberaceae

Ms. Amrutha Balagopal/Vellore Institute of Technology University/2011/
Guide: Dr. D. Prasath

In the present study, eight putative PR 5 genes, were amplified from different species of *Zingiberaceae* (*Elettaria cardamomum, Alpinia luteocarpa, Curcuma amada, Curcuma longa, Zingiber zerumbet, Hedychium coronarium, Curcuma aromatica* and *Zingiber officinale*), which encodes precursor proteins of 215 to 230 amino acid residues and shares high homology with a number of other PR5 genes. The phylogenetic analysis of all the PR5s of Zingiberaceae species showed that all of them share significant evolutionary history. Also, the branch topology of the PR5 phylogenetic tree indicates that the major groups may have evolved from multiple rounds of gene duplication. The secondary and three- dimensional structure comparison of these eight proteins with already reported three PR5s (CaPR5, ZoPR5 and ZzPR5) revealed many striking similarities. The present study has also led to the modeling of their three-dimensional structure, the docking interactions between PR5 and the ligand, Beta 1, 4 D glucan and the analysis of various protein properties which has helped in determining the efficiency of PR5 proteins. It has further led to the determination of antimicrobial peptides which remain conserved among all the cloned PR5 genes. This can be used in determining specifically. the antifungal or antibacterial activity if the exact mechanism of action of the antimicrobial peptide is elucidated. Further studies on the analyses of PR5 and its promoter functions using transgenic plants will reveal the exact roles and functions of PR 5 protein involved in the basal defense in different species of Zingiberaceae.

20. Studies on ginger based value added products

Ms. F. P. Evangelin/Karunya University/2011/Guide: Dr. E. Jayashree

In the present study two value added products from ginger namely ginger sauce and ginger extrudates were standardised. Five different types of sauces namely ginger, ginger black pepper, ginger kokum and ginger nutmeg kokum sauces were formulated and processed. They were pasteurized at 80° C for 20 minutes, bottled in sterilize

bottles, crown corked manually and stored for 135 days at room temperature. During storage period the sauces were analysed for physical, biochemical, rheological, microbiological and sensory properties. The physical properties like total soluble solids and colour were stable during the storage period. Biochemical properties such as pH, titratable acidity, crude fibre, ash, moisture content, amino acids, proteins, carbohydrates, starches, fats, essential oils, oleoresins, calcium and sodium remained constant. But in the five sauces studied, the reducing sugars showed a decreasing trend, non - reducing sugars showed an increasing trend and the total sugars remained constant during the storage period. Total sugars for ginger, ginger black pepper, ginger, kokum, ginger nutmeg and ginger nutmeg kokum sauces corresponded to 18.33, 22.41, 20.83, 19.81 and 20.15 g. Microbial analysis showed that the sauces were free from contamination. As there was no microbial contamination, the rheological characteristics of the sauces remained unaffected during the storage period. On using Herschel-Bulkley model it was observed that all the sauces exhibited a non-Newtonian flow. During the storage period, sensory evaluation showed that ginger sauce had highest acceptability score of 8.5, ginger black pepper with 7.5 and ginger kokum with 6.5. Ginger nutmeg and ginger nutmeg kokum sauces had a similar acceptability score of 5.5. From the results obtained it is understood that all the sauces were shelf stable for 135 days. Out of the five sauces studied, ginger sauce was the best in terms of sensory analysis. Ginger powder was blended with cassava and extruded at four detet temperatures of 180, 190, 200 and 210 °C at three extruder screw speeds of 75, 80 and 85 rpm in a Brabender single screw extruder. The extrusion die temperature was optimised at 190°C. Ginger powder was blended with black gram flour and green gram flour and extruded at the optimized delete temperature of 190 °C for various extruder screw speeds of 75, 80 and 85 rpm. The physical, functional, textural, biochemical and sensory properties of the extrudates were determined. Extrudates obtained from cassava ginger blend was found to be the most suitable one as it had the highest acceptability score of 6.5 on sensory analysis. Extruder screw speed was optimised as 85 rpm as the cassava ginger extrudate obtained had the highest acceptability score of 6.8. The study concluded that ginger was a suitable spice for both thermal processing and high-pressure processing.

21. Studies on the effects of plant growth promoting rhizobacteria on biochemical and microbial properties of soils under ginger

Ms. K.P. Subila/University of Calicut/2010/Guide: Dr. R. Dinesh

Physico-chemical, biochemical and microbial properties were studied in soils collected from a greenhouse study conducted at the Indian Institute of Spices Research, Kozhikode on ginger under the ICAR network project on Application of Microorganisms in Agriculture and Allied Sectors (AMAAS). The treatments involved shortlisted PGPR isolates (GRB-25, -36, -38, -70) and different levels of NPK fertilizers (0, 75% and 100%) applied alone or in combinations. The results on physico-chemical properties of soils revealed that PGPR or NPK had little effects on soil pH, while mineral N, Bray P, exchangeable-K, -Ca & -Mg and SOC accumulated at significantly greater levels in treatments with combined application of PGPRs and NPK fertilizers. Dissolved organic C (DOC) levels in soils were also markedly influenced by the combined application of NPK and PGPRs, while dissolved organic N (DON) did not vary markedly between control and treatments with sole application of PGPRs. However, DON levels increased significantly with increasing N levels and were significantly greater in the treatments with combined application of PGPRs and NPK [100% N + 100% P + 100% K + GRB 36 (32.1 μg g^{-1})]. The data on biochemical/ microbial parameters revealed that microbial biomass-C (CMIC), -N (NMIC) and -P (PMIC) accumulated at greater levels in treatments involving combined application of PGPRs and NPK. The treatments with the greatest levels of CMIC, NMIC and PMIC were 100% N + 100% P + 100% K + GRB 38 (521 μg g^{-1}), 100% N + 100% P + 100% K + GRB 38 (39 μg g^{-1}) and 100% N + 100% P + 100% K + GRB 25 (22.5 μg g^{-1}) respectively. Similarly, greatest NMIN rates were observed in the treatment 100% N + 100% P + 75% K + GRB 70 (179 mg N kg^{-1} per 10 days). Soil respiration (SR) did not vary considerably in treatments with only PGPRs or with sole application of NPK and the greatest SR rate was registered in the treatment involving both PGPRs and NPK i.e. 100% N + 100% P + 100% K + GRB 25 (39 μg CO_2-C g^{-1} day^{-1}). Soil enzymes like dehydrogenase (DH), urease (UR), acid phosphatase (Ac-P), β-glucosidase (BG), aryl sulphatase (AS) were also activated to different degrees by the treatments. DH activity was greatest in the treatment with 100% N + 100% P + 100% K + GRB 38 (311 nmol TPF g^{-1} soil h^{-1}), UR activity in the treatment involving 100% N + 100% P + 100% K + GRB 36 (8.3 μmol NH3-N g^{-1} h^{-1}), Ac-P activity in the treatment with 100% N + 75% P + 100% K + GRB 38 (14.4 μmol p-nitrophenol g^{-1} h^{-1}), BG activity in the treatment with

100% N + 100% P + 100% K + GRB 25 (5.7 µmol p-nitrophenol $g^{-1} h^{-1}$), and AS activity in the treatment with 100% N + 100% P + 100% K + GRB 70 (0.64 µmol p-nitrophenol $g^{-1} h^{-1}$).

22. Studies on the gut micro flora of the shoot borer, *Conogethes punctiferalis* Guenee (Lepidoptera: Pyralidae) infesting ginger, turmeric and cardamom

Ms. M.S. Anuji/University of Calicut/2010/Guide: Dr. T. K. Jacob

The study of the gut micro flora of shoot borer (*Conogethes punctiferalis*) infesting ginger, turmeric and cardamom revealed the presenceof six bacterial and five fungal associates. The microbial diversity was narrow compared to vertebrate guts. The distribution of the flora differed based on the host plant. The mid -guts of the borers harbored maximum bacterial communities irrespective of the host plants. Attempt was also made to characterize some of the bacterial isolates through molecular techniques. The results of molecular characterization showed variations but were largely inconformity with the biochemical characterization. Uses of conventional techniques that relay on the culturing of microorganisms on defined media have its - own disadvantages. Even though a complex of microorganisms are present in the gut, only culturable microorganisms can be studied by this method, while the uncultivable microbes are totally ignored, providing a biased scenario of the structure and dynamics of the microbial communities.

23. Exploring Ginger - (*Zingiber officinale*) transcriptome using Expressed Sequence Tags (ESTs)

Ms. T.K. B. Keerthiga/Bharathhidasan University/2010/
Guide: Dr. Santhosh J. Eapen

In this study annotation was performed for *Z. officianale* ESTs which are rich sources of information for gene discovery. Since the complete genome of *Z. officianale* is not available yet, the functional annotation was performed for understanding various functions of various genes in ginger. For this, around 38, 139 EST sequences were downloaded from NCB I and sequence cleaning process was performed using VecScreen and SeqClean. These ESTs were assembled using CAP3 program and 7,099 contigs and 4,881 singletons were obtained. BLASTX sequence similarity search was done in order to annotate the EST sequences and the result showed that among the 11,980 unigenes, 176 sequences were having E-value "0". Out of these, 69% were novel genes and others have been classified based on their biochemical functions like defense, cell division, metabolism, energy, transcription and translation, ribosomal proteins,

stress response, photosynthesis, cytochrome, signal transduction, plant growth, cell death and aging, carbohydrate metabolism, amino acid biosynthesis process, proteolytic degradation, transport facilitators, binding protein etc. The GO (Gene Ontology) terms were assigned for 176 putative transcripts having E-value: "0". In many cases multiple gene ontology terms could be assigned to the same transcript, resulting in 162 assignments to the molecular function, 155 to the biological process, and finally 147 to the cellular component classes. This preliminary study using the available EST sequences gave insight into some of the functional aspects of ginger. EST analysis provides a powerful and rapid means of restructuring the transcriptome and remains a useful means of gene discovery.

24. Evaluation of Phosphate Solubilizing Bacteria (PSB) for soil nutrient mobilization in ginger

Ms. P. K. Princy/Bharathiar University/2010/Guide: Dr. V. Srinivasan

A pot culture experiment was conducted to study the influence of phosphate solubilizing biofertilizers in integrated nutrient management system on nutrient availability and microbial parameters in ginger rhizosphere. The treatment includes organic manures *viz*.,Vermicompost (150 g pot), Farm yard manure (2.50 g pot) and no manure, with 0% Phosphorus (3.50 g Rock Phosphate), 50% Phosphorus (1.75 g Rock Phosphate) and 100 % Phosphorus (3.50 g Rock Phosphate) and with four efficient plant growth promoting phosphate solubilizing bacteria IISR6 (*Pseudomonas aeruginosa*), IISR 51 (*Pseudomonas aeruginosa*), IISR 26, PB 21a and no organisms. The findings revealed that the application of farm yard manure and vermicompost had favourable impact on available soil phosphorus, potassium, acid and alkaline phosphatases and dehydrogenase enzyme activities. The highest activity by PB2 1a was found at 120 days both for soil P and acid phosphatases activity. Soil phosphorus and potassium availability was higher with the application of IISR6 and IISR 51 + Farm yard manure. The soil nutrient parameters pH, phosphorus, potassium showed positive correlation with the soil enzyme activity. The present study indicated that the application of IISR6, IISR51 and PB21a with farmyard manure and vermicompost along with fertilizer phosphorus-maintained soil fertility and resulted in higher yield of ginger.

25. Influence of processing techniques on the quality traits of ginger and turmeric rhizomes

Ms. K. Latha Rani/Bharathiyar University/2009/Guide: Dr. John Zachariah

In the present study cv Varada is harvested at full maturity and processed for

different drying regimes. One set of samples were peeled and dried in sun, hot air oven and sun dried in polythene bag. Another set is not peeled and dried in the same manner as peeled samples.The dried ginger samples were evaluated for primary metabolites such as total carbohydrates, starch, total proteins, free amino acids and phenol. The same samples were evaluated for crude fibre, oil, oleoresin and pungent principles such as gingerols. The essential oil obtained from the sesamples was subjected for GC and GC-MS evaluation. Results indicated that expect for the long duration of drying in peeled and non peeled samples, the different drying regimes did not drastically influence the primary and secondary metabolites. However non peeled samples took 28 days for drying compared to 14 days of peeled samples. Under a fixed drying condition, these samples be haved uniformly under sun dried and hot air oven. Marginal influence of poly bag drying was observed in gingerol, Z-citral and zingiberene in the present study, turmeric rhizomes (cv Prathibha) were processed into two forms *viz*, slicing and curing. The processed rhizomes were dried under sun and hot air oven. The dried samples were evaluated for primary metabolites such as total carbohydrates, starch, total proteins, free amino acids and phenol and secondary metabolites like oil, oleoresin and curcumin. It was found that sun drying of sliced samples took 5 days for complete drying of a moisture level of 10 % while oven drying at 66 °C took 7 days. However boiled/cured samples took 7 days for sun drying and 9 days for ovendrying. The study revealed that slicing turmeric into chips form did not change oleoresin and curcumin. Fifty percent reduction was observed in essential oil content in the sliced turmeric samples use.

26. Geneticdiversity of ginger (*Zingiber officinale* Rosc.) germplasm assessed by simple Sequence Repeat (SSR) Markers

Ms. R. Suparna/Cochin University of Science and Technology/2009/
Guide: Dr. D. Prasath

The present study was undertaken to assess the genetic variability among the 17 ginger germplasm conserved at Indian lnstitute of Spices Research, Calicut, using SSR markers. The modified protocol used for the isolation of DNA was found to be suitable for ginger which gave very good quality DNA from the leaves. The PCR profile involves one cycle of initial denaturation cycle at 94°C for 3 min followed by cycle denaturation at 94°C for 30 sec, annealing at different temperature for 45 sec according to the primer, extension at noc for 45 sec, and a final extension at noc for 20 min gave good amplification. SSR profile have been developed for 17 ginger accessions *viz.,* ACC 12, ACC 18, ACC 22, ACC30, ACC 31, ACC 139, ACC 144, ACC 246, ACC 282, ACC

294, ACC 295, ACC 510, ACC 654, ACC 731, IISR Varada, *Zingiber* sp. and *Zingiber zerumbet*. Eight primers were tested for amplification such as DP-O1, DP-02, DP-03, DP-04, DP-OS, DP-06, DP-07 and DP -OB. These primers were used for assessing the polymorphism/similarities within and among the ginger germplasms. Among the eight SSR primers tested, six primers were polymorphic and amplified all the seventeen ginger accessions, while two were monomorphic. The UPGMA dendrogram separated 17 genotypes into two major clusters where all the cultivated types were clustered together in one, the other contained primitive and related *Zingiber* sp. The four accessions *viz*., Jaggijan, Kunduli local, H-687 and Dehradun were found to be 100% similar while the wild species such as *Zingiber zerumbet*, Pink Ginger and *Zingiber* sp. did not cluster with any of the cultivated accessions. Further, the dendrogram revealed *Z. zerumbet* had 63% similarity with cultivated accessions, where as the other *Zingiber* sp. had only 51% similarity. The results indicate the existence of moderate level of genetic diversity among the ginger accessions genotyped with eight SSR markers. The results also indicate these SSR markers were useful for studying the genetic diversity among cultivated and related species of ginger.

27. Evaluation of storage methods of ginger rhizomes (*Zingiber officinale*)

Ms. P. Durgadeth/University of Calicut/2009/Guide: Dr. C.K. Thankamani

A trial was conducted to find out best lining material and best method of storing ginger rhizome at Peruvannamuzhi farm. Maximum healthy rhizome was obtained when rhizomes were stored with granite powder as filling material that was on par with sand and sawdust. Under different storage methods, pit method of storage had maximum healthy rhizome production, minimum shrivelled and infected rhizomes that was on par with storing the rhizomes in boxes made up of wooden material with granite powder as filling material. Under different storage methods, maximum height and number of leaf production was observed in pit method of storage that was on par with storing the ginger rhizomes with granite powder as filling material in boxes made up of wooden material. The quantity of rhizomes that can be stored in pit method is less and chances of spoilage is high in low lying areas. It is seen that use of healthy seed material has paramount role in increasing the yield. Timely availability of sand and sawdust is limited and sand is expensive also. Substitution of sand by granite powder, a cheaper substitute obtained from sand quarries will decrease the material cost for storage. Since the aim is to produce healthy quality planting material, storing the pesticide treated rhizomes with granite powder/ sawdust / sand as filling material and storing the rhizomes in structure

made up of wooden material may be suggested for large scale storage of ginger rhizomes.

28. Computational mining of ginger ESTs for simple sequence repeats and putative genes

Ms. M. S. Manjula/Bharathidasan Univeristy/2008/Guide: Dr. Santhosh J. Eapen

The ginger family is a tropical group especially abundant in Indo-Malaysia, consisting of more than 1200 plant species in 53 genera. The genus *Zingiber* includes about 85 species of aromatic herbs from East Asia and tropical Australia. ESTs become a tool to refine the predicted transcripts for those genes, which leads to predictions of their protein products, and eventually of their function. The objectives of the work are to study the importance of EST resources for functional genomics of ginger, to develop molecular markers from the EST sequences, to perform comparative analysis of different SSR tools and to find out the putative genes in ginger from assembled EST sequences. Five methods MISA, SSRIT and TROLL were used to identify SSRs. MISA can elucidate the compound repeats from sequence. Quality primers were designed from SSR derived. 56 quality checked primers including 10 primers for dinucleotides, 29 for tri nucleotides, 9 for tetra nucleotides, 5 for penta nucleotides and 3 for hexa nucleotides were also identified.

29. Molecular characterization of primitive, elite and exotic ginger genotypes to protect the biowealth of elite ginger accessions

Mr. Jithin Prem/Periyar University/2007/Guide: Dr. B. Sasikumar

A total of twenty two random decamer primer were selected for RAPD analysis. Out of the twenty two random decamer primers,only six produced unique bands in the four different genotypes. Maximum number of unique bands were observed in Pink ginger. Three primers *viz.,* OPB-19,OPC- 13,OPE-11 generated discrete bands in this genotype.OPJ-07 produced a unique band in Varada. While the primers OPA-08 and OPB-05 generated bands specific to 'Kintoki' and 'Kozhikalan' respectively. Seven ISSR primers produced unique bands in four of the genotypes studied, out of the fourteen ISSR primers used. Maximum number of unique bands were observed in Pink ginger in this study also. The ISSR primers which discriminated Pink ginger were 53 ISSR-J and ISSR-6, ISSR-8 and ISSR-10. The primer ISSR-9 was discriminatory in case of Kintoki. The primer ISSR-13 and ISSR-3 produced Varada specific bands. UPGMA dendrogram produced by RAPD, ISSR and combined RAPD and ISSR markers revealed a general pattern, all the four primitive (putative wild) types forming a single

cluster having two groups each. and the other genotypes forming separate nodes. The percentage affinity between or among the primitive types were high as compared to the elite types exotic accession or the Pink ginger. However, the exotic materials Kintoki had a high similarity with primitive types than Pink ginger. Varada showed a high similarity with primitive types than with other genotypes. Though the existence of ginger (*Zingiber officinale* Roscoe) in the wild habitat still being debated. The comparatively high degree of affinity observed between the elite variety Varada and the primitive types suggests that the elite varieties may have evolved from these primitive types over a course of time.

30. Molecular and biochemical studies on the effect of various nutrient management regimes on microbial community structure of soils under ginger

Ms. P. Vidya/Bharathidasan Univeristy/2007/Guide: Dr. T.E. Sheeja

Soil samples (0-20 cm) collected from the respective beds subjected to chemical, INM and organic nutrient management regimes were analysed for relevant physico-chemical, biochemical/ microbial parameters prior to determination of microbial community structure by RFLP techniques. Soil samples obtained from control treatment were also included for the study. The pH of the soil under ginger does not vary by any nutrient management treatment. The decreased levels of biologically available substrates and organic matter lead to simultaneous decrease in microbial activity in soils. The nutrient management regimes varied in their influence on microbial activity in soils and, therefore, would cause shifts in the soil microbial community structure. Nucleic acid based cultural independent methods, like targeting SSU rRNA and ITS regions coupled with rDNA fragment analyses by genetic finger printing approaches (DGE, ARDRA/PCR RFLP, SSCP, TGGE) are considered more accurate ones to assess the composition of soil microbial communities. PCR RFLP when compared with the denaturing gel electrophoresis was found to yield better results in terms of time and labor. Denaturing polyacrylamide gels can resolve oligonucleotides from 2 to 300 bases, depending on the percentage of polyacrylamide used. Touch down protocol employed in conjugation with denaturing PAGE helped to improve the resolution and number of bands.

31. PCR based identification of *Pythium* species causing soft rot disease of ginger

Ms. Reeja Susan Thomas/Bharathidasan University/2006/Guide: Dr. A. Kumar

Pythium spp. causing, soft rot in ginger is a large oomycete genus with more than 120 described species. The identification of *Phythium* species is a problem in

several laboratories, still eludes solution, due to the complication arising in morphological characterization, since most of the species are delineated on minor differences. Molecular techniques have come up these days to facilitate the rapid identification of plant pathogenic microorganisms. In present study, a PCR based approach was sought for the identification of 29 isolates of *Phythium*, causing soft rot ginger. Among the 29 isolates, 14 produced an amplicon of 150 bp when PCR was carried out at an annealing temperature of 57°C with the primers Pmy5 and ITS2.The result established these isolates *Pythium myriotylum*. These isolates represented the states Assam, Sikkim, Uttar Pradesh, Kerala and Karnataka. The dominance of the pathogenic species in these areas established so forth. It was found that the isolates from the well separated geographical regions were *P. myriotylum.*

32. Molecular characterization of ginger varieties/cultivars using RAPD Markers
Ms. K. Heeba/Bharathidasan University/ 2006/Guide: Dr. B. Sasikumar

The present work is an attempt to study the level of genetic diversity among ten ginger varieties/cultivars, based on DNA(RAPD) markers. Good quality DNA was isolated from the leaves of different ginger varieties cultivars using CTAB method. Polymerase chain reaction was carded out with 2Sng DNA. The mixture also contained I unit Taq DNA polymerase, 200M dNTPs, 2mM $MgCl_2$ and 10 picomoles of primer. The concentration of assay buffer usedwas IX. Twenty five selected decamer primers were used to amplify the genomic DNA s of ten ginger varieties/cultivars. Out of these only OPC-08 produced a discriminatory band of size OPC-08 (1875) in the variety Suruchi. The primers OPB-06 and OPD-OS also showed some differential banding patterns in the varieties. Ginger is a vegetatively propagated spice. Morphology of the rhizomes in many cases is not sufficient enough to identify variety/cultivar. Molecular markers may be useful in such cases. However, in the present study, the twenty five random primers could not produce major discriminatory bands in varieties/cultivars studied. This can be due to limited number of primers used in the study. However, the lack of polymorphism need not imply the lack of genetic diversity.

TURMERIC

1. **A comparative study on the nutritional composition and nematotoxic property of three *Curcuma* species and their spent residues**
Ms. P. Nishida/Kerala University of Fisheries and Ocean Studies/2022/
Guide: Dr. N.K. Leela

This study was focused on comparison of nutritional composition of these three *Curcuma* species and their spents as first step of value addition for utilization of spice spents in various functional food formulations. In the present study, the moisture content, curcumin content, carbohydrate content, starch content, protein content, fat content, crude fibre, ash content and mineral composition of these three *Curcuma* species and their spents were evaluated. The results of the analyses indicated that the spice spents contain appreciable and high quantities of carbohydrate, starch, protein, crude fibre and ash content than that of corresponding spices. Besides the nutritional composition, the nematotoxic property of the aqueous extract of spent residues and oleoresin extract of these curcuma species were evaluated. The study revealed that the nematodes were immobile at the tested concentrations of oleoresin extracts of three *Curcuma* species and the aqueous extracts of spent residues. Finally, it can be concluded from the results that the spice spents are highly nutritious and can be a good source of carbohydrate, starch and fibre, which make them potentially useful in value addition of food products thus making them highly beneficial for health and also for the environment in reducing pollution.

2. ***In vitro* establishment of different varieties of turmeric (*Curcuma longa* L.)**
Ms. Shinsi Fathima/Bharathiar University/2022/Guide: Dr. Sharon Aravind

The results reveals the existence of significant variation among different turmeric varieties when cultured under *in vitro* conditions. In general, the seven varieties (IISR Prathibha, IISR Alleppey Supreme, IISR Pragati, Duggirala Red, NDH 98, Suroma and Ranga) of turmeric used in the present study regenerated efficiently in the culture medium consisting MS medium supplemented with BAP (3.0 mg/L) and NAA (0.5 mg/ L). The turmeric varieties *viz*., IISR Prathibha, IISR Alleppey Supreme and Ranga could produce multiple shoots rapidly. The maximum shoot length and root length were produced by the varieties *viz*., IISR Pragati, IISR Prathibha and Suroma. The varieties like NDH 98 and Duggirala Red could produce spontaneous rooting. Also,

more leaves were observed in the turmeric varieties like NDH 98 and Suroma. The turmeric variety, Ranga recorded the lowest number of days for leaf emergence after first (8.37 days) and second sub culture (4.57 days) when compared to other varieties used in the study. The variation in the response of turmeric varieties under *in vitro* condition in general due to the difference in the genetic potential of these varieties to perform under controlled conditions. The efficient micro propagation technique described here may be highly useful for raising disease free quality planting material of different elite varieties of turmeric for commercial production.

3. Study of antioxidant and antimicrobial activities of crude polysaccharides and crude proteins from black turmeric (*Curcuma caesia* Roxb.) and mango ginger (*Curcuma amada* Roxb.)

Mr. S. Sujith/Mahatma Gandhi University/2022/Guide: Ms. R. Sivaranjani

The present study was envisaged to analyze the antioxidant and antifungal activities of crude polysaccharides a crude protein extracted from black Turmeric (*Curcuma caesia* Roxb.) and mango ginger (*Curcuma amada* Roxb.) using several *in vitro* assays. In our study, we found that *C. caesia* polysaccharide extracted from fresh rhizome has shown 0.02 mg/mL, 0.28 mg/mL, 0.27 mg/mL of glucose, sucrose and maltose equivalents of total carbohydrates whereas *C. caesia* polysaccharide extracted from dry rhizome has shown 0.026 mg/mL, 0.36 mg/mL, 0.35 mg/mL of glucose, sucrose and maltose equivalents. *Curcuma amada* polysaccharide extracted from fresh rhizome has shown 0.036 mg/mL, 0.48 mg/mL, 0.47 mg/mL of glucose, sucrose and maltose equivalents of total carbohydrates. *C.amada* polysaccharides has the highest total phenolic content with 244 mg GAE/g and polysaccharide extracted from *C. caesia* dry samples has the highest total flavonoid content of 26 mg QE/g. The antioxidant activity was assayed using four methods: DPPH radical scavenging activity, ABTS radical scavenging activity, Ferric reducing antioxidant power (FRAP) assay and Ferrous chelating activity assay. Overall, we found that *C. caesia* polysaccharides extracted from dry samples has showed best antioxidant activity followed by *C. amada* polysaccharide. The antioxidant activities of protein samples from these two species are found to be the least. In case of antifungal activity assay, among all extracts, *C. caesia* polysaccharide extract has shown highest inhibitory activity with 12.0 %, 16.2 %, 23.8 % and 15.5 % in first, second, third and fourth day respectively in *Macrophomia phaseolina* culture. In case of *Fusarium oxysporum* f.sp. *vanillae* isolate 1, among all extracts, *C. caesia* polysaccharide extract has shown highest inhibitory activity with

31.3 %, 16.3 %, 23.8 % and 28.8 % in first, second, third and fourth day respectively. In *Fusarium oxysporum* f. sp. *vanillae* isolate 2 also, *C. caesia* polysaccharide extract has shown highest inhibitory activity with 20.4 %, 20.2 %, 28.5 % and 39.6 % in first, second, third and fourth day respectively. We conclude that crude polysaccharides extracted from *C. caesia* and *C. amada* has antioxidant and antifungal activities.

4. Functional validation of a MYB transcription factor in turmeric (*Curcuma longa* L.) by yeast one – hybrid assay

Ms. Bhavya Sankar/Bharathiar University/2022/ Dr. T. E. Sheeja/

In this study, a MYB TF from turmeric was selected for the functional analysis, Moreover, the structural genes involved in the curcumin biosynthesis identified MYB binding sites on their promoter sequence. The putative transcription factors binding sites on structural genes were predicted using *in silico* analysis. We have mined the promoter regions of nine important structural genes involved in curcumin biosynthesis and blast analysis with turmeric reference genome was carried out. From this we have also, listed out the putative cis-acting regulating elements in the sense strand (positive and negative) of upstream sequence of those nine structural genes.The pray and bait vector construction was successfully done but the subsequent transformation of these vectors into yeast was not successful. Thus, further optimization studies are required for the successful transformation into yeast cells. However, the functional validation of MYB TF identified in the study will definitely be useful especially since its function was validated by yeast one hybrid analysis, this work will add as an important step in the further studies on molecular analysis of protein DNA interactions in turmeric

5. Evaluation of multi trait PGPR, *Bacillus safensis* for zinc solubilization, yield and quality in turmeric

Ms. K. B. Proxima/Cochin University of Science and Technology/2021/
Guide:Dr. R. Praveena

In the present study on evaluation of multi trait PGPR, *Bacillus safensis* for zinc solubilization yield and quality in turmeric the promising ZSB, *B. safensis* was evaluated for its zinc solubilization efficiency and plant growth promotion traits in turmeric under pot culture conditions. Different treatments were imposed at varying levels of ZnO (0, 2.5, 5 and10 ppm) individually and combination with *B. safensis.* The efficiency of bacteria was compared with treatments involving ZnO alone at different levels and also with reference strain of ZnSB. The results of the study showed that the treatment combined application of ZnO (5ppm) + *B. safensis* was found to be superior

with increase in physico – chemical parameters *viz*., organic carbon, available nitrogen etc. and microbial parameters like microbial biomass C and N. Addition of *B. safensis* alone & along with ZnO (2.5 & 5ppm), increased yield and improved the quality parameters (oil, oleoresin, curcumin) of turmeric rhizome. The present study indicated that, *B. safensis* can be used solubilize Zn compounds in soil which is cost-effective and environmentally and there by the use chemical fertilizers can be reduced. Based on the current state of basic and applied research, it appears that the use of ZSB may be a promising technique for solubilizing inaccessible Zn reserves in the soil and facilitating fast Zn accessibility to plants, resulting in increased plant growth with minimal Zn fertilizer application.

6. Effect of different freeze-drying methods on quality of freeze-dried turmeric juice powder

Ms. Nimmy Babu/Mahatma Gandhi University/2021/Guide: Dr. K. Anees

Turmeric variety obtained from Research Farm at ICAR-IISR, Kozhikode was used for extraction of juice through screw press. After extraction juice and press residue was obtained. The juice was subjected to five different freeze-drying temperature and time regime (T1 to T5) to find optimum freeze-drying conditions. The press residue was subjected to tray drying (T6) and fresh turmeric rhizome where sliced (T7) and kept for drying at 70 for 2 days to act as a control. Biochemical profiling of powders obtained from T1 to T7 was carried out. The juice recovery from fresh turmeric was found to vary between 52-57 percent. Press residue obtained varied between 43-48 percent. Freeze dried powder has exhibited significantly lower moisture content compared with normal tray dried turmeric powder. The moisture content of freeze-dried turmeric juice powders ranged from 2.6-4.48 percent. Highest carbohydrate content of 40.15 ± 4.48 percent was obtained for freeze-dried powder from T2 and the lowest carbohydrate content of 24.29 ± 2.47 percent in case of T5. T1 recorded maximum protein content of 2.70 ± 0.059 percent while, T2 recorded minimum protein content of 0.74 ± 0.033 percent. The SDS-PAGE image revealed that the diversity of protein content present in freeze-dried samples were more than that present in T6 and T7. For the freeze-dried powder of different treatments from T1 to T5 the highest fat content was found in T4 (2.54%) and the lowest fat content of 0.77 percent was obtained in T3. The crude fiber percentage of sliced turmeric powder and the press residue powder were 5.51 and 7.22 percent respectively. The essential oil content was maximum (2 %) for T1 and T4 and minimum (1.2 %) was recorded in T2 and T5. There was a significant variation in oleoresin content

for freeze dried and tray dried samples. The curcumin content of freeze-dried turmeric juice ranged from 2.09 to 2.690 %. The maximum phenol content was observed in T1 and minimum in T2. The current study showed maximum antioxidant activity by T3 with an IC50 value of 13.32 µg/mL and the minimum antioxidant activity was exhibited by for T2 with an IC50 value of 19.67 µg/mL among freeze-dried samples. While, the tray dried T6 and T7 samples exhibited IC50 value of 11.20 and 10.22 µg/mL respectively.

7. Evaluation of multitrait PGPR *Bacillus safensis* for plant growth promotion and zinc solubilization in turmeric

Ms. Anitta Abraham/Kerala University of Fisheries and Ocean Studies/2021/
Guide:Dr.V. Srinivasan

In the present study on evaluation of multitrait PGPR, *Bacillus safensis* for plant growth promotion and zinc solubilization in turmeric the promising ZSB, *B. Safensis* was evaluated for its zinc solubilization efficiency and plant growth promotion traits in turmeric under pot culture conditions. Different treatments were imposed at varying levels ofZnO (0, 2.5, 5 and10 ppm) individually and combination with *B. safensis*. The efficiency ofbacteria was compared with treatments involving ZnO alone at different levels and also withreference strain of ZSB. The results of the study showed that the treatment combinedapplication of ZnO (5ppm) + *B. safensis* was found to be superior with increase in physico –chemical parameters *viz*., organic carbon, available nitrogen etc. and microbial parameterslike microbial biomass C, N, and dehydrogenase enzyme activity in soil. The application of*B. safensis* alone also recorded significant results. A separate *in vitro* study was also conducted in which, bacteria were isolated from the soils of Munnar, Thekkadi and Idukki districts of Kerala and they were shortlisted for plant growth promoting traits and Zn solubilization potential under *in vitro* conditions. Nine bacterial isolates were found to be promising (IISR ZSB11 - *Acinetobacter*; IISR ZSB12 -*Acinetobacterbaumanii*; IISR ZSB13 - *Burkholderiadiffusa*; IISR ZSB14 - Unidentified;IISR ZSB15 - *Acinetobacterseifertii*; IISR ZSB16 - unidentified; IISR ZSB17 -*Acinetobacter*; IISR ZSB18 - *Burkholderiaambifaria*; IISR ZSB19 –*Acinetobactercalcoaceticus*), of which, IISR ZSB12 (*Acinetobacterbaumanii*) was found to be the most promising isolate owing to its enhanced Zn solubilization capacity exhibited in qualitative assays. The identified bacteria (*A. baumanii*) hold a great potential in developing bacterial consortia using already identified ZSB strains and can be integrated in future evaluation trials. The

future line of work should focus on further validation of pot experiment results of *B. safensis* under field conditions to understand whether the positive effects are reflected under field conditions in multiple locations.

8. Identification, cloning and characterization of a putative transcription factor from turmeric (*Curcuma longa.* L)

Ms. J. Mridula/Bharathiar University/2021/Guide:Dr. T. E. Sheeja

The role of MYB TF genes in biosynthesis of secondary metabolites is well established in many plants. One MYB TF that showed similarity to the already reported MYB TFs from the *Vitis, Arabidopsis thaliana*, *Brassica* sp. and *Musa* was selected for our study. The selected MYB was having a negative log 2 fold change value when a high curcumin rhizome transcriptome was compared with a low curcumin background. To validate the role of this gene, real time PCR technique was used. The expression of the MYB was compared in high curcumin and low curcumin accessions to evaluate and confirm its involvement in regulation of curcumin biosynthesis. The identified MYB TF was cloned and sequenced. Sequences were analyzed in ORF finder to obtain a sequence of 751 nucleotides, including a full-length open reading frame (ORF) encoding 251 amino acids. Phylogenetic tree representing the relationship between the MYB and other reported MYBs were analysed. The 3-D structure of the protein was predicted through the SWISS MODEL that showed similarity to the already reported MYB TF available in the protein data bank (PDB). Structural model identified in the PDB was analysed and validated by Ramachandran plot analysis.

9. Antioxidant and antidiabetic potential of sequential extracts of *Curcuma caesia* Roxb.

Ms. Ashika P. Hassan/Mahatma Gandhi University/2020/Guide: Dr.N.K. Leela

The objective of the study was to determine the antioxidant and antidiabetic activity of sequential extracts of *Curcuma caesia* and correlate with its total phenolic content. It is also envisaged to study the composition of the petroleum ether extract and its fractions by GC-MS analysis. Antioxidant activity of petroleum ether and methanol extracts of *Curcuma caesia* were compared by DPPH free radical scavenging assay and ferric reducing power assay and also with that of synthetic antioxidants, namely, butylated hydroxy anisole (BHA) and ascorbic acid. Methanol extract showed higher antioxidant and α- glucosidase inhibitory activity and total phenolic content. GC-MS analysis of hexane soluble fraction of petroleum ether extract indicated curzerenone (18.22%), 1, 8-cineole (12.08%), camphor (4.02%), β-elemene (3.73%), germacrone

(2.44%), isobornyl acetate (2.27%), germacrene D (1.56%), isoborneol (1.30%) and β-selinene (1.21%) as major components. Petroleum ether extract was fractionated by column chromatography and the major compounds in each fraction were identified.

10. Studies on application of turmeric (*Curcuma longa* L.) leaf essential oil for control of *Aspergillus flavus*

Ms. K. Manasa/University of Calicut/2020/Guide: Dr. E. Jayashree

The fresh turmeric (*Curcuma longa* L.) leaf samples at dried as well as mature state were obtained from ICAR-Indian Institute of Spices Research (ICAR-IISR), Experimental Farm, Peruvannamuzhi, Kozhikode, Kerala. The harvested leaf samples were cleaned well and the mature leaf sample then subjected to shade drying in the IISR –Experimental farm, Peruvannamuzhi. Moisture analyses of both samples were conducted in triplicate by means of Sartorious Moisture Analyser. Essential oils of both sun dried and shade dried leaf samples were extracted by hydro-distillation of powdered leaf sample by Lee and Ogg Method (ASTA, 1968). Essential oil samples were then fractionated by size exclusion column chromatography with a stationary phase of anhydrous silica gel and n-hexane mobile phase. The fractions eluted out through the column were then subjected to UV-Visible absorption spectroscopy in Genesys 50 UV-Visible spectrophotometer at 490nm. The data obtained on absorbance of fractions were plotted graphically. Based on conclusions obtained from graphical data, the fractions were pooled in to five. The pooled fractions were then concentrated in Heidolph Rotary Vaccum Flash Evaporator separating out the extra solvent. The concentrated essential oil samples of all twelve different genotypes as well as the five fractions of dried leaves were introduced in as such concentrated state and mature leaf essential at a concentration of 10%, 25% and 50% dilution in ethanol were introduced in Potato Dextrose Agar plates on sterilized filter paper discs (diffusion discs) on either side of the radially growing fungal culture. The radial mycelial growth was measured and compared with that of control containing ethanol and absolute control. The percent inhibition of mycelial growth over control was calculated. 0.5%, 1% and 5% dilutions of concentrated dried EO samples in ethanol were prepared and inoculated to Potato Dextrose Broth test tubes containing *Aspergillus flavus* to examine if there is any inhibitory property to the EO samples of dried leaves. The GC-MS analysis conducted for determining the composition of both dried as well as mature leaf essential oil samples differed from each other. The GC-MS profile of dried and mature leaf EO samples identified 14 and 21 different constituents respectively. The volatile

compounds specific to dried turmeric leaf oil were α-pinene (3.72%), 3-carene (1.83%), p-cymene (19.12%), γ-terpinene (2.15%) and sabinyl acetate (1.09%) and, the compounds specific to mature turmeric leaf oil were α-Santalene (4.49%), caryophyllene (2.72%), α-bergamotene (1.26%), (Z)-β-farnesene (2.49%), Ar-curcumene (1.11%), α-zingiberene (16.01%), β-bisabolene (3.42%), β-sesquiphellandrene (8.16%), germacrene-B (2.08%) and germacron (3.65%). Hence from the experiment conducted, is was concluded that, aromatic organic compounds of the volatile essential oil collected from mature turmeric leaves have some compounds responsible for chemopreventive action against aflatoxin producing strains of *Aspergillus flavus* and the essential oil sample collected from dried leaves does not have the specific property.

11. Functional validation and characterization of a novel Zn transporter gene from *Curcuma longa* L. using molecular approaches

Ms. C. T. Nandana/Mangalore University/2020/Guide: Dr. T.E. Sheeja

Turmeric was chosen as a model plant for our study due to its immense importance in global trade in the area of pharmaceuticals, cosmetics and oil and oleoresin industries. *Curcuma* transcriptome was mined to identify Zn transporter gene. The totals of 17 unigenes were retrieved from the transcriptome which showed similarity to the already reported transporter genes from the *Vitis, Arabidopsis thaliana, Brassica* sp., and *Thlapsi* sp. Unigenes showing highest fold change were used for gene expression. To validate Zn transporter gene real time PCR & *in vitro* screening technique used. The healthy plantlets were inoculated to Murashige & Skoog medium with varying concentration 0 ppm, 10 ppm and 50 ppm ZnO. The bacterial isolate was inoculated in to the plantlet and the plants grew without contamination and showed positive response to treatment of ZSB to the medium. The expression of Zn transporter gene vis a vis presence and absence of ZSB was evaluated. Gene expression analysis was done using qRT-PCR. We also studied the effect of potential Zn solubilizing bacteria on gene expression to validate its role in transportation of Zn to plants. The gene was cloned and sequenced. Sequence were analyzed in ORF finder and obtained an ORF of 957 nucleotides, including a full-length open reading frame (ORF) encoding 318 amino acids. Phylogenetic tree representing the relationship between family of known Zn transporters was depicted, and finally the 3-D structure of the protein predicted through the SWISS MODEL, and showing similarity to the already reported membrane protein in the protein data bank. structural model identified in the PDB were analysed and validated by Ramachandran plot analysis.

12. Effect of Nano CuO on soil microbial properties in turmeric rhizosphere

Ms. O. Dilruba/Mahatma Gandhi University/2019/Guide: Dr. V. Srinivasan

Application of increased levels of CuO (0 to 100 ppm) as nano and bulk with or without FYM has not affected the soil pH, available phosphorus, calcium, magnesium, iron and manganese. Application of increased levels of CuO (0 to 100 ppm) as bulk and nano without FYM has significantly reduced the soil electrical conductivity and organic carbon content, but increased the availability of potassium, zinc and copper when applied in combination with FYM. The application of organic amendments such as farmyard manure significantly increased the availability of all the essential nutrients, pH and organic carbon content as compared to no FYM application. Application of increasing levels of CuO as bulk and nano in the soil without or with FYM significantly increased the soil copper availability and the rate of increase was higher in nano CuO application than bulk source. Increased levels of CuO in bulk and nano forms with or without FYM did not affect the essential soil microbial properties like microbial respiration, microbial biomass carbon, enzyme activities of dehydrogenase, alkaline phosphatese, β-glucosidase and urease enzymes. The major microbial assisted enzyme activities are unaffected even at different levels of CuO or application of nano forms indicates the genuine response of a stimulated microbial population with no evidence for negative effects of NPs on the microbial processes. The application of organic amendments such as farmyard manure significantly increased the microbial activity as a steady C substrate and also helped to detoxify the antimicrobial action of nano or bulk CuO. Application of increasing levels of CuO as bulk or nano only found to significantly reduce the acid phosphatase enzyme activity and the magnitude of reduction is higher in nano CuO as compared to bulk form. This may be due to the selective toxicity of CuO NP to a group of microbial community, which needs further confirmation with more detail ed assessment of parameters.

13. Expression analysis of a novel zinc transporter gene from turmeric (*Curcuma longa* L.) mediated by a promising soil zinc solubilizing bacteria

Ms. Hridya Vijay/Mahatma Gandhi University/2019/Guide: Dr. T. E. Sheeja

From a series of experiments related to the PGPR activity, apromising Zn solubilizing bacteria ZSB-1 isolate was considered to be the best promising Zn solubilizer from all other potential Zn solubilizers isolated from the rhizosphere soil of a tropical spice crop. The activity of isolated promising Zn solubilizer ZSB-1, was validated by the expression analysis of Zn transporter gene, isolated from the turmeric

variety IISR- Pratibha, micropropogated *in vitro* and was observed that as the concentration of Zn level increases in the soil, the Zn transporter gets down regulated. This gene can thus be used as an indicator to study the Zn availability in soil. From the phylogenetic tree data, we could conclude that, the isolated Zn transporter gene from turmeric is a novel gene which has close relatedness with Zn tranporters in *Vitis vinifera*. The difference in expression patterns in the treatment with and without ZSB-1 isolate also claims that the ZSB-1 is indeed a potential Zn solubilizer with PGPR activity and this can be used as novel technology in under green manure.

14. Management of nematode, *Pratylenchus* spp. infecting in turmeric (*Curcuma longa* L.) through different strategy

Ms. Disney Joy/Mahatma Gandhi University/2019/Guide: Dr. C. Sellaperumal

We attempted to find out the management strategy such as new generation chemical molecules, biocontrol agents, and fumigants were tested. Screening of biocontrol isolates showed in the range of 34 to 100% mortality, among them Iso1 and Iso2 showed 94% mortality over media control which is considered to be very effective on lesion nematode, *Pratylenchus* spp. This can be utilized to control *Pratylenchus* spp in field condition after evaluation of field screening as well. Fumigants and other nematicides *viz*., Dazomate, Metham sodium, Marshal, Neem cake were tested, all of them caused reduced nematode population, weed growth and pathogen population and increased beneficial microbes after treatments. Different new chemical molecules were tested at different concentrations *viz*. 100 ppm, 250 ppm and 500 ppm. At 100 ppm, chemicals 3L 84%, 4B 79% mortality, at 250 ppm 3E showed 83% and 3F 82%, at 500 ppm resulted mortality rate % showed by 3F, 3G, 3m, 4k after 24h exposure periods which was highest % of mortality among the tested chemicals.

15. Water purification using turmeric powder-based cartridges

Ms. Athira Mohanan/Mahatma Gandhi University/2019/Guide: Dr. K. Anees

In the present study, turmeric powder in combination with activated charcoal is used without sterilizing the column, the microbial load in the water increases beyond counting limit. Due to sterilization of the turmeric powder-based cartridge, even though, reduction in microbial load was achieved the level of microbial load in the processed water was significantly higher than the input water. $KMnO_4$ treatment of turmeric was highly successful in reducing the microbial load by 66%. The $KMnO_4$ treatment was found to be the best with respect to conductivity, and microbial load reduction during water purification. Since $KMnO_4$ treatment used spent turmeric, this opens up a new area of utilization of industrial waste of turmeric.

16. Screening of biocontrol against lesion nematode *Pratylenchus* spp. in turmeric

Ms. Evelyn Mariet Josy/Mahatma Gandhi University/2019/
Guide: Dr. C. Sellaperumal

We attempted to find out the management strategy of lesion nematodes in storage condition, and it was revealed that 100% reduction in the number of nematodes was recorded after 35 days in cold storage which is one option to reduce population during off season. But many things are needed to be sorted out, such as cold stored rhizome germination ability and whether any other pathogen can survive in cold storage along with nematodes. Screening of biocontrol agents showed very good results ranging from 0.0% to 100% mortality of *Pratylenchus* spp. with different strains of actinomycetes, streptomyces, endophytic bacteria and *Bacillus* spp. This can be utilized to control *Pratylenchus* spp. in field condition after evaluation of field screening as well.

17. Effect of curing methods and drying temperature on the quality of sliced turmeric (*Curcuma longa* L.)

Mr. N. K. Muhammed Nisar/Maharaja Ranjit Singh Punjab Technical University/2019/Guide: Dr. E. Jayashree

Turmeric (IISR-Alleppy Supreme) was harvested from the fields of IISR-Experimental farm, Peruvannamuzhi. The harvested rhizomes were cleaned manually to remove the roots and other adhering materials. Turmeric rhizomes of about 3 Kg were cured by traditional water boiling method in aluminum pot for 60 min, sliced in a mechanical slicer (Pilotsmith India Pvt. Ltd., Thrissur, Kerala) and dried by spreading on the trays (size of 44.8 × 38 cm) of the hot air oven (Labline) to a drying bed thickness of 5 cm at varying temperatures of 50, 60,70, 80, 90 and 100°C till constant mass was obtained. Sun drying and solar tunnel drying of turmeric served as control. Another 3 kg of fresh rhizomes were sliced mechanically without curing and dried in the hot air oven at the same drying temperatures with sun drying and solar tunnel drying as control. The trays were shuffled alternatively every 2 h. Sun drying was carried out by spreading the rhizomes on a clean shade net. The ambient temperature during the period of sun drying varied between 30°C and 36°C, relative humidity varied from 35% to 61%. Solar tunnel drying was conducted by spreading the sliced turmeric on the trays inside the drying chamber. The temperature inside the solar tunnel dryer varied between 36°C and 55°C. The loss in mass during was recorded periodically and the drying characteristics were calculated. The physical and biochemical properties of the dried turmeric were determined and the quality of cured and uncured sliced turmeric dried at various temperatures was evaluated.

18. Comparative study of five varieties of *Curcuma* species at different stages of maturation

Ms. C. V. Gayathri/Bharathiar University/2019/ Guide: Dr. N. K. Leela

The five-*Curcuma* species in 6-8 months after planting were analysed for its chemical properties, antioxidant activity and their antifungal properties. Curcumin content and its curcuminoid profiling, oleoresin content, essential oil content and its GC profiling, starch content were done in the chemoprofiling of *Curcuma* species. The amount of curcumin was high in the month of January in *C. longa* and *C. zedoaria*. Both the two species contains curcumin 1 as its principal curcuminoid followed by BDMC in *C. longa* and DMC in *C. zedoaria*. All the other species contains lesser curcumin and only curcumin 1 is predominant in its curcuminiod profiling. The oleoresin content was found to be decreasing in the three months and the oil content also showed slight variations. The starch content in *C. longa* and *C. zedoaria* shows an inverse proportion with its curcumin content and in *C. caesia* and *C. amada*, the starch content shows proportionate increase with curcumin content. The antioxidant potential was high in *C. zedoaria* rhizome oil (7.58 µg/mL) and the lesser activity was shown by *C. longa* oil (19. µg/mL). The antifungal activity was determined using well diffusion method and the higher antifungal activity was shown by rhizome oils of *C. longa* and *C. zedoaria* whereas the lower activity was observed in *C. aromatica*.

19. Molecular marker based genetic diversity of turmeric (*Curcuma longa* L.) genotype and selfed seedling progenies using ISSR and SSR markers

Ms. V. Priyadharshini/Bharathiar University/2019/Guide: Dr. S. Aarthi

The present study on genetic diversity among the selected turmeric genotypes predominantly cultivated in India using ISSR primers and the genetic diversity of selfed seedling progenies using SSR primers were carried out to elucidate the genetic diversity/relatedness among the genotypes and selfed seedling progenies, reveals that the distance of genotype at genetic level was significant with polymorphism within the genotypes. The genotypes representing from the major turmeric growing areas were characterized using ISSR markers segregated the selected genotypes in to five clusters with Cluster V was found to be the largest with six genotypes falling in that cluster. The morphological character of this genotype was bold rhizome except for SC 61. The next cluster IV was having four genotypes *viz*, Megha Turmeric 1, Rajapuri, Suvarna, Varna. ACC 849 and Narendra Haldi 98 clustered separately. Duggirala Red and BSR 2 were clustered separately. IISR Prathiba was separated in single cluster. This shows the diversity at molecular level within the genotypes. The twelve selfed seedling progenies

of 69/5/22 was compared at molecular level with the mother and two checks namely, IISR Prathiba and IISR Pragati using SSR markers resulted in four cluster with eleven seedling progenies and the mother genotype fell in same cluster revealing the closeness of the progenies. The selfed seedling 69/5/22/I8 formed a separate cluster. The two checks IISR Pragati and IISR Prathiba also formed a separate cluster III and I respectively. The molecular markers should be combined with reliable morphological descriptors of qualitative nature and pedigree details of the genotype to get strength to the diversity analysis and in turn germplasm management. The present study is an attempt to employ the possibility of using molecular markers to assess genetic diversity in vegetative propagated crops. But the accuracy of molecular characterization can be enhanced by screening more number of markers and genotypes. Identification of genotype specific molecular markers may help to maintain the integrity of the genotypes by identifying duplicates. Hence, there is a need to apply these tools to gain more insight into the genotype at molecular level.

20. Molecular characterization of turmeric (*Curcuma longa* L.) and its wild relatives
Ms. Simi Yohannan/Bharathiar University/2019/Guide: Dr. D. Prasath

The present study was undertaken to assess the genetic variability of turmeric and its wild relatives using the SSR molecular markers. The protocol used for the isolation of DNA was found to be suitable for turmeric which gave good quality DNA from the rhizome and leaves. The PCR profile gave good amplification. Among the twenty SSR primers five polymorphic and nine monomorphic bands were found. The UPGMA dendrogram separated 12 genotypes into five clusters indicating 95% similarity between Acc. 849 and NDH 98, 88% similarity between *C. xanthorrhiza* and *C. zedoaria* and 78% similarity between IISR Pragati and IISR Prathiba.

21. Influence of two processing methods on quality attributes of turmeric (*Curcuma longa* L.) and chemoprofiling of selected genotypes
Ms. K. R. Rintumol/Kerala University of Fisheries and Ocean Studies/2018/
Guide: Dr. N. K. Leela

In the present study the effect of two processing methods on the quality attributes of turmeric, namely, oleoresin, total curcuminoids and essential oil was investigated in four genotypes. The results indicated that by slicing rhizomes and drying yielded higher oleoresin and total curcuminoids compared to traditional curing method. HPLC analysis indicated that the major pigment of turmeric, curcumin contributed 48.5-66.9% of total curcuminoids. Estimation of curcuminoids in fifteen seedling

progenies (0.97-5.5%) as well as primary rhizomes of selected released varieties (1.7-8.6%) indicated wide variation. Among the genotypes studied No.65/12 was found to be unique with high level of oleoresin and curcumin in mother rhizome and higher level of turmerones in essential oil. Among the four genotypes curcumin level was high in mother rhizomes of the genotype No. 65/12 and in primary rhizomes of other three genotypes.

22. Comparison and quality profiling of freeze dried turmeric solubles from different varieties of *Curcuma longa* L.

Ms. Laya Liz Kuriakose/Karunya University/2018/Guide: Dr. E. Jayashree

Turmeric variety (IISR-Prabha) was obtained from farmer's plot at Koorachundu, Kozhikode and IISR-Pragathi, Chintapalli 1 year crop and Chintapalli 2 year crop were obtained from farmer's field at Chintapalli district, Andhra Pradesh. 2 kg of turmeric from each variety was sliced and dried in the hot air oven. The dried turmeric was used for further quality evaluation. Experiments on freeze drying were done at Biowin Agro Research Centre, Mananthawady, Wayanad. Fresh turmeric juice was extracted from about 20 kg of each variety in a twin screw expeller (make: Global Kitchen: 1 HP) with a capacity of 60 kg/h and filtered through a fine sieve to remove the fibrous particles. The juice was frozen at -47°C for 4 hours and then freeze dried at -75 C for 9 hours (Freeze dryer make: SANSHON, capacity 12 L) under vacuum (0.1 MPa), till complete moisture was removed. The residue obtained after the juice extraction was dried in an oven (make: LABLINE) and was taken for lab analysis. The oven dried and freeze-dried turmeric powders were analyzed for various Physical, biochemical and medicinal properties. Profiling of essential oil was done using GC-MS (GC-2010, SHIMADZU) equipped with mass spectroscope (Shimadzu QP -2010) and capillary column. The experiments were replicated thrice. The mean dry recovery for oven dried turmeric powder was 18.69 per cent. It was found that the average moisture content of oven dried turmeric powder obtained was 9.39 per cent. The oven dried turmeric powder from the variety Chintapalli (2 year crop) showed maximum yield in case of its essential oil, oleoresin and curcumin content with values of 10.02, 21.50 and 7.49 per cent, respectively. The dry recovery of freeze dried (FD) turmeric juice powder varied from 4.61 to 5.88 per cent. The mean value of moisture content for FD turmeric juice powder was 1.91 per cent. The Hunter colour value, L*(lightness Vs darkness) obtained for FD turmeric juice powders varied from 47.14 per cent in IISR-Pragathi to 50.10 per cent in IISR-Prabha, a*(red Vs green) value varied from 17.07 per cent in Chintapalli (1 year

crop) to 21.98 per cent in Chintapalli (2 year crop) and b*(yellow Vs blue) value varied from 55.22 in IISR-Prabha to 61.37 per cent in Chintapalli (2 year crop). The mean value of bulk density for FD turmeric juice powder was 0.57 g/cc. The primary metabolites like total carbohydrates were maximum in the variety IISR-Pragathi (34.82 per cent) and minimum in IISR-Prabha (17.80 per cent). The protein content ranged from 9.83 per cent in Chintapalli (2 year crop) to16.68 per cent for IISR- Prabha. The mean fat content observed was 0.24 per cent whereas the crude fibre content was 0.54 per cent. The essential oil content of FD turmeric juice powders from Chintapalli (1 year crop), Chintapalli (2 year crop), IISR-Pragathi and IISR-Prabha were 4.37, 2.08, 2.12 and 1.93 per cent, respectively; the corresponding oleoresin content was 5.29, 10.13, 5.26 and 2.71 per cent. Curcumin content for Chintapalli (1 year crop), Chintapalli (2 year crop), IISR-Pragathi and IISR-Prabha were 2.77, 4.75, 2.82 and 1.93 per cent respectively. The phenol content of Chintapalli (1 year crop), Chintapalli (2 year crop), IISR Pragathi and IISR-Prabha for FD turmeric juice powders were 17.45, 22.22, 21.48 and 13.21 mg/dL and their corresponding anti-diabetic property was 4.43, 4.57, 4.34 and 3.93 mg/mL, respectively. The variety IISR-Prabha showed higher anti-diabetic property. Four major volatile oil constituents present in FD turmeric oil were β-sesquiphellandrene, turmerone, curlone and ar- turmerone. Among the four varieties studied, the freeze dried turmeric juice powder from Chintapalli (2 year crop) was considered superior in terms of its oleoresin and curcumin content with values of 10.13 and 4.75 per cent respectively, whereas Chintapalli (1 year crop) was considered superior in terms of its essential oil content with 4.37 per cent.

23. Curcumin enriched edible oils: quality profile during storage

Ms. A. Chithra/University of Calicut/2018/Guide: Dr. T. John Zachariah

The polyphenolic content of turmeric, extracted as an orange yellow crystalline substance, with a green fluorescence is known as curcumin. It is obtained by solvent extraction of ground turmeric rhizome. Functional use of curcumin as a food additive is its application as a colorant and nutraceutical. It has powerful anti-inflammatory property and is a very strong antioxidant. Turmeric variety (IISR- Prabha), virgin coconut oil and olive oil were used for extraction of curcumin. Curcumin was extracted into VCO and olive oil were carried out by an optimised process of heating 1 g powdered turmeric sample mixed in 75 mL edible oil (virgin coconut oil and olive oil) and heated over a boiling water bath for 1 h and the oil was filtered and made up to 200 mL oil. The optimised procedure was adopted for extraction of curcumin into the oil using oven

dried turmeric powder, freeze dried turmeric juice powder and turmeric press residue. The physio-chemical properties in curcumin enriched virgin coconut oil and olive oil like free fatty acid, acid value, saponification value, peroxide value, iodine value, total phenolic content, oil profiling and antioxidant activity during the storage processes has been evaluated during the storage period at 30 days interval up to sixty days. The curcumin content of curcumin enriched VCO with turmeric powder, freeze dried turmeric juice powder and press cake powders showed a reduction during storage for 60 days as 20%, 65% and 13%, respectively. Free fatty acid value is a relative measure of rancidity as free fatty acids are normally formed during decomposition of triglycerides. The initial free fatty acid content of VCO and curcumin enriched VCO with turmeric powder, freeze dried turmeric juice powder and press cake powder were 0.34 mg KOH/g oil and it showed an increase of about 35% after storage of the oil for 60 days.

24. Quality evaluation of turmeric press residue – an industrial by product from freeze drying of turmeric juice

Ms. P. K. Shakkira/University of Calicut/2018/Guide: Dr. E. Jayashree

Turmeric variety Chintapalli (1year crop) and Chintapalli (2 year crop) and IISR-Pragathi was procured from farmer's field at Chintapalli district in Andhra Pradesh and IISR-Prabha was procured from farmer's field at Koorachundu, Kozhikode. The turmeric procured was cleaned and whole turmeric rhizomes were subjected to extraction process in a twin-screw expeller to obtain turmeric juice and press residue. Fresh turmeric juice was extracted was filtered through a fine sieve to remove the fibrous particles. The juice so obtained is freeze dried to obtain the soluble turmeric freeze-dried powder while the press residue which is considered as a by-product of this industry was taken for the present study with the aim to find new alternatives for the commercialization of spice crops. In the present study turmeric press residues from four important varieties *viz*., Chintapalli (1year crop), Chintapalli (2 year crop), IISR-Pragathi and IISR-Prabha were obtained after the juice extraction. The dry recovery of the press residue based on initial weight of fresh turmeric varied from 8.07 to 9.70 per cent. While, the dry recovery based on the initial weight of wet press residue after juice extraction varied from 14.52 to 20.61 per cent. The moisture content of dry turmeric press residue of varieties Chintapalli (1 year crop), Chintapalli (2 year crop), IISR-Pragathi and IISR-Prabha were 8.08, 7.98, 8.21 and 8.03 per cent, respectively. There was significant variation in the Hunter colour values L*, a* and b* between the varieties. The L* value of turmeric press residues of varieties Chintapalli (1 year crop),

Chintapalli (2 year crop), IISR-Pragathi and IISR-Prabha varied as 36.48, 36.79, 39.36 and 40.37, respectively and the corresponding bulk density varied as 0.30, 0.28, 0.27 and 0.25 g/cc, respectively. There was significant variation in the total carbohydrate, protein, fat and crude fibre content of turmeric press residues of the four varieties studied. The essential oil content for the turmeric press residues of varieties Chintapalli (1 year crop), Chintapalli (2 year crop), IISR-Pragathi and IISR-Prabha varied as 5.86, 6.30, 5.06 and 5.33 per cent, respectively. There was significant variation in the oleoresin content between the varieties and the corresponding values were 9.66, 11.92, 10.76 and 15.53 per cent, respectively. The curcumin content of turmeric press residues of varieties Chintapalli (1 year crop), Chintapalli (2 year crop), IISR-Pragathi and IISR-Prabha were 3.28, 3.26, 5.13 and 5.21 per cent, respectively. The maximum phenol content was obtained in the variety Chintapalli (2 year crop) (17.57 mg/mL) followed by Chintapalli (1 year crop) (16.01mg/mL), IISR-Pragathi (12.70mg/mL) and IISR-Prabha (12.67 mg/mL). The anti-diabetic property of the press residues, which was measured in terms of alpha- glucosidase activity and expressed as IC50 (mg/mL) varied from 2.17 to 3.63 mg/mL. The variety IISR-Pragathi showed higher anti-diabetic property. GC-MS analysis revealed that the major volatile oil constituents identified were ar-turmerone, turmerone, curlone, β-sesquiphellandrene and zingiberene. The study revealed that the turmeric press residue, an industrial by-product from freeze drying of turmeric.

25. Studies on drying of turmeric (*Curcuma longa* L.) in solar dryer

Mr. P. T. Naseeb/Sri Dev Suman Utharakhand University/2018/

Guide: Dr. E. Jayashree

Turmeric (IISR-Prathiba) was obtained from the fields of IISR-Experimental farm, Peruvannamuzhi. The harvested rhizomes were cleaned and 10 kg each of turmeric rhizomes were cured by 10 kg each of turmeric rhizomes were cured by three different methods like i) traditional water boiling for 60 minutes, ii) steam cooking for 60 minutes in TNAU boiler, iii) pressure cooking for 60 minutes in solar cooker developed by ICAR-IISR, Kozhikode. The fourth method followed was slicing the turmeric rhizomes to 5mm thick followed by drying. Cured turmeric rhizomes were dried in a solar dryer and under open sun. The solar dryer was developed by IISR, Kozhikode and is installed at IISR, Experimental farm, Perunnamuzhi. The loss in mass during drying was recorded periodically and the drying characteristics were calculated. The ambient weather parameters like temperature, relative humidity and sun light

intensity were recorded at an interval of 1 h. The physical and biochemical properties of the dried turmeric were evaluated and the quality of solar dryer dried turmeric was compared to that of sun dried turmeric. From the above experiments, it was observed that drying time was higher for drying in solar dryer compared to sun drying. Minimum drying time was reported for sliced turmeric as 7 days in sun drying and 10 days in solar drying. TNAU boiled turmeric recorded maximum drying time as it took 12 days for both solar drying and sun drying. Dry recovery and final moisture content of turmeric were not affected by different curing and drying methods. Drying characteristics curves showed that moisture content during drying decreased exponentially with time and drying occurred in the falling rate period and no constant rate period was observed during both sun drying and solar drying. Sliced turmeric recorded considerably higher values of primary metabolites like protein, fat and crude fibre, compared to turmeric subjected to heat treatments. Retention of primary metabolites was higher in solar dryer dried turmeric compared to sun dried turmeric, with a maximum protein content of 6.44 per cent, fat content of 6.62 per cent and crude fibre content of 8.13 per cent for sliced turmeric dried in solar dryer. Essential oil content of solar dryer dried turmeric was higher compared to sun dried turmeric with maximum value of 6.40 per cent for sliced turmeric. Turmeric cured by all the heat treatments reported the lowest essential oil content of 5.20 per cent when subjected to sun drying. Curcumin content of dried turmeric recorded a maximum value of 4.61 per cent for solar dried sliced turmeric. Lowest curcumin content (3.89 per cent) was reported for sun dried turmeric cured in solar cooker. Highest oleoresin content was also observed in sliced turmeric. GC-MS analysis of turmeric essential oil revealed that its major constituents are turmerone, curlone and ar-turmerone. zingeberene, beta-sesquiphellandrene, curcumene and alpha-phellandrene were also found to be present in considerable concentrations in the essential oil of turmeric. It was concluded from the study that slicing of turmeric to 5 mm thickness was considered the better pretreatment method compared to heat treatments in terms of retention of primary and secondary metabolites. Solar dryer is superior over sun drying for the production of dried turmeric with better physical and biochemical quality.

26. Comparison of crystalline curcumin and its nano formulation for bioactivity

Ms. Shilpa Sivadas/Vellore Institute of Technology University/2018/

Guide: Dr. T. John Zachariah & Dr. K. Anees

To enhance the bioavailability of curcumin, it was made into nano scale by subjecting the crystals to ultra sonication in the presence of a stabiliser, Tween-80. A

dilute curcumin solution was prepared in ethanol, and it was mixed Tween-80 for stabilising. This mixture was subjected to ultrasonication at regular intervals to obtain nanoparticles. The mixture thus obtained was visualised under Scanning Electron Microscopy (SEM) of field emission type, from which its shape and size were analysed. The crystals were globular in structure in the solution made with Tween-80, with sizes ranging from 40-80 nm. The optimum concentration for complete solubility in water and virgin coconut oil were found, and these solutions were tested for their antioxidant property using DPPH assay. Nanocurcumin in virgin coconut oil possessed enhanced antioxidant property, and could potentially provide better bioavailability in the body due to complete solubility in the oil.

27. Extractability of *Curcuma* in virgin coconuts oil and olive oil

Ms. Mabel Rachel Jacob/Karunya University/2018/Guide: Dr. T. John Zachariah

In the current study, turmeric powder (variety Prabha), freeze dried fresh turmeric juice powder and residual press cake were compared with authentic curcumin for its solubility in Virgin Coconut Oil (VCO) and Olive oil. The strong antioxidant properties of both virgin coconut oil and olive oil make these oils a fine choice for this experimental study. Optimisation studies were carried out by varying time, temperature and quantity of the samples while heating, powdered samples mixed in the oils, over a boiling water bath. Extractability was more prominent in hot oils compared to ambient temperature. Solubility of curcumin was measured by quantifying the curcumin content in oil by UV Spectrophotometer using acetone extraction method as control. The results indicated that about 82-85, 95 -100 and 70-80% of curcumin was getting extracted from oven dried turmeric powder, freeze dried turmeric juice powder and press cake powder, respectively, into oils with respect to that in acetone. Curcumin extracted in VCO and olive oil is expected to show more availability compared to normal curcumin intake.

28. Chemoprofiling of turmeric (*Curcuma longa* L.)

Ms. Geethu P. Raj/Kerala University of Fisheries and Ocean Studies/2017/
Guide: Dr. N.K Leela

In the present study the influence of processing on the quality profile of Suguna, Sudarshana and Suranjana and accession No. 38911 was studied. The results indicated that quality of the rhizomes of chopped and dried turmeric was superior in terms of essential oil, oleoresin and total curcuminoids. In turmeric loss of curcuminoids was observed during boiling rhizomes with water. Curcuminoid profile of turmeric showed that the major component was curcumin which was followed by BOMC and OMC. In the accession 38911 curcumin level was in the order primary>secondary>mother. The

essential oil composition of eight turmeric varieties and nineteen accessions were determined by GC - MS analysis and thirty constituents contributing to characteristic aroma of the oil were identified.

29. Quality evaluation of turmeric cured in concentrated solar thermal turmeric curing unit

Ms. Rakhi Rajendran/Mahatma Gandhi University/2017/
Guide: Dr. T. John Zachariah

In the present study, turmeric variety-Alleppey Supreme obtained from ICAR-Indian Institute of Spices Research, Experimental Farm, Peruvannamuzhi, Kozhikode was used to study the effect of curing on the quality of cured turmeric. Turmeric was cured in the newly developed concentrated solar thermal turmeric curing unit utilizing solar renewable energy. Turmeric cured in concentrated solar thermal curing unit fitted with cooking vessel-II, the time required for drying turmeric cured for 15 min was 25 days (600 h) and minimum period of 10 days (240 h) was required for drying turmeric when it was cured for 90 min. Maximum retention of quality was obtained when turmeric was cured for 15 min in concentrated solar thermal curing unit. The biochemical analysis of turmeric cured for 15 min indicated that the primary metabolites like carbohydrate, fat, protein and starch content varied as 46.69, 2.45, 3.10 and 41.69 per cent, respectively and the secondary metabolites like essential oil, oleoresin and curcumin content varied as 5.30, 10.17 and 3.08 per cent, respectively. GC MS profiling of essential oil revealed five important constituents and they were ar-turmerone (44.25 per cent) p-sesquiphell andrene (3.32 per cent), a -phellandrene (3.60 per cent), ar-curcumene (1.36 per cent) and turmerone (0.61 per cent). The study concluded that curing of turmeric in concentrated solar thermal turmeric curing unit fitted with cooking vessel-I for 60 min was considered optimum based on the maximum retention in the essential oil content and the minimum drying time required. In case of turmeric cured in concentrated solar thermal tunneric curing unit fitted with cooking vessel-II, the optimum cooking time was 60 min and time required for complete drying was15 days (360 h) so that maximum quality of turmeric is retained.

30. A study on the impact of ZnO Nanoparticie on soil microbial properties in the rhizosphere of turmeric

Ms. Arathi P. Raj/Vellore Institute of Technology University/2015/
Guide: Dr. R. Dinesh

The primary objective of the study were to assess the impact of nano ZnO on soil microbial biomass carbon; microbial biomass phosphorus, microbial biomass nitrogen, soil respiration rates and metabolic quotient. The secondary objective was to assess the impact of ZnO-NP on hydrolytic enzyme activities (urease, acid phosphatase, alkaline phosphatase, aryl sulphatase, p-glucosidase) involved in soil nutrient transformations and dehydrogenase activity (oxi-reductase enzyme). The inter-relationships between relevant physico-chemical parameters, biochemical and microbial parameters in these soils were also examined. For this purpose, soils were collected from a green house experiment on turmeric conducted at the ICAR- Indian Institute of Spices Research, Kozhikode, Kerala. For comparison, ZnO was also applied as bulk ZnO. The five levels of Zn applied either as nano ZnO or Bulk ZnO were 5, 10, 50, 100, 250 mg/kg. One such set was treated with farmyard manure (FYM) and another set was devoid of FYM. Suitable controls, with no Zn application were also maintained. Clearly demonstrated the strong inhibitory effect of ZnO NP on 5MB (CMIC, NML C & PMIC) and enzyme activities in soils, and the effects were significant at 100 and 250 mg/kg. In treatments without FYM addition, with low organic matter content the effect of the ZnO NP was markedly higher than in the treatments with FYM addition. Bulk ZnO also decreased 5MB (CMIC, NMIC & PMIC) and enzyme activities in soils but the degree of inhibition was lower and less obvious than that observed in treatments with nano-ZnO. Our findings thus showed that ZnO NP was clearly toxic to the soil ecosystem, especially at higher levels.

31. Studies on quality variation during polishing of turmeric (*Curcuma longa* L.) in power operated turmeric polisher

Ms. Nejma Basheer/Mahatma Gandhi University/2014/Guide: Dr. E. Jayashree

Turmeric (variety Prathiba) was procured from a farmer from Naalammayil, Wayanad. The cleaned rhizomes were cured for 1 h in a steam operated turmeric boiler of capacity 100 kg per batch which was developed by Tamil Nadu Agricultural University, Coimbatore. Cured turmeric rhizomes were spread in single layer and dried under open sun on cemented concrete floor and in a solar tunnel drier of size 9 x 4 x 2.6 m until constant weight was obtained. Turmeric rhizomes obtained from solar dried and sun dried turmeric were subjected to polishing in a power operated turmeric polisher to

obtain clean and yellow coloured turmeric. About 50 kg of dried turmeric was loaded in to the turmeric polisher and initially polished and polishing was carried out for different time intervals like 0, 15, 30, 45 and 60 min. The degree of polish was checked by unloading the material from the polisher and recording the weight loss. The partially polished turmeric was reloaded into the polisher and the experiment was continued for 30, 45 and 60 min of polishing. Solar tunnel dried and sun dried turmeric rhizomes were polished separately and the experiment was replicated three times for each drying method. The effect of polishing on different drying methods and on quality of polished turmeric produced and the dust obtained were determined.

32. Drying characteristics of turmeric (*Curcuma longa* L.) dried under different methods and its quality evaluation

Ms. Divya D. Das/Mahatma Gandhi University/2014/Guide: Dr. E. Jayashree

Two methods were followed to determine the drying characteristics of turmeric. The first method was by drying the cleaned, washed and cured turmeric rhizomes of Prathibha variety in the solar tunnel drier, and the second was drying sample by conventional sun drying method. Three trials of drying were performed in the solar tunnel drier with 200 kg of samples each for a single trial and simultaneously three trials were performed with 5 kg of samples by traditionals un drying. The temperature, relative humidity and sun shine intensity of both inside and outside of the solar tunnel drier were measured every one hour interval of the day. The relative humidity of solar tunnel drier seems to be less compared to the atmospheric humidity. The temperature was also higher inside the solar tunnel drier. These characteristic features of solar tunnel drier made the drying process faster. The drying characteristics, physical and biochemical parameters were analyzed. Drying characteristics curves were plotted for moisture content, moisture ratio and drying rate. Physical parameters including moisture content, bulk density and colour were also recorded. Primary metabolites such as carbohydrates, proteins, fat and secondary metabolites such as oil, oleoresin and curcumin were analyzed. Identification of volatile oil constituents were also perform gas chromatography equipped with mass spectroscope (Shimadzu QP- 2010). The drying characteristics of both sun and solar drying methods indicate that the moisture content of turmeric sample decreased constantly with increase in drying time. The drying process in solar tunnel drier was completed in 213 h where as in sun drying, took 10 days (235 h) to dry completely. In solar tunnel drying of turmeric the moisture ratio was reduced to 0.024 towards the end of drying time (213 h) where as in sun drying of

turmeric the moisture ratio reduced to 0.023 at the end of the drying time (235 h). The drying rate of solar and sun-dried turmeric was observed to be 0. 023 kg /h and 0 .018 kg/h towards the end of drying time 213 hand 235 h respectively. The mean collector efficiency, heat transfer efficiency and overall efficiency of solar tunnel drier was found to be 1.95,70.90 and 1.14 percent, respectively. Quality parameters of solar tunnel dried and sun-dried turmeric were compared by physical, biochemical and microbial analysis. It was observed that the physical and biochemical parameters of' solar dried samples were on par with sun dried samples. Sun dried samples showed microbial load of 9x 10^4 cfu/mL and solar dried samples have microbial load or 7x10^4 cfu/mL. The result indicated that the microbial load of solar tunnel dried turmeric was lower than the sun dried turmeric sample.

33. Effect of varying temperature on stored turmeric powder (*Curcuma longa)*

Ms. P. Aiswarya/Karunya University/2014/Guide: Dr. E. Jayashree

Whole dry turmeric rhizome of Prathibha variety was pulverized in a hammer mill to obtain turmeric powder of size 250 microns. This turmeric powder obtained was packaged in three layered metalized polyester covers (12 polyester + 12 metalized polyester + 80 polyethylene) and stored at different temperatures like 30, 40 and 50°C with control stored at ambient conditions for a storage period of 10 weeks. The stored turmeric powder were analysed for various physical and biochemical properties at weekly interval. The microbial load of the turmeric powder was determined at the beginning and end of storage period. The physical properties like moisture content and water activity increased when stored at ambient storage conditions while it decreased with increase in storage temperature. The Hunter colour value of L*, a* and b* increased from 45.62 to 49.82, 21.69 to 24.54 and 46.91 ~, to 64.63 at 50°C at the end of storage period. The biochemical properties studied include primary and secondary metabolites. Primary metabolitcs like carbohydrate, fat, protein and starch present in turmeric powder were 67.24, 9.75, 3.37 and 59.53% respectively. There was no considerable change in primary metabolites during the storage period of 10 weeks. The secondary metabolites of stored turmeric powder such as essential oil, oleoresin and curcumin content were determined. There was no significant change in essential oil content at ambient and 30°C of storage. While at the end of storage period for ten weeks, the essential oil content reduced by 10 % when it was stored at 40 and 50°C and the oleoresin content decreased by 8.50% when it was stored at ambient conditions. At the end of storage period, curcumin content reduced by 4.58% and 10.38% when turmeric

powder was stored at ambient and 50°C, respectively. Volatile constituents of essential oil were determined using GC-MS. The constituents like α-terpineol, zingiberene and tumerone decreased while ar- curcumine and curlone increased at the end of 10th week of storage. The total plate count or stored turmeric powder decreased from 5.4 x 10^4 cfu/mL to 1.2 x 10^4 cfu/mL at 50°C towards the end of storage period. From the current study, it was found that temperature had a significant effect on storage of turmeric powder. Ambient temperature was found better for storage period of turmeric powder up to 10 weeks since it retains essential oil with a minimum reduction in oleoresin and curcumin content.

34. Development of DNA markers for identification of turmeric (*Curcuma longa* L.) varieties

Ms. R. Swedha/Vellore Institute of Technology University/2011/
Guide: Dr. K. Nirmal Babu

The present study aims at developing diagnostic molecular markers for varietal identification of improved varieties of turmeric based on ISSR (Inter Simple Sequence Repeats) profiling. The varieties Suguna and Sudarshana can be differentiated by using the primer UEC 834b and T 06. The varieties Alleppey Supreme and Kedaram could be differentiated by using the primers UBC 808 and ISSR 4 and T 06. Suvarna and Suguna be differentiated using the primers UBC 808, UBC 816, UBC 817. UBC 834a, ISSR and ISSR 4. The varieties Prabha and Prathiba could be separated by using the primers UBC 834b and 844b.The varieties Prabha and Sudarshana could be differentiated by the primers UBC 808, UBC 817 and 834b.The identified markers can later be converted in more robust SCAR (Sequence Characterized Amplified Regions) markers. This helps in huge planting material production programme under National Horticulture Mission to ensure the supply of quality and genetically pure certified planting materials for cultivators. In addition the markers developed can also be used for checking adulteration at industry level.

35. Cloning of Phenylalanine Ammonia-Lyase (PAL) gene from turmeric (*Curcuma longa* L.)

Ms. R. Supriya/Bharathidasan University/2010/Guide: Dr. T. E. Sheeja

This is the first report on cloning of phenylalanine ammonia-lyase gene from turmeric. Since this enzyme catalyses the conversion of L-PHE to trans cinnamic acid in plants, it attains more significance in the relevant field. High quality DNA was isolated from the developing rhizome and the purity was checked by gel method. The

absorbance ratio was found to be 1.92, which indicates less contamination of proteins and RNA. The isolated DNA was then amplified enzymatically by peR using specific primers to PAL genes and the amplified fragment had an appropriate length of 522 bp. The fragments were then cloned using TOPOTA cloning vector and the desired clones were sequenced. Sequences were aligned manually and analyzed using BLAST to check the identity with other sequences available in Gen Bank. The analysis shows 94.3% similarity with the PAL gene from *Zingiber officinale* (Phenylalanine ammonia-lyase mRNA, partial cds-526 bp product). Here these similarities confirm results that our sequenced results were PAL gene sequences.

36. Study of the role of key phenylpropanoid enzymes in the partitioning of primary and secondary metabolites and effect of temperature on antioxidant potential in high and low curcumin turmeric accessions

Ms. Vani Chithra/Bharathidasan University/2009/Guide: Shamina Azeez

This study also attempts to substantiate the antioxidant properties of turmeric rhizomes and compare the effect of temperature on the same. The coloring pigment in turmeric, the curcuminoids, is well known for their antioxidant activity. However, they are readily decomposed on exposure to bright light, high temperatures or oxidative conditions. Considerable decrease in the concentration of curcumin, has been observed during the heat processing of turmeric. They have characterized three major molecules among the several degradation compounds of curcumin: ferulic acid, vanillin and vanillic acid. The diketone bridge in the curcumin molecule is vulnerable to heat. In addition, formation of vanillic acid and vanillin indicated that the molecule is sensitive to heat at the first carbon atom of the alkyl chain which connects the two phenyl moieties. In addition to the curcuminoids, other compounds possessing antioxidant properties in turmeric include: Y-terpinene, ascorbic acid, beta-carotene, beta-sitosterol, caffeic acid, campestrol, camphene, dehydrocurdione, eugenol, p-coumaric acid, protocatechuic acid, stigma sterol, syringic acid, turmerin, turmeronol -a, turmeronol -band vanillic acid. The volatile components are easily lost during the process of preparing hot extracts, as observed from our results. Turmerin contains three residues of methionine which are partly responsible for the observed antioxidant activity. Thus the present study gives demonstrable proof of the antioxidant property of turmeric rhizomes, though the cold extracts were more efficient than the hot extracts.

37. Intraclonal variability study in alleppey finger turmeric accessions using molecular tools

Ms. Athulya Aravind/Bharathiyar University/2008/Guide: Dr. B. Sasikumar

In the present work an attempt is made to study the intraclonal variability in twenty two Alleppey Finger Turmeric accessions using RAPD and ISSR markers. Good quality DNA was isolated from fresh leaves of the twenty-two Alleppey Finger Turmeric accessions using CTAB method. The DNA yield varied from 45 to 140.5 µg/g of fresh leaf tissue. RAPD reaction was carried out with 30 ng DNA in a 25 µl reaction mixture, 1 unit of Taq DNA polymerase, 0.2 mM dNTPs, 2 mM $MgCI_2$,10X concentration of assay buffer and 10 picomoles of primer. In the ISSR reaction used 25 ng genomic DNA and 60 picomoles primer concentration were used. Concentration of $MgCl_2$, dNTPs and Taq DNA polymerase used were same as that of the RAPD reaction: However, the annealing temperature was raised to 50°C and number of cycle repeats was 32. A total of eighteen random decamer primers were selected for RAPD analysis. The extent of RAPD polymorphism ranged from 41.18 to 94.44%. Nine primers produced unique bands in the two of the twenty-two genotypes studied. Maximum number of unique bands was observed in the Acc.588 collected from Kadappara. The primers which produced discrete bands in this Acc.OPA-02, OPA-04, OPA- 18, OPAJ9, QPB-07, OPC -0 6, OPC-20 and OPD- 15.OPC-06 produced two unique bands in the Acc. 584 while OPE-07 produced only oneunique band in this Acc. The extent of ISSR polymorphism ranged from 33.33 to 90%. Out of the ten ISSR primers studied, only one primer, ISSR- 8 was discriminatory in the Acc. 588. The UPGMA dendrogram constructed based on the similarity coefficients showed a single cluster with five groups. In this cluster, the maximum similarity (99. 13%) was observed between the Acc. 577 and 579 which were collected from Puthencruz and Vengola, respectively. Acc.588 displayed a unique genetic architecture among the Acc studied by generating maximum number of unique bands in both RAPD and ISSR markers. The study indicated moderate intraclonal variability in 'Alleppey Finger Turmeric' thereby reinforcing the fact that 'AFT' is not single genotype rather a mixture of few genetically distinct but morphologically not so distinct Acc.

38. Computational analysis and annotation of *Curcuma longa* L. ESTs

Ms. P. H. Remya/Bharathidasan University/2008/Guide: Dr. Santhosh J. Eapen

Genomic studies on turmeric can provide several insights into many of these properties. One such important study is EST analysis which may facilitate gene finding in turmeric. An EST is a tiny portion of an entire gene that can be used to help to identify

unknown genes and to map their positions within a genome. The EST sequences in a genome are represented with the help of genetic markers. A genetic marker is known that pieces of DNA that lie near each other on a chromosome that tend to inherit together. Among the genetic markers, the Simple Sequence Repeat markers are becoming the most important markers in both plants and animals as they have several advantages over other molecular markers. From the EST analysis of *C. longa*, it is concluded that CAP3 is the best web tool forthe sequence assembly. CAP3 produced the maximum number of contigs with minimum errors. An unusual feature of CAP3 is the use of forward-reverse constraints in the construction of contigs. The CAP3 result was analyzed with five different SSR finding tools and found out that SSR tools such as MISA, SSRIT and WEBTROLL are the best ones. Primers were designed for these results and validated to find out high quality primers using Primer 3 and Fast PCR. These are being analyzed in the wet lab for cloning. The results are annotated with BLASJX and this gave many functional aspects which are useful to the scientific community who are working in the area turmeric genomics.

39. Changes in quality traits of turmeric under different drying conditions

Ms. K. Anu Chacko/Mahatma Gandhi University/2007/Guide:Dr.T. John Zachariah

In the present study, cured samples whether dried for 6 hours or drying for three hours followed by an interval of one hour and again dried for three hours took about eighteen hours (three days) in hot air oven, forty-two hours (seven days) in reverse flow drier, forty-eight hours (eight days) in agricultural waste drier and sixty hours (ten days) in sun drying for complete drying. Non-cured samples took thirty-six hours (six days) in hot air oven, sixty hours (ten days) inreverse flow drier, sixty hours (ten days) in agricultural waste drier and eighty-four-hours (fourteen days) in sun drying. The interval of one hour after three hours continuous drying did not show any advantage or disadvantage in the drying time. Irrespective of drier or drying time non-cured samples yield more than 45% oil compared to cured samples. In oleoresin recovery also, non-cured samples yield more than 15% compared to cured samples. The colouring principle curcumin did not show much variation due to curing or drying conditions. The essential oil constituents were evaluated by Gas chromatography. Major constituents in turmeric oil were ar-turmerone and beta turmerone. The percentage composition of both these compounds did not show significant variation due to the drying regimes. To get maximum recovery of oil and oleoresin mechanical drying without curing is more beneficial.

NUTMEG

1. **Studies on decline and die-back disease of nutmeg (*Myristica fragrans* Houtt.)**

Ms. A. Fadla Basima/University of Calicut/2019/Guide: Dr. C. N. Biju

The isolate under investigation was morphologically characterized and PCR conditiontargeting ITS region was standardized leading to the generation of expected amplicon. Molecular analysis placed the pathogenic isolate in the species, *Lasiodiplodia theobromae*. *In planta* pathogenicity studies indicated that, the isolate could infect nutmeg inducing symptoms similar which was observed under field conditions. Among the nine fungicides evaluated, Bordeaux mixture (0.1%), carbendazim- mancozeb (0.1%), and metalaxyl-mancozeb (0.125%) were completely inhibited mycelia growth of the pathogen.

2. **Chemoprofiling of selected nutmeg accessions and nutritional evaluation of value-added products from nutmeg**

Ms. Athira Vijayan/Kerala University of Fisheries and Ocean Studies/2018/
Guide: Dr. N. K. Leela

Essential oil of 12 different nutmeg samples were extracted using hydro-distillation method. The average yield of the oil is varied between 7-23%. In comparison to *M. fragrans*, *M. prainii* yielded highest amount of butter and oleoresin. The compounds present in the samples were analyzed by GC-MS method the major components present in the mace oils are α-pinene, sabinene, β-pinene, safrole and myristicin. Butter and oleoresin were extracted by cold percolation method using petroleum ether and acetone respectively the amount of butter in the samples analysed varied from 28.76-37.07%. Nutmeg seeds yielded 1.46% -3.62% oleoresin. The compounds present in the butter were esterified and then analyzed using GC-MS for fatty acid methyl ester profiling. It was observed that myristic acid methyl ester and palmitic acid methyl esters were the two important compounds present in the methyl ester of nutmeg butter. Two important value added products nutmeg candy and nutmeg herbal tea were prepared from nutmeg and were evaluated for quality. In the case of nutmeg candy, the overall acceptability of the product was good. The general comment on the taste of the product was that it had high pungency which needs to be addressed in future studies. The taste of the product is mainly determined by its maturity, where the

matured fruits give a better taste. A herbal tea preparation was also made using dried nutmeg leaves and the study reveals that the nutmeg leaves improve the antioxidant activity of tea.

3. Isolation and characterization of aflatoxigenic *Aspergillus* spp. from nutmeg

Ms. S. Amrutha/Bharathiar University/2018/Guide: Dr. C. Sarathambal

In the present study,we found that seventy-five presumptive *Aspergillus* spp. has been isolated from 45 nutmeg and mace samples collected from markets. Morphological features observed on most PDA plates revealed that *A. flavus* formed white mycelia with spreading dark green, green or yellow colonies.The conidia crowns were olive green with some being over laid by olive yellow colonies. The fungus produced brown sclerotia in some samples. The microscopic morphology starts with the typical large, swollen vesicle. From the vesicle, both uniseriate and biseriate phialides form in a loosely radiate fashion surrounding most of the vesicle. The reverse colours of the ADM plates ranged from weak orange colour to a bright orange colour were observed as a indication of aflatoxin production. Forty isolates of aflatoxin producing *Aspergillus* spp. screened according to the orange colour pigmentation.

4. Studies on chemopreventive activities of *Pimenta dioica* (L.) leaf essential oil on aflatoxigenic *Aspergillus flavus* associated with nutmeg

Ms. Rona Viswanathan/Kannur University/2018/Guide: Dr. C. Sarathambal

Present study describes the antifungal activity, essential oil composition, and antioxidant capacity of leaves of *Pimenta dioica*. The study analyses the potential of the extracts of the leaves of *P. dioica* in inhibiting the growth of selected *Aspergillus* fungi.The phytochemical analysis of the essential oil which showed the highest antifungal activity and separation and localization of the bioactive compounds by TLC and bioautography were also done. Based on the present study, it could be concluded that essential oil from allspice possess fungi toxic activities inhibiting the growth of *Aspergillus flavus* leading to irreversible deleterious morphological alterations and thus it is worth exploiting for the biomanagement of *Aspergillus flavus*. This study also revealed important antioxidant and phenolic properties of methanolic extracts from allspice leaf essential oil. Further studies are needed to identify and characterize the structure of the bioactive compound responsible for the antifungal activity. The study has laid the foundation for discovering a potent phytochemical with possibly lower side effects to treat fungal infections at post harvest level.

5. Nutritional composition and antioxidant potential of value-added products from nutmeg (*Myristica fragrans* Houtt.) and kokkum (*Garcinia indica* Choisy) fruits

Ms. N. Prajisha/Kerala University of Fisheries and Ocean Studies/2017/
Guide: Dr. N.K. Leela

The dried nut and mace contained 8.8 and 12.8% essential oil respectively and rind contained 0.2% essential oil on fresh weight basis. Nut and mace contained 23.5% and 22.0% oleoresin respectively. Nutmeg seed contained 39% butter. The chief components of the mace oil were α-pinene (5.65%), sabinene (4.45%), limonene (8.62%), terpinen-4-ol (9.94%), myristicin (6.53%) and elemicin (11.97%). The chief components of the seed oil were γ-terpinene (6. 80%), terpinen-4-ol (16.84%) and γ-terpineol (8.42%). Pericarp oil contained a- terpinene (6.51%), limonene (6.98%), γ-terpinene (10.04%), telpinolene (7.81%), t-terpinen (26.52%) and α- terpineol (12.04%).The physico-chemical property of the nutmeg candy, nutmeg jam and kokum jam. There was not much variation in pH acidity and anthocyanin content after storage for I month in all the three products. But TSS of the jam showed higher value after I month storage.The antioxidant activity determined by DPPH assay was expressed as IC50 values, and the value denotes the concentration of sample required to scavenge 50% of free radicals. IC50 values of kokum jam, nutmeg jam and nutmeg candy were 5.2 mg/mL, 12.6 mg/mL and 24.5 mg/mL respectively. Low IC50 value indicates high antioxidant potential. Among the three products kokum jam showed higher antioxidant activity which could be due to its high anthocyanin content. Organoleptic evaluation of the jam and candy carried out on a 9 point hedonic scale among 30 persons. Kokum jam: More than 70% persons rated 'color' and 'appearance' as above 7 of the 9 point hedonic scale. More than 60% members rated consistency above 7. 70% members rated texture above 6. The data indicate that there is scope for improving the texture and consistency of kokum jam for better acceptability. Nutmeg jam: Above 70% evaluators rated the appearance of nutmeg jam as above 7 on 9 point hedonic scale and 80% members gave score above six. More than 80% evaluators gave score 6 and above for texture and consistency. 75% evaluators rated above 6 for overall acceptability. Nutmeg candy: More than 70% evaluators rated 'color' and 'appearance' as above 7 of the 9 point hedonic scale; 60% members rated consistency above 6; 70% members rated texture above 6. Overall acceptability of the product was gold but only 56% members gave the score 8. The data indicate that the texture and consistency needs improvisation. Organoleptic evaluation of the products indicates that acceptability of nutmeg jam was better than nutmeg candy and kokum jam. Kokum jam possesses very attractive color

but the taste and consistency was rated low compared to other products by the evaluators. All the products were received well by the consumers; however there was minor disparity in the scoring of various parameters. All three products developed were found to be well accepted by the evaluators; but there is still scope for improvement.

6. Drying characteristics of nutmeg and mace (*Myristica fragrans*) dried under different methods and its quality evaluation

Mr. Desmond Joseph/University of Calicut/2014/Guide: Dr. E. Jayashree

Drying of nutmeg and mace was conducted by four different methods like drying in solar tunnel drier with a biomass back up and mechanical drying at drying temperatures of 45, 50 and 55°C. The experiments on drying of nutmeg was conducted during July 2014 by spreading 2 kg of fresh nutmeg in stainless steel trays of size 90.5 x 90.5 cm which was obtained from a fanner from Pullurampara village, Kozhikode, Kerala. While 0.5 kg mace (variety Vishwasree from ICAR- Indian Institute of Spices Research, Kozhikode) was spread over the stainless trays (90.5 x 90.5 cm) and placed for drying inside Solar tunnel drier with biomass back up. In case of mechanical drying of nutmeg and mace, weighed quantities were placed inside the drier and drying was performed at varying temperatures of 45, 50 and 55˚C. The loss in weight during drying was recorded every 3h and drying was continued till constant weight was obtained. Three such replicates were maintained for all the experiments. During the drying period from 9.00 to 18.00 h weather parameters like temperature, relative humidity and solar intensity were measured both in side and outside the solar tunnel drier. A single factor completely randomized block design was followed to determine the effects of drying methods on physical and biochemical constituents of dried nutmeg and mace. Drying characteristic curves were plotted for moisture content, moisture ratio and drying rate verses drying time. Physical parameters including moisture content, bulk density, dry recovery shell recovery, kernel recovery and dimensions of kernel and nutmeg were recorded. Primary metabolites such as carbohydrates, proteins, fat and secondary metabolites such as oil, oleoresin and lycopene were analyzed. Identification of volatile oil constituents were also performed by gas chromatography (Shimadzu GC 2010) equipped with mass spectroscope (Shimadzu QP-2010).The drying characteristics curves indicated that the moisture content of nutmeg and mace decreased constantly with increase in drying time. The process of drying nutmeg in solar tunnel drier was completed in 147 h and mace in 33 h. In mechanical drier, at drying temperatures of 45, 50 and 55°C, nutmeg drying was completed in 105,102 and 78 h and mace drying was completed in 9, DH and 6 h, respectively.

7. Chemical profiling and antioxidant potential of a few selected accessions of nutmeg (*Myristica fragrans* Houtt.)

Ms. G. Anjana/University of Calicut/2013/Guide: Dr. N.K. Leela

In the present study fourteen accessions of nut and mace were evaluated for essential oil constituents, oleoresin content and antioxidant potential. Major essential oil constituents of nut and mace were sabinene, a-pinene, p-pinene, limonene, 4-terpineol, myristicin, elemicin and safrole. Wide variability was observed among the accessions with respect to the essential oil constituents such as sabinene, myristicin, elemicin and safrole Among the studied accessions a few unique accessions were observed: essential oil from mace of IC 645944 contained high safrole (18.2%) and high elemicin (11.0%) where as IC 548918 contained high safrole (14%) and high myristic in (13.2%). IC 548917 recorded maximum myristicin (26. 3%). In yellow mace elemicin level was high (18%). The essential oil from nut also was dominated by sabinene, a-pinene, p-pinene, limonene, 4-terpineol, myristicin, elemicin and safrole. But the percentage composition of the oils was substantially different. The oil from IC 548915 had high elemicin (18%) with low myristicin (7.7%). IC 548916 had 17.72% elemicin and 13.19% myristicin. IC 548881 had high safrole (17.8%) and 12.7% myristicin. IC548917 recorded 22.6% sabinene and 22.9% p- pinene. In IC 548917 p-pinene was very high compared to others « I0%). Nuts of fruits with yellow mace were also dominated by elemicin (14-16%) with low myristicin and safrole. Nut contained 24-38% oleoresin with the highest in IC 548923. *M. fragrans* seeds are reported to possess antilipid-peroxidant properties. Antioxidant activities of mace and nut oil were determined by DPPH method and Phospho molybdenum method using BHT as standard. The results indicated that antioxidant potential of the essential oils were positively correlated with myristicin and negatively correlated with elemicin. The findings emphasize the medicinal value of essential oils from nut and mace.

8. Studies on drying characteristics of nutmeg (*Myristica fragrans)*

Mr. Anup George/Karunya Univeristy/2012/Guide: Dr. E. Jayashree

In the first stage study the fresh nutmeg were harvested from a private farm and studied the different drying patterns such as sun drying, traditional drying, and commercial drying. The samples obtained from different samples were taken and biochemical analysis studied. During the sun drying the biochemical parameters was good. For the traditional dying the temperature 45°C was good to obtain the maximum biochemical parameters, and for commercial drying the temperature 55°C was recorded

the best among all the temperatures. Now for the commercial drying the samples of nut and mace were studied under various treatments of different temperatures.

9. Molecular characterization of *Myristica* species based on 18s rDNA gene sequence analysis

Ms. K. V. Fijida/University of Calicut/2009/Guide: Dr. T.E. Sheeja

Choice of appropriate tissue is very important for DNA isolation. Third leaves from the shoot tip of *Myristica* were found to give good quality DNA without much polysaccharide contamination and low level of RNA. The yield was maximum using CTAB buffer at pH 7. Efficient removal of secondary metabolites, polysaccharides and proteins is very important for extraction of good quality, high molecular DNA which is essential for most molecular biology techniques. The three wild endangered species of *Myristica* has been studied for morphological and genetic variations. A lot of morphological variations were observed in the leaf and floral characters of these three species.

10. Identification of sex specific molecular markers in nutmeg (*Myristica fragrans* Houtt) by RAPD and ISSR

Ms. Shibina Nazir/Periyar University/2008/Guide: Dr. T.E. Sheeja

The present study indicates that there exists clear cut variation in the PCR profiles between male and female plants in *Myristica fragrans*. Out of the three methods *viz*. RAPD, ISSR and denaturing PAGE tried to reveal polymorphism; it was observed that RAPD is the most appropriate technique to detect them. Even though a number of polymorphic bands were identified and confirmed further experimentation involving analysis of individual DNA is warranted to exactly pinpoint the sex specific nature. Future experiments should also involve more number of female and male trees to be involved in screening. programme to clearly say the frequency of abundance of this band and whether it can be exploited to develop SCAR markers for detecting sex specific markers in *Myristica fragrans.*

11. Studies on the essential oil profile and other biochemical constituents in nutmeg

Ms. V. Angayarkanni/Bharathiyar University/2008/Guide: Dr.N.K. Leela

The present study is an attempt to compare the essential oil composition of fresh and dried nutmeg and mace and that of leaves of male, female and bisexual nutmeg trees. Essential oil composition of fresh and dry nutmeg and mace showed some differences. The chief constituents of fresh nut oil were sabinene and 4-terpineol, where as that of mace was sabinene. But, the pericarp oil was dominated by 4-terpineol and1-

terpineol. Major components of the essential oils of dried nut and mace were I-pinene, sabinene and myristicin, which together accounted for 42% of the oils. But, mace oil contained substantial quantity of safrole (18.66%) also. Safrole content was less in nut oil compared to that of mace. No clear- cut difference was observed in the essential oil composition of leaves of male, female and bisexual nutmeg. But, myristicin content in the essential oil of leaves of male nutmeg was low (0.3-1.2%) compared to that of majority of female and bisexual plants. However, this has to be evaluated in a larger population before drawing a conclusion. Interestingly, sabinene was absent in pericarp oil and it contained relatively higher levels of 1-terpinene,1-terpinolene and caryophyllene compared to the oils from nut and mace.

12. Towards sex differentiation in dioecious *Myristica fragrans* Houtt. using ISSR and RAPD methods

Ms. K. Vidya/Bharathidasan Universit/2007/Guide: Dr. T. E. Sheeja

In nutmeg, sex determination at juvenile stage is very important because that takes about 5 to 8 years to come to flowering. Characters like size of leaves and type of branches can be used for determining sex of fully grown trees, but in case of trees it is difficult. In the present study Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR) primers were used for sex determination. Six male and six female leaf samples were used for the ISSR technique. At the same time 5 male and 5 female trees were used for the RAPD technique. 50 RAPD primers and 12 ISSR primers were used for the same. ISSR primer failed to identify any polymorphism, however one of the RAPD primer OPA 07 showed polymorphism in the female bulk and in one of the individual female sample (A9/11). So we can further use this method for the sex differentiation in nutmeg.

13. Optimization of PCR parameters and characterization of elite nutmeg (*Myristica fragrans* Houtt) accessions by RAPD

Ms. Jessy Jerome/Bharathidasan University/2006/Guide: Dr. T. E. Sheeja

The present study, good quality DNA was isolated from the third leaf in young shoots of nutmeg. The yield obtained was found to be maximum with CTAB at pH 9. Each new PCR application is likely to require optimization. In nutmeg PCR optimization was carried out to influence the final yield of amplification products. Various optimization conditions including DNA concentrations, $MgCl_2$ concentration, different annealing temperature, different Taq Polymerase, different dNTP and Taq concentrations, different thermocyclers and primer combinations were carried out. The

PCR protocol of each experiment resulted in reproducible patterns of amplification products. Thus in nutmeg optimization of PCR parameters was done.

14. Molecular characterization of wild and related genera of *Myristica* by RAPD, ISSR and 18Sr DNA - RELP Techniques

Ms. M. Lakshmi/Acharya Nagaraja University/2006/Guide: Dr. T. E. Sheeja

This is the first report on molecular characterization of *Myristica* using RAPD and ISSR markers Good quality DNA was isolated from the third leaf in young shoots of nutmeg. The yield obtained was found to be maximum with CTAB. DNA isolated was of high molecular weight (- 23 Kb) showed no shearing and gave clear bands. DNA yield ranged from 25-300 microgram per gram fresh tissue. Maximum yield was obtained from *K. andamanica*, *M. malabarica*, *M. fragrans* and the unidentified species.

CINNAMON

1. *In vitro* assay guided fractionation of compounds from cinnamon bark (*Cinnamomum verum* J. Presl) for its antidiabetic activity

Ms. Drisya Mol Babu/University of Calicut/2018/Guide: Ms. R. Sivaranjani

The present study investigated the antidiabetic potential of compounds isolated from cinnamon bark methanol extract by studying its α-glucosidase inhibitory potential. The results demonstrated that partially purified fractions collected from crude cinnamon extracts inhibited α- glucosidase enzyme more efficiently than standard acarbose in *in vitro* based assay. There is no correlation between the sequence and its α-glucosidase inhibitory potential. It could be implied that antidiabetic compounds spread over different polarity group in cinnamon bark. Structural studies confirmed the sequence of 21 amino acids in the active site of the enzyme which forms additional sugar subsites (+ 2 and + 3 subsites), accounting for the preference for longer substrates of MAGM-C compared with that of MGAM-N. These binding pockets might very well used by large phenolics of cinnamon to inhibit the activity of glucosidase. Earlier studies suggested that cinnamon's polyphenolic composition mainly tannins (cinnamon tannins) and proanthocyanidins as major compounds. Both of these compounds have complex structure with many hydroxyl groups freely available for bonding with enzymes active site pockets. This might be the reason for the higher α-glucosidase

inhibitory activities of cinnamon fractions than acarbose. Overall, the *in vitro* assays suggest that cinnamon fractions have clear advantage over acarbose to reduce post-prandial glucose increase by inhibiting α-glucosidase enzyme. The inhibition of starch degrading enzymes like α-glucosidase and α-amylase is the simplest and important step in reducing the blood glucose level after a meal. Further work of identifying the structures of the compounds from these fractions would help in partial synthesis of the compounds for potential antidiabetic activity.

2. Molecular characterization and species inter relationships of Cinnamomum using RAPD polymorphism

Ms. R. Jensy/Bharatidasan University/
Guide: Dr. K. Nirmal Babu & Dr. B. Krishnamoorthy

Molecular markers will augment the conventional phenotypic and biochemical characterization as they can detect even minute differences, irrespective of the climatic effects. Very few reports were available in molecular characterization of cinnamon. The present study was initiated in this background to use RAPD polymorphism as an index for genetic variability and interrelation ships among sevens pecies of cinnamon occurring mainly in south India and a few from north India available at the germplasm repository of Indian Institute of Spices Research.

3. Fungitoxic effect of essential oil of cinnamon bark (*Cinnamomum cassia*)

Ms. N.P. Dhuwaraga/Periyar University/2008/Guide: Dr. B. Chembakam

The present study focused on the potential use and efficacy of the volatile fraction of cinnamon bark (*Cinnamomum cassia*) against storage fungi of spices. Resultsindicated that both mycelial growth and release of an alflatoxins by *A. flavus* and by *A. parasiticus* were affected by the essential oil and the active ingredient, cinnamaldehyde. Such modifications induced by essential oils may be related to the interference of essential oil components with enzymatic reactions of wall synthesis, which affects fungal morphogenesis and growth.

4. Biochemical characterization and isozyme profiles of four cinnamomum species

Mr. T. M. Vipin/Bharathidasan University/2006/Guide: Dr. N. K. Leela

Present study is an attempt to understand the intra- and inter-specific variations in the genus *Cinnamomum* with regard to their biochemical constituents and enzyme activities. Four *Cinnamomum* species namely, *C. cassia, C. camphora, C. verum* and *C.tamala* and also few accessions of *C. verum* were used in the studies. The contents of

total carbohydrates, starch, reducing sugars, proteins, amino acids and phenols were estimated in the leaves of *Cinnamomum* germplasm collections maintained at Indian Institute of Spices Research, Calicut. The enzymes studied were peroxidase, polyphenol oxidase and superoxide dismutase. The profiles of volatile and non-volatile constituents in the leaves of the four species were also compared.

GARCINIA

1. **Study on the use of anthocyanin pigments from kokum (*Garcinia indica* Choisy) rind for staining protein and DNA**

Ms. Pooja Sabu Thomas/University of Delhi/2021/Guide: Dr. K. Anees

The brilliant hue of anthocyanin extract from kokum fruit rind promises its use as a natural dye and thus, an analysis on the potentiality of anthocyanin extract from Kokum rind as a natural alternative to the dyes used in protein and DNA staining in SDS-PAGE and Agarose Gel electrophoresis respectively were studied. A preliminary test was done by making use of the kokum anthocyanin extract as a dye for cytological staining of a few species of Zingiberaceae, for which the dye was found to be useful when the stained species were viewed under a microscope. For analyzing its utility in staining protein and DNA in Gel electrophoresis – the experimental techniques used for the study being SDS-PAGE for protein and Agarose Gel Electrophoresis for DNA - 1% kokum anthocyanin extract was obtained from the dried fruit rind, using water as the extracting solvent. The potentiality of the kokum anthocyanin extract as a Pre and Post - electrophoretic staining agent for protein in SDS-PAGE was investigated in the study, with the latter being further evaluated under different physiological conditions such as pH and temperature. Pre-electrophoretic staining was employed by adding the kokum anthocyanin extract in polyacrylamide, and agarose gels respectively, during their preparation, following which the bands were visualized under UV-Light after running the gel. Staining using the conventional Coomassie Brilliant Blue protocol in SDS-PAGE, and ethidium bromide in DNA Agarose Gel Electrophoresis was also done concomitantly, to compare the extent of separation of the sample bands, and its sensitivity with the ones stained using the kokum anthocyanin extract. The present study showed that the anthocyanin extract from the kokum fruit rind, stained protein, and

DNA in gel electrophoresis. Further studies are needed for the development of a proper staining protocol.

2. **Extraction, fractionation and isolation of antioxidant principle from *Garcinia indica* (Choisy)**

Ms. Tesna Mathew/University of Calicut/2018/Guide: Dr. N. K. Leela

Sequential extracts of *Garcinia indica* fruit rind and seed were prepared using hexane, chloroform, methanol and water as solvents by soxhlet extraction and methanol yielded the maximum residue from fruit rind and hexane yielded the maximum residue from kokum seed. The antioxidant potential was evaluated by DPPH scavenging assay. And total phenolic content was determined by Folin-Ciocalteau method. Hexane extract of fruit rind exhibited highest activity which also had highest total phenols. The chief constituent of the hexane extract was isolated by column chromatography as yellow crystals. The purity of compound was tested by High Performance Liquid Chromatography (HPLC) and its antioxidant potential was compared with that of known antioxidants such as curcumin, eugenol, BHA. Antidiabetic potential of the extracts and the isolated compound was determined by α- glucosidase assay, which revealed that antidiabetic activity of the isolated compound in terms of IC 50 value was 502.70 µg/mL where as that of acarbose was 311.06 µg/mL. The above data provides evidence that the fruit extracts of *G. indica* and isolated compound are rich in natural anti-oxidants and thus justify its use in folk medicine especially in the management of free radical mediated disorders. Kokum butter was extracted using hexane and its fatty acid profile was determined by GC-MS analysis and the major compounds were identified as stearic acid oleic acid, palmitic acid.

3. **Shelf life evaluation of ready to serve kokum juice**

Ms. Rithu Susanna/Mahatma Gandhi University/2017/Guide: Dr. E. Jayashree

Freshly harvested matured kokum fruits obtained from ICAR- Indian Institute of Spices Research (ICAR- IISR), Chelavoor farm, Kozhikode, Kerala was selected for the study. The fruits were washed in running tap water to remove the dust and dirt adhering on to the surface. Ready to Serve (RTS) kokum juice was prepared from concentrated kokum syrup by adding boiled and cooled water in the ratio 1:6. In order to raise the concentration of kokum juice to 15°Brix, sugar was added and the juice was stirred and cooled. After cooling, potassium meta bisulphite (KMS) at therate 0.1 per cent was added as preservative and stirred thoroughly. 100 mL of kokum juice was then filled in to the polyethylene terephthalate bottles (PET) and stored under ambient and

refrigerated storage conditions. A three factor Completely Randomized Block Design was followed to determine the effect of the three treatment combinations of kokum RTS juice on various quality parameters during storage. The juices were kept under ambient and refrigerated storage conditions for a period of 75 days.

4. Micropropagation and molecular characterization of three species of *Garcinia*
Ms. Simi Mohan/Bharathidasan University/2010/Guide: Dr. Utpala Parthasarathy

In the present studyis to develop a simple and efficient method for high frequency plant regeneration fromthe seeds of these three species. Two approaches *viz*. Micropropagation and *in vivo* germination of seed fragments were attempted. Apomictic nature of *Garcinia* seeds makes them the ideal explants for *in vitro* establishment and multiplication of selected superior clones for obtaining true-to-type plants. Micropropagation studies conducted in the preset investigation has shown that MS medium + 2.5 mg/litre BAP gave best response and induced multiple shoot initiation. Root initiation took place in MS medium with or without NAA but root elongation was faster in MS medium supplemented with 2 mg/litre of NAA. The highest number of multiple shoots obtained in this medium was twenty in *G. indica*, followed by eight in *G. gummigutta*. *G. tinctoria* did not respond to the above media and gave only one shoot. This indicates species differences in *in vitro* response of *Garcinia*, *G. indica* being the most responsive and *G. tinctoria* is the least responsive. The cultured plants were hardened and planted out with over 95% success. *In vivo* seed germination: The seed of *Garcinia* is reported to be diffused and hence fragments of seed instead of whole seed could be used for propagation. Studies on *in vivo* seed germination using whole seeds as well as fragments of seeds resulted in 100 % gennination. However species differences were notices in germination pattern and plant development. In *G. indica* most of the seeds germinated with roots and shoot in 15 days, adventitious roots developed with in 27 days and leaves were developed with in 45 days. In *G. gummigutta* took double the time for seed germination with roots and shoot (32 days), adventitious roots developed with in 50 days. In *G. tinctoria* the results were similar to those of *G. indica* in that the seeds germinated with roots and shoot in 20 days, adventitious roots developed with in 23 days. But leaf development was slow and took 60 days. When the seeds were cut into three fragments and placed for gemination plants developed from all the three fragments *G. indica.* In *G. gummigutta* and *G. tinctoria* both the terminal fragments gave plantlets while the middle fragment did not develop any plant. The plant development pattern was same as in the case of whole seeds. Thus the multiplication

rate of *Garcinia* was doubled in *G. gummigutta* and *G. tinctoria* and tripled in the case of *G. indica* using seed fragments. Genetic fidelity of micropropagated plants In order to ensure that the plantlets developed through micropropagation did not show somaclonal variation, the genetic stability of cultured material was tested using RAPD profiling. Twelve RAPD primers were used and all RAPD profiles developed were monomorphic and are similar to those of field grown mother plants. No variation was detected within the micropropagated plants and this indicates that the micropropagation plants are genetically stable. Thus efficient methods for multiplication were developed for two species of *Garcinia viz*. *G. indica* and *G. gummigutta* through micropropagation and all the three species *viz*. *G. indica*. *G. gummigutta* and *G. tinctoria* through low cost multiplication using seed fragments. The plantiets developed were genetically stable and are similar to the mother plant. This technology can be used for multiplication of elite genotypes and conservation of *Garcinia* species.

5. Standardisation of RAPD profiling of *Garcinia* species of western ghats

Mr. S. Umamaheswara Rao/Acharya Nagarjuna University/2009/
Guide: Dr. Utpala Pathasarathy

RAPD PCR technique has been widely used in the analysis of genetic diversity in plants. In the present study this technique was used to analyze the genetic diversity among four indian species of *Garcinia*. In this study 50 ng of DNA was used for amplification which was found to be optimum. Taq DNA of 1 unit gave good amplification and sufficient amount of desired product. Very good amplification was obtained with a concentration of 10 mM dNTP concentration depends on the length and composition of the target sequence. Lower concentration increases the specificity and fidelity of PCR by preventing mispriming at non target sites. $MgCl_2$ concentration (50 mM) affects primer annealing, strand dissociation temperature and enzyme activity All the primers were used at a concentration of 10 Pico moles. Higher concentration may promote mispriming and generate primer dimers. The standard buffer containing 50mM KCI, 10 mM Tris HCI, 1.5 mM $MgCl_2$ and 100 mL/mg of gelatin will be adequate for majority of genomic PCR.

6. Comparison of *Tamarindus indica* and *Garcinia gummigutta* by biochemical analysis and RAPD technique

Ms. Azneeta/Periyar University/2008/Guide: Dr. Shamina Azeez

In the present study, both ethanol and water extract of tamarind and *Garcinia* exhibited antioxidant properties, as determined by DPPH assay, TBARS assay and by the phosphomolybdenum method. Ethanol extract of *Garcinia* demonstrated higher

antioxidant activity by the DPPH assay, followed by the ethanol extract of tamarind. The antioxidant potential as measured by the TBARS assay was maximum the ethanol extract of *Garcinia*, almost twice that of any other extract. Water and ethanol extracts of tamarind have much greater total antioxidant capacity asmeasured by the phosphomolybdenum method, than the *Garcinia* extracts. In general, compared to the water extracts, ethanol extracts had greater antioxidant property, and compared to tamarind, *Garcinia* was a better antioxidant. Compared to *Garcinia*, tamarind had more total phenolic content in both water and ethanol extracts. No correlation was observed between phenol content and antioxidant capacity. Therefore, in our materials, it may be assumed that the mechanism of radical scavenging activity may be different, *viz*., metal (iron (II)) chelation, ability of extracts to donate electrons or hydrogen atoms, the ability to protect carbohydrate structures etc.The HCA content in *Garcinia gummigutta* was 12.33% and in tamarind a negligible 0.23%. HCA, the principal acid in *Garcinia gummigutta*, is not the main component in tamarind.

VANILLA

1. *In vitro* seed germination of *Vanilla planifolia* and molecular characterization of vanilla germplasm using SSR and ISSR primers

Ms. P. Sneha Venugopal/AmritaUniversity/2021/Guide: Dr. S. Aarthi

In vitro regeneration of the vanilla plant from seeds were carried out in the study. *V. planifolia* mature seed was utilized as an initial material. T4 and T5 (Knudson + 4.44 M BAP + 2.68 M NAA) had the best response to seed explant regeneration (80%-85%) with 35 days of first response. T7 and T8 (Vacin and Went + 4.44 M BAP + 2.68 M NAA) produced the least response. The molecular characterization of *V. planifolia* using SSR and ISSR primers is the next step of the research, for which we employed 22 leaf samples from accessions of *V. planifolia* and other species. Details of monomorphic/polymorphic primers produced by the 18 ISSR and 11 ISSR primers are furnished. ISSR and SSR primers generated 143 and 48 scoreable alleles. The polymorphism information content (PIC) provides an estimate of discriminatory power of a locus by considering not only the number of alleles expressed, but also the relative frequency of those alleles. A dendrogram was constructed for 22 genotypes using Jaccard's similarity index values using the NTSYS-pc version 2.02 using ISSR and SSR primers data. ISSR and SSR markers were used to separate the genotypes into discrete

clusters, confirming the similarities and differences between the genotypes investigated.

2. Molecular and anatomical characterization of vanilla species

Ms. P. Ahalya/Mangalore University/2020/Guide: Dr. S. Aarthi

The present study has documented the anatomical and molecular variability of the genus, Vanilla collected from the Andaman and Nicobar Island compared with *Vanilla planifolia* collections from Kerala. The anatomy was examined in six species *V. planifoila, V. andamanica, V. wightiana, V. aphylla, V. pilifera, V. tahitensis*. Anatomy of stem, root and leaves revealed some variability among species. The structural organisation shows some taxonomic significance and analysed by fluorescence microscope. Molecular characterization of *V. planifolia* and Andaman collections using ISSR markers revealed that the extents of genetic diversity in a set of 16 genotypes were used in this study. A total of 196 scorable alleles were generated by 34 ISSR primers, of which 185 were polymorphic among the genotypes and 11 were monomorphic. The Polymorphic Information Content (PIC) value as a relative measure of polymorphism level among the polymorphic markers ranged between 0.61 to 0.88. Allele size ranged from 250 bp to 3500 bp.The species under study were diverse and show a wide range of variability. Dendrogram was constructed using Jaccard's similarity coefficient. The similarity coefficients based on ISSR markers ranged from 0.074 to 01.00. Anatomical features are important characters to support identification and classification of different species. The "leafy" species *V. andamanica* is more related to the "leafless" species *V. aphylla* and *V. wightiana*. *V. planifolia* has persistent pericycle ring and all the species have large sized hyaline water storage cells in the ground tissue. Anatomical molecular characterisation of species helps in easy identification of species and avoids duplication in germplasm.

3. Plant growth promoting and molecular characterization of *Chaetomium* spp. isolated from vanilla

Ms. Sreebala Mohan/Mahatma Gandhi University/2019/
Guide: Dr. Muhammed Faisal Peeran

In present investigation, *C. globosum* were cellulose degrading and even able to solubilize zinc. Mineral solubilizing capacity of *Chaetomium* helps in the conversion of insoluble zinc into soluble form and this helps in the growth promotion. It has been found that *C. globosum* strains inhibit certain plant pathogens. *C. globosum* processing the enzymatic activity coupled with the above growth promotion characters and antagonistic traits makes it is an excellent candidate for biocontrol of plant pathogens.

4. **Comparison of *in vitro* shoot proliferation potential of four species of vanilla on SH medium supplemented with different levels of 6-benzylaminopurine (BAP)**
Ms. P. Deepthi/University of Calicut/2009/Guide: Dr. R. Ramakrishnan Nair

In vitro response of nodal segments with axillary buds of four Vanilla species namely *V. planifolia, V. aphylla, V. pilifera* and *Vanilla* sp. (A&N Islands) was tested on SH medium supplemented with different levels of BAP (0.0 - 3.0 mg/L). A BAP concentration between 2.0-3.0 mg/L was found to be ideal for inducing shoot proliferation. Among the species studied *V. planifolia* was having highest shoot proliferation potential followed by *V. pilifera*, *V. aphylla* and *Vanilla* sp. (A&N Islands) at optimum BAP concentration of 3.0 mg/L. Analysis of variance indicated significant influence of BAP concentration, species difference and their interaction in determining the shoot proliferation. The histological analysis showed the production of multiple shoots by axillary branching from the pre-existing meristem as well as *de novo* from the base of the elongating axillary shoots. Influence of genotypic difference and BAP concentration in determining *in vitro* shoot proliferation is discussed.

5. **Influence of different basal media on seed germination of vanilla (*Vanilla planifolia* Andrews) *in vitro***
Ms. Priya Treesa Tom/University of Calicut/2008/Guide: Dr. R. Ramakrishnan Nair

In vitro seed germination of vanilla (*Vanilla planifolia* Andrews) is tested in four popular tissue culture media namely MS, B5, SH and White's for assessing their comparative efficiency. Forits of 4-5 months maturity after pollination were used as source of the seeds for culturing. SH medium found to be ideal for seed germination with highest mean number of seeds germinated (89.00) followed by B5 (58.50) and MS (46.30) after 80 days of culture. White's medium was not suitable for germinating vanilla seeds as the number of seeds germinated on the same was very few (0.70). Morphological and histological analysis of germinating seeds confirmed the normal process of seed germination and protocol development. Influence of media composition and seed maturity on *in vitro* germination of vanilla seeds is discussed.

6. **Investigations for biocontrol organisms against pathogens of vanilla (*Vanilla Planifolia* Andrews)**
Ms. B. Remya/Bharathidasan University/2006/Guide: Dr. R. Suseela Bhai

Fusarium and *Colletotrichum* are the main organisms isolated from different parts of infected vanilla plants. Though *Pseudomonas* is found to be inhibitory under *in vitro* conditions, there was no control of the pathogen under *in vivo* conditions which is evidenced from the infection of vanilla plants by *Fusarium* under artificial inoculation.

Penicillium sp. and *Trichoderma* sp. are found to be controlling the infection due to *Fusarium*. *Rhizoctonia* was isolated from aerial roots which shows its association as a mycorrhizal colonizer Thus from the study it is concluded that the two fungal isolates *viz*., *Penicillium* and *Trichoderma* sp. can be promising as biocontrol agents against *Fusarium* disease of vanilla. More studies are warranted to establish the biocontrol potential of these two bioagents.

7. Investigations on the occurrence of fungal diseases on vanilla (*Vanilla planifolia* Andews) in wayanad region

Ms. Jithya Danesh/Bharathiar University/2006/Guide: Dr. R. Suseela Bhai

Sixty-four isolates of fungi were isolated from thirty- two samples collected from Wayanad region. Among them, thirteen isolates were found to be pathogenic under artificial inoculation on the vanilla leaves and stem. The pathogenic isolates belongs to *Colletrotrichum*, *Fusarium* and *Mucor* sp. All the non pathogens were screened against two isolates of *Fusarium* and *Mucor racemosus* obtained as pathogens. Among the screened isolates, *Trichoderma* showed inhibitory effect against all the pathogen tested (5 8-70%). Thus from the present study, it can be concluded that *Colletrotrichum, Fusarium*, and *Mucor* are the main pathogens associated with vanilla in the Wayanad region. *Trichoderma* sp. obtained as a non pathogen is found to be inhibitory to the pathogens under *in vitro* conditions. To exploit the potential of *Trichoderma* sp, an intensive study is required to confirm its potentiality under field conditions. Usually more or less host specific natural enemies are screened to ensure that non-target organisms of economic importance or of conservation value are not harmed. In this way undesirable side effects are avoided and biological control has a minimum impact on the environment.

PAPRIKA

1. Molecular characterization of *Capsicum* species of different locations using random amplified polymorphic DNA (RAPD) markers

Mr. K. N. V. Sivakumar/Acharya Nagarjuna University/2009/
Guide: Dr. Utpala Parthasarathy

In the present study, RAPD is a better method to study the intra species relationship. Though the species of *Capsicum frutescens* are collected from the different region, but their heterogeneity index is very low, even though the locational variation is

there, it may not influence the genetic setup of the plant. RAPD gives a clear idea of the genus specific band of the *Capsicum* species that is lying in between 450-500 bp in this study. All parts of the same plant (leaf and fruit) give the same genetic profile. Three primers are showing good segregation of bands of *Capsicum*, they are OPA-OS, OPB-07, OP. All These three primers can be used for the futher studying of the *Capsicum* characters effectively.

2. Molecular characterization of paprika germplasm (*Capsicum* sp.) using random amplified polymorphic DNA (RAPD) markers

Mr. R. Prabhaharan/Bharathidasan University/2008/Guide: Dr. D. Prasath

The present study was undertaken to assess the genetic variability among the 22 Paprika germplasms conserved at Indian Institute of Spices Research, Calicut, using RAPD markers. The modified protocol used for the isolation of DNA was found to be suitable for Paprika which gave very good quality DNA from the leaves. Among the different combinations tried, IU of Taq and 25 mM $MgCl_2$ concentration gave good amplification with clear bands without non-specific banding. The PCR profile involves one cycle of initial denaturation cycle at 94°C for 3 min followed by cycle denaturation at 94°C for 1 min, annealing at 37°C for 1 min, extension for 2 min, and a final extensionfor 7 min gave good amplification. RAPD profile have been developed for twenty two Paprika accessions such as ICBO 01, ICBO 02, ICBO 03, ICBO 04, ICBO OS, ICBO 06, ICBO 07, ICBO 08, ICBO 09, ICBO 10, ICBO II, ICBO 12, ICBO 15, ICBO 16, ICBO 17, ICBO 18, ICBO 19, ICBO 20, CC 01, CC 02, KtPI-19, EC 01 and EC 02. Twenty two primers were tested for random ampli fication such as OPA- 04, OPA- OS, OPA- 06, OPA- II, OPA- 16, OPA- 17, OPA- 18, OPA- 19, OPB- 01, OPB- 06, OPB- 07, OPB- 09, OPB- II, OPB- 15, OPB- 19, OPC- 08, OPC- 09, OPC- 10, OPC- 16 OPC- 18, OPC- 20 and OPO- 08. Among the primers, six polymorphic primers (OPA-04, OPA- OS, OPA- 06, OPA- II, OPB- 07 and OPO- 02) were used for assessing the polymorphism/similarities within and among the Paprika gennplasms. Cluster dendrogram revealed maximum similarities (100%) among 16 ICBO accession. The ICBO 03 had 76% similarity with other ICBO lines. The CC 01 had comparatively low similarity with ICBO forms (30%), followed by EC 01 (28%), EC 02 (33%), CC 02 (35%), and Kt.PI (60%). The similarity between EC 0 I and EC 02 was 54%. Kt.PI - 19 showed different similarities such as CC 01 (41%), CC 02, EC 01 (38%), EC 02 (29%) and ICBO 03 (40%).

3. Cloning and sequencing of coat protein gene of cucumber mosaic virus infecting paprika (*Capsicum annuum* Linn.)

Ms. Anju George/Bharathidasan University/2007/Guide: Dr. A. Ishwara Bhat

Cucumber mosaic virus (CMV) causing mosaic, leaf distortion, stunting and fruit malformation of paprika (*Capsicum annuum* Linn.) in India was characterized on the basis of serological and coat protein (CP) nucleotide properties. Direct antigen coating-enzyme linked immunosorbent assay was done for the detection of CMV. CP gene of the virus was amplified using reverse transcription-polymerase chain reaction (RT-PCR), cloned and sequenced. Sequenced region contained a single open reading frame of 657 nucleotides potentially coding for 218 amino acids. Sequence analyses with available five CMV infecting chilli isolates from other parts of world showed an identity ranging from 93-97 % and 97-99 % at nucleotide and amino acid respectively except for one isolate from Korea which showed an identity of 82 & 76 %. Analyses with CMV isolates from other hosts revealed the greatest identity with Sarpagandha and black pepper isolates of CMV and the phylogram clearly showed that CMV infecting paprika belongs to subgroup lB. Dot blot hybridization for the detection of CMV in paprika was standardized. This is the first report on molecular characterization of CMV infecting paprika in India.

4. Distribution of metabolites in chilli tissues - relation to the colour value

Mr. C. K. Anoop/Bharathiyar University/2006/Guide: Dr.T. John Zachariah

In the study ten chilli germplasm samples and three market samples were separated into pericarp, placenta and seeds. All these three tissues were evaluated for major biochemical constituent. Such as starch, carbohydrate, reducing sugar, protein amino acid and total phenol content. Variation was observed between the three tissue samples as well as between the germplasm and the market sample. Contradicting results from available literature also were obtained in the study. The commercial quality index, ASTA colour value was found to be very low in the germplasm sample compared to the market sample. Pungency is very low in the germplasm sample compared to the market sample. Oleoresin level is on par' in both the groups. The SDS-PAGE did not show any variability in the protein pattern of germplasm sample and TLC of pigments showed uniformity in both the germplasm and market sample.

OTHERS

1. **Analysis of adulteration in spice essential oil using optical properties**
Ms. O.P. Sajinas/University of Calicut/2022/Guide: Dr. K. Anees

The simplest, fastest and non destructive way to measure the quality standards of essential oil is to measure the physical parameters like specific rotation and optical rotation by using polarimeter. At first, measurements were made of the optical rotation of 14 distinct essential oils at various temperatures and concentrations. The potential adulterants were blended, and the trials for adulteration detection using optical characteristics were repeated. The existence of adulterants can be determined based on differences in the specific rotations that are analysed from the graph in relation to different specific rotations that relate to potential adulterants in the sample. The present study was successful in developing optical property-based method for detecting mixing of cinnamon leaf oil in cinnamon bark oil as well as turmeric leaf oil in turmeric rhizome oil.

2. **Development of chitosan based packaging films impregnated with essential oil for aflatoxin management**
Ms. P. P. Aswini/Kerala University of Fisheries and Ocean Studies/2022/
Guide: Dr. K. Anees

The goal of this work is to create bioplastic films made of chitosan that are impregnated with essential oils and characterize them. To create a clear, flexible, beautiful film that can be used as an active packaging material, three plasticizers were applied in various ratios, including glycerol, sorbitol, and polyethylene glycol along with chitosan. The physical, chemical, mechanical, and biological properties of the formed films were investigated. To evaluate and study the film's quality, the anti-oxidant and total phenol tests were conducted. The soil burying technique was used to illustrate the film's biodegradability. The impregnation of clove essential oil dramatically improved the antimicrobial and antioxidant capabilities. An *in vitro* test in 96% ethanol (v/v) was used to explore the release of clove essential oil from chitosan membrane, and a UV-VIS spectrophotometer was used to characterize the essential oil release kinetics. The antibacterial properties of the film make it a superior packaging material since it can effectively prevent food contamination or spoilage. This work demonstrates that the incorporation of EO in to food packaging polymeric matrices is an interesting approach

for two reasons: first, it lowers the amount of EO required for food preservation, there by reducing costs and second, it helps to release the EO in a controlled manner so that release is sustained for longer duration. Biodegradable chitosan based edible films with EO also have the potential to transform the spice packaging industry for controlling aflatoxin contamination and there by enhancing both environmental and human health.

3. **Potassium solubilizing bacteria: isolation, characterization and organic acid production during solubilization of recalcitrant K compounds**

Ms. Ciciliya Cyril/Kerala University of Fisheries and Ocean Studies/2021/
Guide: Dr. R. Dinesh

In the present study on Potassium solubilizing bacteria: Isolation, characterization and organic acid production during solubilization of recalcitrant K compounds. KSB strains were isolated from forest sites of Idukki, Kozhikode, and Wayanad districts of Kerala and 34 isolates were shortlisted for K solubilization potential under *in vitro* conditions. In addition to the K solubilizing potential, the shortlisted KSB isolates were also screened for their PGP traits. *In vitro* study showed that IISR KSB1 (*Pantoea cypripedii)* & IISR KSB3 (*Pseudomonas tolassi*) were the most potent strains with enhanced K solubilization capacity. In growth promotion traits, all the shortlisted isolates were positive for IAA production. Except two isolates, all the strains showed NH_3 production. IISR KSB13 was the only isolate, which was able to produce HCN, while 25 isolates showed siderophore production. All cultures showed negative results with respect to amylase production but only 2 Isolates IISR KSB2 (*Acinetobacter*) & IISR KSB28 (*Bulkholderia tropica*) showed pectinase production. Among the shortlisted ones, 25 isolates were able to produce cellulase and 12 isolates showed protease production. Based on *in vitro* study & tests on PGP traits, 10 isolates *viz*., IISR KSB1- *Pantoea cypripedii*, IISR KSB2 - *Acinetobacter*, IISR KSB3 - *Pseudomonas tolassi*, IISR KSB28 – *Bulkholderia tropica*, IISR KSB32 - *Serratia*, IISR KSB34 - *Bacillus marisflavi.* IISR KSB9, IISR KSB15, IISR KSB 17 & IISR KSB33 - identified as *Enterobacter* sp.) were found to be promising, of which IISR KSB1 (*P. cypripedii*), IISR KSB2 (*Acinetobacter*) & IISR KSB3 (*P. tolassi*) were the most potent strains owing to its enhanced K solubilization efficiency along with their multiple PGP traits. In quantitative studies efficiency of promising strains, IISR KSB1 (*P. cypripedii*), IISR KSB2 (*Acinetobacter*) and IISR KSB3 (*P. tolassi*) were compared with non-solubilising strains (NSB).The results of the study showed that the treatments with IISR KSB strains was found to be superior with significantly higher K release.The

liquid assay showed that markedly higher available K release was registered by treatment with isolate IISR KSB1 (*Pantoea cypripedii)* on the 7th day of incubation and the dissolution rate was inversely proportional to the pH of the medium. While the NSB isolates showed a low dissolution rate with increased pH. The total organic acid production levels were also greatest with *P. cypripedii*. While NSB isolates showed a least production of organic acid with increased pH. The K release pattern in soils showed that *Acinetobacter* (IISR KSB2) had the greatest K releaserate on the 15thday and also had a decrease in pH at all days of incubation. This was attributed to decreased pH may owing to their high organic acid production capacity. Based on the data, it could be inferred that the isolated strain IISR KSB1 (*Pantoea cypripedii)* had multiple PGP traits and considerable K releasing ability from insoluble form in liquid media and in soil. So, this isolate has the potential for enhanced soil K solubilization and plant growth promotion. Further studies on the mechanism by which KSB solubilized K and the effectiveness of their use in the field are needed to promote evergreen agriculture.

4. Isolation and characterisation of nitrogen fixing bacteria with multiple plant growth promoting traits and biocontrol potential

Ms. C. S. Rosemary/Kerala University of Fisheries and Ocean Studies/2021/
Guide: Dr. R. Praveena

The present study was designed to isolate and characterize nitrogen fixing bacteria with multiple plant growth promoting traits and biocontrol potential. NFB strains were isolated from collected from forest sites of Idukki, Kozhikode, and Wayanad districts of Kerala and 50 isolates were shortlisted for N solubilization potential under *in vitro* conditions. From the present study, IISR NFB9- *Pantoea agglomerans*, IISR NFB24 – *Burkholderia tropica*, IISR NFB27 - *Burkholderia stabilis*, IISR NFB28- *Burkholderia cepacia* IISR NFB31- *Raoultella terrigena*, IISR NFB45- *Stenotrophomonas maltophila*, IISR NFB50 - *Bacillus marisflavi* were identified as promising nitrogen fixing bacteria with PGP traits. All the 50 NFB isolates were found to be positive for IAA production, the maximum concentration of IAA production was observed by the isolate IISR NFB45 (*Stenotrophomonas maltophila*). All the tested isolates, showed NH_3 production also. Three isolates IISR NFB17, IISR NFB32, IISR NFB40, were able to produce HCN, while 47 isolates were able to produce siderophore. Among the shortlisted isolates, 12 isolates, showed the ability to produce amylase. The isolate IISR NFB9 (*Pantoea agglomerans*) showed maximum zone of clearance. 37 isolates showed protease activity. Besides, 13 isolates produced

pectinase, of which IISR NFB31 (*Raoultella terrigena*) strains showed maximum production. With regard to cellulase production, 20 isolates tested positive. The two isolates IISR NFB27 (*Burkholderia stabilis*) & IISR NFB28 (*Burkholderia cepacia*) showing biocontrol against *Phytophthora capsici* and *Fusarium oxysporum.* IISR NFB31 (*Raoultella terrigena*), IISR NFB38, IISR NFB50 (*Bacillus marisflavi*) showed antagonism against *Pythium myriotylum*. Among the shortlisted isolates, the most promising NFB strains was identified by 16S rRNA as IISR NFB1 and IISR NFB29 - *Lelliottia amnigena*, IISR NFB4 - *Citrobacter gilleni*, IISR NFB9- *Pantoea agglomerans*, IISR NFB24 - *Burkholderia tropica*, IISR NFB27 -*Burkholderia stabilis*, IISR NFB28- *Burkholderia cepacia* IISR NFB31- *Raoultella terrigena*, IISR NFB45- *Stenotrophomonas maltophila*, IISR NFB50 - *Bacillus marisflavi*. IISR NFB16 and IISR NFB38 were identified as *Enterobacter* sp. The nif gene were amplified for two isolates which further confirmed the nitrogen fixing ability. Out of the two isolates, one was identified as *Raoultella terrigena* (IISR NFB31) and the isolate IISR NFB6 yet to identified. The two isolates IISR NFB27, IISR NFB28 showing biocontrol against *Phytophthora capsici* and *Fusarium oxysporum*. IISR NFB31, IISR NFB38, IISR NFB50 showed antagonism against *Pythium myriotylum*. In summary, the present study indicates the occurrence of various strains of NFB with plant growth promoting traits and biocontrol potential. The use of effective nitrogen-fixing microorganisms helps to improve crop production in addition to maintaining soil structure and fertility. Further studies need to be carried out to test the efficacy of the selected strains under greenhouse and field conditions.

5. Evaluation of antifungal activity of eugenol on aflatoxigenic *Aspergillus* spp. associated with spices

Ms. R. Reshma/Mahatma Gandhi University/2021/Guide: Dr. C. Sarathambal

Eugenol is found in a variety of plants including clove buds, cinnamon bark and leaves, tulsi leaves, turmeric, pepper, ginger, oregano and thyme. In addition, several other aromatic herbs including basil, bay, mace and nutmeg are also claimed to have significant quantity of eugenol. The study aims to understand the role of eugenol in inhibiting the growth of *Aspergillus* spp. found in spices. We collected five spices such as dried ginger, pepper, chilli, nutmeg and turmeric from market and serial diluted in sterile distilled water and plated on potato dextrose agar (PDA). *Aspergillus* spp. was morphologically identified under Potato Dextrose Agar medium and microscopically confirmed. After 7 days of incubation, fungal development was effectively inhibited at a

concentration of 0.04% eugenol. Fungicidal activity was discovered at this concentration. *Aspergillus* spp. radial growth reduced in response to eugenol concentrations ranging from 0 to 0.04%. IC50 was recorded as 0.027%. Germination of spore decreased as concentration of eugenol increased in the media. Maximum inhibition was shown at 0.04% of eugenol after 24 hours of incubation. Based on the results of this study, it can be stated that eugenol has antifungal properties, preventing the growth of *Aspergillus* spp. and causing irreversible detrimental morphological changes, and thus it is worth pursuing for *Aspergillus* spp. bio management. The findings pave the way for the discovery of a powerful phytochemical with fewer adverse effects that might be used to treat fungal infections at the post-harvest phase.

6. Effect of pretreatments of finger millet flour on development of spice-based finger millet cookies

Ms. P. Bhagyalekshmi/Calicut University/2021/Guide: Dr. E. Jayashree

The present study is aimed to evaluate the effect of various pretreatments given to finger millet flour and its effect on the quality of the cookies developed. The results of the study indicated that the cookies developed by the pretreatment method steaming had higher over all acceptability than the other two pretreatment methods. Experiments on steaming of finger millet flour were carried out at at emperature of 60°C for three steaming durations of 5, 10 and 15 min for five concentrations of finger millet flour *viz*., 30, 40, 50, 60 and 70%. Based on the overall acceptability of cookies prepared, it was found that cookies with the highest overall acceptability of 8.9 was obtained when 40% concentrated and 10 min steamed finger millet flour was used. The moisture content, hardness and spread ratio of the optimized cookies were 2.87%, 8.79 N and 4.4, respectively. Among the three spices used for improving the flavour of finger millet cookies, it was found that cookies with addition of 40% finger millet flour and 4% cardamom seed powder obtained the highest overall acceptability score of 8.9 with moisture content of 4.29%, hardness of 10.89 N, spread ratio 4.2 and ranked first. Similarly, cookies with addition of 40% millet and 12.5% black pepper showed overall acceptability score of 7.8 with moisture content of 4.1%, hardness of 9.81 N, spread ratio 4.2 and ranked second and spice blend (masala) addition of 8% in 40% finger millet cookies showed the overall acceptability score of 6.8 with moisturecontent of 4.15%, hardness 10.86 N, spread ratio 4.6 and ranked third. Thus, the study indicated that steaming as pretreatment to finger millet flour for 10 min and concentration up to 40% finger millet flour as replacement to refined wheat flour with addition of cardamom

seed powder (4%) could be optimized based with highest overall acceptability obtained (8.9).The optimized finger millet cookies were evaluated for its nutritional parameters such as carbohydrate, protein, fat, crude fibre and moisture content and the result showed that, the supplemented cookies developed with cereal-millet mix of refined wheat flour and finger millet flour (10 min steamed) in the ratio of 60:40 had the nutritional composition of carbohydrate (60.65%), protein (9.79%), fat (25.13%) and crude fibre (3.96%). The moisture content and hardness of the developed finger millet cookies was 2.87% and 8.79 N respectively.

7. **Optimization and development of spice enriched finger millet cookies**
Ms. A. S. Arya Surendran/Mahatma Gandhi University/2021/
Guide: Dr. E. Jayashree

The present study was aimed to optimize the use of finger millet for the preparation of cookies and to develop spice enriched finger millet cookies. Cookies were prepared by substituting the refined wheat flour with finger millet flour in varying concentrations from 0 to 100% (flour weight, w/w) using the recipe standardized at ICAR-IISR for preparing refined wheat flour cookies. A single factor completely randomized block design (CRD) was followed to determine the effect of variation of finger millet flour on various quality parameters like physical, biochemical and organoleptic properties of the developed cookies. Spice enriched finger millet cookies were prepared by adding different spices at varying concentrations like cardamom (3 to 5% of flour weight, w/w), black pepper (10 to15% of flour weight, w/w), cinnamon (3 to 5% of flour weight, w/w), nutmeg (4 to 6% of flour weight, w/w), curry leaves (5 to 9% of flour weight, w/w), bird's eye chilly (1 to 3% of flour weight, w/w), ginger (30 to 40% of flour weight, w/w) and spice blend (6 to 10% of flour weight, w/w) and the spice concentration was optimized to get unique flavour of each spice. A two factor completely randomized block design was followed to determine the effect of addition of each spice in various quality parameters of the developed cookies. The effect of addition of each spice on physical, biochemical and organoleptic characteristics of developed cookies were evaluated.

8. **Optimisation of production of spice based non –dairy oats milk and studies on its nutraceutical properties**
Ms. M. J. Angel Jasmine/Kerala University of Fisheries and Ocean Studies/2021/
Guide: Dr. E. Jayashree

The present study is aimed to optimize the concentration of oats for the preparation of oats milk and development of spice enriched oats milk. Spice flavoured

oats milk was prepared for concentration of oats varying from 1 to 10%. Oats milk was prepared by mixing oats powder to 100 mL water and homogenised in the mixer grinder. The milk was then filtered using a double layer muslin cloth. The filtered milk was boiled on an induction stove (Prestige PIC 6.0V3) at 2000 W for 1 min and cooked at 900 W for 5 min. Two sets of milk, for each concentration were prepared and to the second set, sugar (9 g/100 mL oats milk) was added and cooked. A two factor Completely Randomized Block Design was followed to determine the effect of concentration of oats *viz*., 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% with and without addition of sugar. Three replicates of each treatment were taken for the evaluation of its physical and organoleptic properties. The oats milk prepared for various concentrations were compared with dairy milk (1.5 and 3% fat content) and evaluated for various quality parameters. The concentration of oats milk was optimised based on the overall acceptability score obtained in the sensory evaluation tests. The spices in the form of oleoresin were added to the oats milk with sugar to obtain the spice flavoured oats milk. The spice oleoresins of turmeric (curcumin content 10%), ginger (gingerol content 14%) and cinnamon powder (powdered to 60 mesh) were added to obtain the flavoured oats milk. Oats milk (300 mL) was prepared as mentioned above and spice oleoresins were added before cooking of oats milk along with sugar (9 g/100 mL) and boiled for 5 min. The concentration of turmeric oleoresin was varied from 40 to 60 mg and its effect on the development of turmeric enriched, spice flavoured oatsmilk was studied. The concentration of other spices like ginger oleoresin (10 mg) and cinnamon powder (50 mg) were maintained constant for all the concentrations of oats milk prepared. A three factor Completely Randomized Block Design was followed to determine the effect of concentration of oats *viz*. 1, 2 and 3%, for five different concentrations of turmeric oleoresin added (40, 45, 50, 55 and 60 mg/100 mL of oats milk) and for two preparation methods followed (unsterilized and sterilized milk). Ginger oleoresin (10 mg/100 mL) and cinnamon powder (50 mg/100 mL) added were maintained constant. Three replicates of each treatment were taken for the evaluation and its physical, biochemical, organoleptic and its antioxidant properties were studied. Based on the overall acceptability and the viscosity; it was observed that the developed oats milk of 2% concentration (with added sugar) was comparable to dairy milk of 1.5% fat (approx.). Hence, oats milk of concentrations from 1 to 3% was used for optimising spice enriched flavoured oats milk. As the concentration of oats in the spice flavoured oats milk increased from 1 to 3%, for a given concentration of turmeric oleoresin (40 mg/100mL),

the viscosity of the milk increased from 20.12 to 31.25 N. sm^2 for unsterilised milk and the corresponding values for sterilised milk varied from 20.11 to 30.13 N.sm^2. Similarly, for concentration of oats milk varying from 1 to 3%, at turmeric oleoresin concentration of 50 mg/100 mL, the overall acceptability of the oats milk varied from 7.27 to 8.3 for unsterilised milk and the corresponding values for sterilised milk varied from 6.33 to 8.37. The highest overall acceptability of 8.37 was obtained for spice flavoured oats milk (unsterilized) prepared at a concentration of 2% oats and turmeric oleoresin concentration of 50 mg/100 mL. At this concentration, the curcumin content varied as 0.00063% for unsterilized and 0.00051% for sterilised oats milk. The antioxidant activity varied as 30.44% for unsterilized and 30.44% for sterilised oatsmilk. The oats milk was also evaluated for other physical properties like-pH, colour values L*, a*, b* and nutritional properties like carbohydrate, protein, fat, and moisture content. From the study, it was concluded that oats milk prepared with 2% concentration of oats powder, enriched with turmeric oleoresin (10% concentration of curcumin) at the rate of 50 mg/100 mL milk, ginger oleoresin (14% concentration of gingerol) at the rate of 10 mg/100 mL of milk and cinnamon powder at the rate 50 mg/100 mL milk, produced the most preferred oats milk with an overall acceptability value of 8.3 (for unsterilised milk) and 8.37 (for sterilised milk).

9. Conversion of solid organic waste into compost using cellulose degrading bacteria and *Trichoderma asperellum*

Ms. C. Aleena/Kerala University of Fisheries and Ocean Studies/2021/
Guide: Dr. C. K. Thankamani

The study revealed that compost produced by cellulose degrading bacteria *Trabulsiella* sp. (CDB4) has less C:N ratio, maximum potassium content, organism is having better nitrogen fixing and phosphorus solubilizing ability and capable of producing IAA. However, maturity period for composting is long. On the other hand, compost produced by *Trichoderma asperellum* found to have Less C:N ratio, maximum potassium content and fungus is having IAA producing ability and maturity period is less. Hence combined inoculation of CDB4 (*Trabulsiella* sp.) and *Trichoderma* sp. to be taken further to reduce the composting period.

10. Studies on the co-inoculation of plant growth promoting rhizobacteria on solubilization of zinc in soil

Ms. P. Akshaya Das/Periyar University/2020/Guide: Dr. R. Dinesh

In vitro, liquid culture and ZnSB-mediated Zn release in soil studies were conducted with two promising Zn solubilizing bacteria isolated for major turmeric and

ginger growing regions of India. *In vitro* study showed that IISRGB7 (3) (*Bacillus cereus*) and IISRTB4 (*Bacillus safensis*) were the most potent strains with enhanced Zn solubilization capacity. The promising ZnSB strain was identified by 16S rRNA as *Bacillus safensis* and *Bacillus cereus*. The sequence data of strains was deposited in GenBank and accession number *B. safensis*-NCBI IISR-TB4 MT192800, *B. cereus*-NCBI- IISR GB7 (3) MT192803 was assigned. The two bacterial isolates were characterized based on phenotypic characters. Phenotypic characters included colony morphology, motility, gram staining, and biochemical characters included indole, MR-VP, and citrate utilization test. Motility results showed that, IISRGB7(3) were non motile and IISRTB4 were motile. In Gram staining test isolates IISRGB7(3) and IISRTB4 are Gram positive. IMVIC test showed that isolate IISRGB7(3) positive in methyl red test and IISRTB4 positive in citrate utilization test. Both isolates showed negative results with regard to indole and VP test. Gelatin hydrolysis test showed that isolate IISRGB7(3) and IISRTB4 were positive results and in Urease test both isolates IISRGB7(3) and IISRTB4 were negative. Nitrate reduction test showed that isolate IISRGB7(3) were positive results and IISRTB4 showed negative results. In growth promotion traits the two isolates were positive for IAA (Indole 3 acetic acid) production and isolate IISRTB4 were showed positive results for NH3 production. Both the isolates showed positive results to HCN production and IISRGB7(3) was negative to siderophore production, while isolate IISRTB4 was positive for siderophore production. Both were negative with respect to amylase production and isolate IISRGB7 (3) was positive for protease production. Isolate IISRTB4 was positive for both pectinase production and cellulose production. By T streak method concluded that IISRGB7(3) and IISRTB4 are compatible to each other. Broth assay and ZnSB-mediated Zn release in soil study showed that markedly higher available Zn release was registered by co-inoculation of the isolates IISRGB7(3) and 45IISRTB4 on the 15th day of incubation. This was attributed to decreased pH owing to its high gluconic acid production capacity. A major drawback with *B. cereus*, is that many strains cause food contamination, eye and respiratory infection, gastrointestinal diseases and have been reported to cause bacteremia in immune compromised patients. Hence, our strain of *B. cereus* [(IISRGB7(3) MT192803)] has to be necessarily studied for its human pathogenicity prior to its use as a PGPR. Therefore, the future line of work should focus on further evaluation of *B. safensis* (NCBI IISR-TB4 MT192800) in the green house and subsequently in multiple locations in ginger and turmeric growing regions of India.

11. *In vitro* studies on effect of different concentrations of copper fungicides on selected isolates of *Trichoderma*

Ms. Merin George/Karpagam Academy of Higher Education/2020/
Guide: Dr. R. Praveena

The present study was conducted to identify the *Trichoderma* cultures which can remove copper in the presence of copper hydroxide and copper oxy chloride. Some cultures showed gro wth even at high centration of copper hydroxide and copper oxychloride. Among the cultures, MTCC, APT-2, KL-2, KL-7, KL-9 and KL-12 strains showed growth at high concentrations on treatment with both copper hydroxide and copper oxychloride. *Trichoderma,* which is a biocontrol agent and a strong competitor can remove the excess copperin the medium as well as in soil. *Trichoderma* also offers mechanisms such as mycoparasitism, antibiosis, competition for nutrients or space, induced resistance, tolerance to stress through enhanced root and plant development. Hence, *Trichoderma* can inhibit the presence of copper to a great extend when sprayed in soil and thus increase the plant yield. Copper is an essential trace element for fungal functioning. It is also required for the plant growth. However, at high concentrations in its free- ionic form, copper is toxic to microbial cell. Excess amount of copper can destroy the plant. In this study, microscopic examination was done to identify the sporulation pattern in *Trichoderma* species. Some branched and unbranched conidia and conidiophores were seen. A comparative study was also done to demonstrate the growth rate pattern graphically in all these species.

12. Studies on antifungal activities and molecular characterisation of selected biostimulant microorganisms

Ms. Archana Sasi/Mahatma Gandhi University/2019/Guide: Dr. C. Sarathambal

Isolation of biostimulant microorganisms from different origins such as compost, vermicompost, vermiwash, jeevamirth, milk and curd were carried out. Forty-five morphologically different of biostimulant isolates were obtained from the MRS agar, YES agar, LBS agar, Minimal medium, and Kings B medium. The selected strains were characterized by cell shape, morphology, Gram reaction. Plant growth promoting traits of all the isolates from origin were analyzed. Among the isolates, fourteen were able to produce IAA, where the isolate S1 registered the highest activity of 26.1 ± 5.12µg/mL. Among the isolates, nine isolates were able to solubilize the phosphorus, four were able to solubilize potassium, and ten were able to solubilize the zinc. Among the isolates twenty were to produce the siderophore in the CAS medium. The maximum siderophore production was recorded in S5 (64.30 ±3.21 µg/ mg protein) followed by

S6 (54.30 ±3.21 µg/mg protein). The isolates with positive plant growth activities were further analyzed for the presence of antifungal activities. The antagonistic activity of all the isolates against four plant pathogenic organism *viz*., *Phytophthora capsici*, *Fusarium oxysporum*, *Pythium myriotylum*, and *Colletotrichum gloeosporioides* were evaluated. Comparative BLAST analyses which include the closest species, and per cent homology of full length 16S rRNA revealed the presence of diversity of Firmicutes and Yeast. The isolate S22 was closely resemble to *Bacillus.* sp. The isolates S26 and S16 which similar to *Bacillus pumilus* and *Dabaromyces hansenii* respectively. In the present work, firmicutes were mainly dominated by different groups of *Bacillus*, which have been isolated from different natural origins.

13. Standardization of the substrate for large-scale production of arbuscular mycorrhizal inoculum

Ms. A. P. Shahana/ University of Calicut/2019/Guide: Dr. C. Sarathambal

Two plant species *viz*., napier grass (*Pennisetum purpureum*) and maize (*Zea mays*) were evaluated with different substrates, *viz*., perlite, vermicompost, coir pith, and FYM @ 10 % in vermiculite medium on growth and multiplication of *Rhizophagus* sp. Napier grass raised in vermicompost substrate had highest mycorrhizal root colonization (80%) and spore numbers (135/ 50 g of the substrate). N uptake was significantly higher in maize plants grown in FYM (2.56 g/plant) followed by napier grass grown in vermicompost (2.01 g/plant). In the case of phosphorus, a significantly higher amount of uptake was observed in both vermicompost and FYM amended napier grass. With the involvement of mycorrhizae, uptake of Mn, Zn, and Cu were also significantly more in napier grass raised in vermicompost. However, uptake of Fe (49.22 mg/plant) was higher in maize amended with vermicompost. Both the hosts (napier grass and maize) amended with FYM showed a significant increase in the shoot length, root length and root biomass over those grown in other substrates. Root colonization positively correlated with root biomass and uptake of major nutrients like nitrogen, phosphorus, micronutrients such as manganese, copper and zinc. The study indicates that vermiculite amended with vermicompost is a viable option to improve the AM inoculum production on a large scale, especially in the case of *Rhizophagus* sp. This nutrient-rich substrate may be helpful in the inoculum production of other AM species as well, as it is a material comprising of beneficial microorganisms that favour both mycorrhizal multiplication and host plant growth. Napier grass is recommended as the best host substitute for maize for the mass multiplication of AM inoculums at the farm level.

14. Antifungal activity of novel indole derivatives and bacterial isolates against fungal pathogens of spices

Ms. Revathy Ramachandran/Mahatma Gandhi University/2019/
Guide: Dr. R. Praveena

The experiments on *in vitro* screening of rhizobacteria for antagonism against *Macrophomina phaseolina* indicate the isolate IISR TB4 (*Bacillus safensis*) exhibited more than 50 per cent inhibition of *Macrophomina phaseolina.* From all isolates, IISR TB4 (*Bacillus safensis*) and IISR TB5 (*Pseudomonas* spp) exhibited more than 60per cent inhibition of *P. myriotylum.* IISR TB1 (*B.pumilus*), IISR TB4 (*Bacillus safensis*) & IISR TB5 (*Pseudomonas* spp) showed more than 50per cent inhibition against *Exserohilum rostratum*. Screening of all isolates through antagonistic activity of bacterial isolates mainly IISR TB1 (*Bacillus pumilus*), IISRTB2 (*Bacillus cereus*), IISR TB3 (*Bacillus* spp), IISR TB4 (*Bacillus safensis*) & IISR TB7 (*Pseudomonas aeuroginosa*) showed more than 60 per cent inhibition against *P. capsici.* Among these seven bacterial isolates, IISR TB4 (*Bacillus safensis*) was found to be more effective against all major spices pathogens and IISR TB6 (*Ochrobacterum intermedium*) did not have any effect on the major pathogens of spices. Under green house conditions also *Bacillus safensis* was effective in preventing diseases development by *P. myriotylum*, which indicates that the bacteria can be further, tested under field conditions for its efficacy. The identified bacteria holds great promise as a viable alternative to chemical inputs and can be integrated into appropriate nutrient management and disease management schedules for ginger.

15. Isolation, purification and evaluation of bioactive secondary metabolites from *Pseudomonas aeruginosa* (IISR6 and IISR853) against major pathogens of spices

Ms. Swathi Nambiar/Bharathiar University/2019/Guide: Dr. C. Sarathambal

In the present study, the production of secondary metabolites, *viz*., IAA, mineral solubilisation siderophore and antifungal activities has been assessed to elucidate the agronomic significance of the soil isolate *Pseudomonas aeruginosa* strain IISR6 and IISR853. Extraction, purification and evaluation of secondary metabolites with antibiotic activity produced by *P. aeruginosa* IISR6 and IISR 853 were also investigated. Maximum amount of IAA was produced by IISR6 (30.80 ±1.14 µg/mL) followed by IISR853 (25.60 ± 2.23 µg/mL). The maximum siderophore production was recorded in IISR6 (45.30 ±3.21µg/mL) followed by IISR853 (32.6 ± 2.14 µg/ mL). The antagonistic activity of *P. aeruginosa* strains against five plant pathogenic organism *viz*., *Phytophthora capsici, Fusarium oxysporum, Pythium myriotylum, Colletotrichum*

gloeosporioides and *Aspergillus flavus* were evaluated and both strains were effective. Mineral solubilization were tested and found the both the strains were ablesolubilise K and Zn. *P. aeruginosa* strains recorded good antioxidant activity with ethyl acetate extract. The bioactivity of compounds produced by *Pseudomonas aeruginosa* (IISR6 and IISR 853), against *P. capsici, P. myriotylum* and *C. gloeosporioides* were investigated *in vitro* and shows good antibiotic activity. *Pseudomonas aeruginosa* strains (IISR6 and IISR 853) are significant disposition plant growth promoting traits and also have the concurrent in suppressing soil-borne phytopathogenic fungi offers great promise for improved and sustainable crop productivity. Secondary metabolites from *P. aeruginosa* strains can be an important and viable alternative to manage several debilitating diseases in spice crops. The recent technological advances in the area of bioactive metabolites are now beginning to lay out important avenue of research focused on the role of metabolites in agriculture.

16. *In vitro* studies on selected isolates of *Trichoderma* spp. to varying levels moisture and temperature stress

Ms. Shalini Muralidharan/Bharathiar University/2019/Guide: Dr. R. Praveena

The objective of the present study was to understand the *In vitro* studies on selected isolates of *Trichoderma* spp to varying levels of moisture and temperature stress. The study included three major aspects *viz*., studies on the growth of *Trichoderma* isolates under different moisture and temperature conditions, *in vitro* compatibility of *Trichoderma* isolates with commonly used fungicides and the molecular characterization of selected *Trichoderma* isolates. Among the ten isolates tested, the isolates KA 15, KL3, TN 3 and MTCC 5179 showed good growth at lower moisture levels of 5, 10 and 20 percent respectively and all isolates tested showed more than 10^{14} cfu/ g of *Trichoderma* population at 40 percent moisture and above.

17. Biochemical characterization of industrial wastes of spice industry

Mr. P. K. Nasik/Kerala University of Fisheries and Ocean Studies/2019/
Guide: Dr. K. Anees

The current study was conducted with an objective of finding the potentiality of the turmeric and cinnamon industrial wastes for using in animal/poultry feed. For this a preliminary investigation was carried out regarding its physical and chemical properties. The salient findings of the study are as follows. Even the protein content was reduced by 60.7 and 76% in case of turmeric and cinnamon respectively, the protein concentration in both cinnamon and turmeric was found to be high enough to be added

as ingredient in feed formulation. The carbohydrate content of turmeric and cinnamon industrial waste was found to be 21 and 45% respectively. There for this forms a good nutritional supplement for animal/poultry feed. Out of the three primary metabolites tested, fat content was greatly reduced due to extraction procedures of both turmeric and cinnamon. Even though about 65-85% reduction of phenol was observed, a good amount of phenol was retained in the industrial wastes of turmeric and cinnamon. It is a good indication that these materials, even after extraction, are potential sources for feed fortification for poultry and cattle. Due to solvent/water extraction of both turmeric and cinnamon, the tannin content has reduced to a low level where they can exhibit its beneficial effect rather than being anti-nutritional. The bulk density of the three different industrial wastes of turmeric and cinnamon varied greatly. It implies its potential for use in different forms of animal/poultry feed *viz*. pellets, powders and flakes. In short, the current study, though a preliminary work, has thrown light in to the potential of spice industrial wastes for finding alternate uses. This will require further investigation regarding the poultry/animal feed trials and extrapolating the same observation to other spices industrial wastes too.

18. Development of arrowroot cookies and studies on its quality

Ms. Aiswarya Sundar/University of Calicut/2019/Guide: Dr. E. Jayashree

The present study is about blending of arrowroot powder and spice (vanilla extract) along with refined flour to produce cookies. Arrowroot cookies were prepared by incorporating 10 % (T1), 30 % (T2) and 50 % (T3) of arrowroot powder and compared with control (T4-cookies without arrowroot powder). The cookies were baked at a temperature of 175 degree Celsius for 8 to 10 minutes using microwave oven. The detailed studies show that there are variations in moisture, carbohydrate, protein, fat and crude fiber. Sensory evaluation based on the overall acceptability of the cookies with 10%, 30%, 50% and cookies without arrowroot powder were carried out on the first day of storage and after 20 days of storage were evaluated. From this study it can be concluded that cookies with 50% of arrowroot powder stored under ambient condition were most preferred cookies with overall acceptability score 7 towards the end of storage.

19. Isolation and characterisation of cellulose degrading bacteria

Ms. V. J. Haritha/Mahatma Gandhi University/2019/Guide: Dr. C. Sarathambal

Cellulose degrading bacteria was successfully isolated from the compost, farmyard manure, mango leaf litter and termites' guts. Among the five isolates showed

halo zones due to cellulose or carboxyl methyl cellulose degradation, which were identified in the genus of CDB2, CDB3 that closely resemble *Trabulsiella odontotermitis* and percent of similarity was found to be 98.3%. The isolates CDB 1 and CDB 4 showed close similarity to *Bacillus* sp whereas CDB 5 exhibited 100% similarity with *Bacillus cereus.* in present study, compatibility assessment between the cellulose degrading bacteria isolates. All the isolate of cellulose degrading bacteria tested was found to be compatible with each other. The antagonistic activity of all the isolates against 4 plant pathogenic organism *viz.*, *Phytophthora capsici, Fusarium oxysporum, Pythium myriotylum,* and *Colletotricum gloeosporioides* were evaluated. It was observed that none of the five isolates had any antagonistic activity against the plant pathogens.

20. Studies on induction of spice flavours into fruits and its storage

Ms. Soorya Balan/Periyar University/2019/Guide:Dr. E. Jayashree

Spice induced candy was prepared using selected fresh raw papaya. About 1kg of fresh papaya was peeled, cut into small cubes (8 mm) which were further boiled in water for 2 to 3 min for softening. Osmotic dehydration process was used to prepare different flavours of candies from papaya with flavour of ginger and pepper induced in it. Sugar syrup was prepared by adding 270 g sugar in 80 mL of water and boiled for 15 min till the sugar dissolves completely. Spices like white pepper or ginger were then added and the solution was heated till a concentration of 30° Brix was obtained. Three concentrations of spices added to sugar syrup were 22, 27 and 32 g of white pepper/80 mL of sugar syrup and ginger juice extracted from 22, 27 and 32 g of fresh ginger. The softened papaya cubes (270 g) were added to each of the spice flavor induced sugar syrup (pepper and ginger flavours) and was kept for 1 h. After an hour, the papaya was drained and the sugar syrup was boiled for about 15 minutes to increase the concentration to 30° Brix and the papaya cubes were soaked in the sugar syrup for 24 h. The concentration was increased by 10° Brix every day till the solution attained 70° Brix. The papaya cubes were dried by placing in a hot air oven at a temperature of 70° C till no stickiness was observed. Ginger candy was prepared from Varada variety of ginger by following the above process which served as the control sample. The dried candy was packaged in air tight polyethylene packaging covers (75 microns) and stored under ambient conditions (Temp max. 32°C, min 23°C and relative humidity max 70 per cent, min 47 per cent).

21. Studies on extrusion characteristics of rice flour blended with selected spices

Ms. P. V. Mufseena/University of Calicut/2019/Guide: Dr. E. Jayashree

For this study, blending of rice flour and spice powder was used to develop rice flour spice extrudates. For extrusion process, the raw materials used constituted of the rice flour and five different spice powders which were each blended in the ratio of 96:4. The spices used were ajwain, black pepper, white pepper, turmeric and dry ginger respectively and conditioned at 4°C for 3 days. Moisture maintained was 13.9%. The extrusion process was carried out in a lab model twin screw extruder at a constant die temperature of 140°C and a screw speed of 350 rpm. Comparison of rice flour alone and extrudates from flour spice blends were done based on their functional, biochemical, physical, textural and sensory properties. Finally, it was concluded that rice flour blended with ajwain and dry ginger would gave better preferred extrudates based on their overall acceptability scores. It was observed that the overall acceptability scores of rice flour blended with ajwain and rice flour blended with dry ginger extrudates would be at a maximum of 6.7 and 6.6 respectively when extruded at a die temperature of 140°C and a screw speed of 350 rpm. Rice flour blended with dry ginger extrudates showed the lowest water absorption index of 4.21 and higher hardness of13.91N. It also would gave the better retention of the active compound (gingerol) with an average value of 10.15 per cent.

22. Spice mixtures: Study on anti-diabetic and anti-oxidant efficacy

Ms. Anusha Baby/Kerala University of Fisheries and Ocean Studies/2018/
Guide: Dr.T. John Zachariah

The aim of the present study was to examine whether the potency will increase as a mixture. Sequential extract using methanol was a different approach from hitherto published literature. The current study clearly established methanol extract of cinnamon possesses the highest concentration of phenol which can be directly correlated to the anti-diabetic and antioxidant activity. However, the present study did not give any conclusive evidence to prove that different combinations like turmeric: pepper: cinnamon (1:1:1), turmeric: pepper: cinnamon (1:2:1), turmeric: pepper: cinnamon (1:1:2) and turmeric: pepper: cinnamon (2:1:1) could enhance the antidiabetic or antioxidant potential. This may have to be studied using a different approach. Further fractionation of the crude methanol extract of cinnamon will help in identification of the active ingredient in the extract.

23. Comparative genomics of selected strains of *Pseudomonas aerugenosa* from diverse ecosystems

Ms. Sangeetha Thomas/Mahatma Gandhi University/2018/
Guide: Dr. Santhosh J. Eapen

In this study, whole genome of a *P. aeruginosa* strain of marine origin was compared with the genomes of both clinical and non-clinical strains using different genome comparison tools, pathogenicity prediction tools and phylogenetic tree construction tools. The comparative genomics of the selected 11 genomes of *P. aeruginosa* did not yield any major genetic differences. The results showed that all the strains (except PA7 and CR1) have very high genetic similarity, since their genomes are almost identical. T3SS and T6SS effector profiles were identical in all strains. However slight differences were noticed only in the T4SS profile. The phylogenetic trees support the result obtained from genome comparison studies. We could not arrive at any clear-cut grouping among clinical and environmental isolates of *P. aeruginosa* by using any of the above tools. The factors governing the clinical nature of these isolates are worth investigating further. Deployment of any of these environmental isolates for agricultural purposes is questionable because of biosafety concerns.

24. Enriched composting of agricultural wastes – a comparative study

Ms. Sariga Martin/Mahatma Gandhi University/2018/Guide: Dr. C. K. Thankamani

In present study, compost produced with Jeevamrutham (JA) + Rock phosphate (RP) and JA + Poultry manure (PM) contains 0.53% to 0.69% N, 0.67 to 2.5% P, 0.25 to 0.44% K, 2% Ca, 0.29% to 0.38% Mg along with micronutrients which are better than FYM which is a commonly used organic manure and the number days for composting was very less. Maximum dehydrogenase activity was also found in JA + RP. It is reported that application of fresh poultry manure causes rapid volatilization of ammonia and toxicity. It is seen that in matured compost heavy metals like Cr, Cd, Ni, Zn, Pb and Cu etc. are well below the standard limits. Hence enriched compost is good for enhancing fertility in the soil and eco-friendly for organic cultivation. Harvested compost was tested for germination study using cowpea as a test crop and germination index (GI) was also worked out. More than 70% GI was observed for the treatments which show compost is free from any toxicants to plants. From these results, it may be concluded that preparation of compost using dairy waste as substrate (grass + paddy straw), JA as inoculum with enrichment of rock phosphate or poultry manure is rapid, highly nutritive an

25. Genomic comparison of *Ralstonia solanacearum* isolates from India

Ms. Sandra Joshy/Bharathiar University/2018/Guide: Dr. Santhosh J. Eapen

Ralstonia solanacearum causes bacterial wilt affecting more than 200 plant species, including many economically important plants. The strains of the *R. solanaeearum* used in the study arc spread across three phylotypes, corresponding roughly to their geographic origin: Asia (phylotype I), the Americas (H) and Indonesia (IV). Whole genome data of II strains of *R. solanacearum* available with ICAR-IISR were compared each other using GMII000 as a reference. For finding the relationships between sequences, several methods were utilized. These methods are average nucleotide identity, genome to genome distance calculation and multiple genome alignment. These methods arc per formed using OrthoAN l, GGDC and Mauve software tools. Results obtained showed that maximum similarity exists among three isolates of *R. solanacearum* collected from Zingiberaceae family belonging to race 4, biovar 3 and phylotype I. *R. solanacarum* isolates from potato, CP R1_ Rs2 and C PRJ_ Rs7S, belonging to Phylotype II and IV showed maximum divergence from phylotype I isolates.

26. Isolation and characterization of *Pseudomonas* species for biocontrol and growth promotion in spice crops

Ms. Ancy Maria Sebastain/University of Calicut/2017/Guide: Dr. R. Suseela Bhai

The present investigation focused towards isolation of *Pseudomonas* species from the rhizosphere soil of spice crops *viz*., black pepper, nutmeg and vanilla for the assessment their antagonistic activity *in vitro* against *Phytophthora capsici*, *Fusarium oxysporum, F. vanillae* and *Sclerotium rolfsii* which causes respectively foot rot (black pepper), root and stem rots (vanilla) and basal rots (black pepper) which reasons reduction in the annual yield.

27. Biochemical and molecular characterization of symbiotic and associated bacteria of entomopathogenic nematode

Ms. Revathi Janardhanan/Kannur University/2014/Guide: Dr. Rashid Pervez

In the present study is to isolate the symbiotic and other associated bacteria from *Helerorhabditis* sp. (IISR-EPN 01) and identify them by phenotypic, biochemical and molecular characterization. EPN, *Helerorhabditis* sp. (IISR-EPN 01) was obtained from the Nematology Laboratory of Indian Institute of Spices Research, Kozhikode and maintained using the insect host *Galleria mellonella*. EPN, *Helerorhabditis* sp. (lISR-EPN 01) was obtained from the Nematology Laboratory of Indian Institute of Spices

Research, Kozhikode and maintained using the insect host *Galleria mellonella*. The isolation of bacteria was done by three methods *viz*., haemolymph drop method, maceration method and incubation method. Six bacterial isolates were recovered using three isolation methods of which three of them were isolated by haemolymph drop method followed by two through incubation method, while one was isolated by maceration method. The biochemical tests *viz*., catalase, oxidase, indole, methyl red, Voges Proskauer, citrate utilization, urease, triple sugar iron (TSI) agar, escullin hydrolysis, tributyrin agar, carbohydrate fermentation test using sugars e.g., glucose, lactose, sucrose, maltose and mannitol were carried out. The results show that, all isolates are catalase, citrate utilization and glucose positive, whereas, urease, esculin hydrolysis and lactose tests negative. However, carbohydrate fermentation of most of the isolates of bacteria were found positive except some isolates, which were found to be negative.

28. Isolation, biochemical and molecular characterization of associate bacteria from entomopathogenic nematode (*Oscheius gingeri*)

Mr. N. Anoop/Bharathidasan University/2014/Guide: Dr. Rashid Pervez

Entomopathogenic nematodes, *Oscheius gingeri* obtained from the Nematology Laboratory of Indian Institute of Spices Research, Kozhikode and maintained using the insect host *Galleria mellanella*. Total eight bacterial isolates were recovered from the nematodes. Among them three each bacterial isolate isolated by maceration and haemolymph drop methods, respectively, while two isolates isolated by incubation method. All the 8 bacteria isolates isolated from *O. gingeri* seven were gram negative. Among the biochemical tests, oxidase, indole, methyl red and urease was negative except indole and methyl red of the IISR-EPN 07H and urease of the IISR-EPN 07E was positive. Among the tested biochemical tests, voges proskaur and citrate utilization of the all isolates were found positive except voges proskaur of the IISR-EPN 07B and citrate utilization of the IISR-EPN 07H was found negative.

29. *In silico* docking studies to identify nematicidal compounds from *Bacillus megaterium* against *Radopholus similis*

Ms. M. N. Gogula/Bharathiar University/2012/Guide: Dr. Santhosh J. Eapen

Bacillus megaterium produce antimicrobial substances that exhibit antagonistic activity against soil -borne pathogens. It produces extracellular proteases which degrade the cuticle of nematodes. Primary and secondary metabolites of *B. megaterium* promotes plant growth and also causes pathogen suppression. The

burrowing nematode *Radopholus similis* an important root pathogen. As the nematode migrates into the plants, it feeds on the cytoplasm of cortex cells, collapsing cell walls, and causing cavities and tunnels which evolve as a necrosis and may extend to the whole cortex. Endo-I,4-beta-glucanases is a protein in *R. smilis* which hydrolyses the fJ-(1,4) glycosidic bonds of cellulose of plants. Exoglucanases and beta-glucosidases are needed to completely break down cellulose into glucose monomer. Endo- J, 4 -beta-glucanase can be modelled using Modeller 9.10. The modelled structure were validated using Ramachandran Plot. The secondary metabolites from *Bacillus megaterium* can be identified by literature search and screening of compounds from Bio Cyc database. There are 281 compounds was predicted having nematicidal activity using PASS Server. Among them 140 compounds were selected for docking studies. Docking is done by using Molegro Virtual Docker tool, from which the higher hydrogen bond interactions are taken. There were 17 secondary metabolites which were validated *in vitro*. These compounds are *in silico* validated compounds inhibit the Endo 1,4-betaglucanase from *Radophollous similis.*

30. Functional annotation of SNPs in *Phytophthora capcisi* and their *in silico* analysis to identify deleterious Non-synonymous SNPs

Ms. J. Jino Blessy/Bharathiyar University/2012/Guide: Dr. Santhosh J. Eapen

In this work, by using computational tools we have analyzed the protein coding SNP's using SNP functional annotation that can alter the expression and function of genes involved in *Phytophthora capsici*. SNPs for the organism *Phytophthora capsici* were collected from db SNP database of NCBI, there were 360 reported SNP's present in the database. These 360 SNP' sequences were aligned with IISR *Phytophthora* genome database using local BLAST analysis to map the location of SNP in the whole genome and to take the scaffolds which contain the SNP's for SNP Functional annotation using NCBI BLAST. Of the 360 SNP's from dbSNP functional protein s were identified in 75 SNP's. To explore possible relationships between genetic mutation and phenotypic variation, computational algorithm tools like Sorting In tolerant from Tolerant (evolutionary- based approach) are used to predict the deleterious non-synonymous SNP's. SNP's which had functional proteins in them were analysed for deleterious effect in SNP and hence there were only one SNP in each of the 75 SNP's no deleterious effect was obtained. The functional annotation data of *Phytophthora* genome database have been analysed for functional proteins containing SNP's. 120 functional proteins with SNP were analysed out of 847 functionally annotated proteins

44 proteins were detected with deleterious index, of which 22 proteins showed complete deleterious effect exhibited a highly deleterious tolerance index score of 0.00.

31. Development of extrudates from cassava flour blended with spices and optimization of extruder parameters for extrusion processing

Mr. Dennis Abraham George/Karunya University/2012/Guide: Dr. E. Jayashree

In the present study, blending of cassava flour and spice powder was experimented to produce cassava flour spice blend extrudates. The experiment was carried out in two stages. In the first stage, ten flour blends were prepared from cassava flour mixed with spice powders. The spices used for blending were mace, turmeric, clove, cinnamon, white pepper, nutmeg, *Curcuma amada*, red chilli, cardamom and black pepper. The cassava flour was mixed with spice power in the ratio of 96:4, respectively and conditioned at 4 °C for 15 days. The ten spice blends with cassava flour as control sample were extruded in a single screw stand alone Brabender extruder to select the blends that were suitable for extrusion process. The flour spice blends were extruded at a constant die temperature 180°C and a screw speed of 80 rpm. The extrudates obtained from the flour spice blends and cassava flour alone was compared based on their physical, functional, textural and sensory properties.

32. EST based secretome analysis of the burrowing nematode (*Radopholus similis*)

Ms. K. Sangeetha/Bharathidasan University/2010/Guide: Dr. Santhosh J. Eapen

On exploring the ESTs of the burrowing nematode (*Radhopholus similus*), 214 secretary proteins were identified. About 45% of these secretory proteins showed similarity to nematodes. Functional annotation of these protein s revealed that about 10% of them was involved in biological processes like transporter the organism and 30% in binding. The Interproscan showed EGF like and IGFBP domains. The present study revealed the role of certain hypothetical proteins involved in signaling, metabolic and degradative pathways. Degradative pathway is involved in utilization of the plant's chemicals by the nematode for its survival. Besides, there are proteins essential for immune response of the nematode and involved in signaling pathway, The proteins that are involved in the metabolic pathways arc responsible for the survival of the nematodes. It was also observed that most of the secretome is involved in metabolic pathways and molecular functions such as growth and nematode larval development.

33. Study on the proliferation of promising biocontrol agents in vermicompost

Ms. Lamya Moideen/University of Calicut/2010/Guide: Dr. R. Suseela Bhai

In the present study, the suitability of vermicompost was evaluated for the proliferation of promising biocontrol agents *viz*., *P. aeruginosa* (IISR 6, llSR 853), *C. luteum* (TC 10), *T. harzianum, P. chlamydosporia* and biofertilizer organism' s *viz*. *Azospirillum* sp. (N_2 fixer), *P*. *fluorescence* (P-solubilisers), potash mobilizers developed at llSR Calicut. *T. harzianum* and BPaz4 showed better growth and proliferation in sterile VC when compared with non-sterile vermicompost. All the other isolates also showed increase in population after 5, 10 and 20 days in sterile vermicompost. *P. chlamidosporia* showed decline in population in both sterile and non-sterile vermicompost, but the decline was comparatively less in sterile VC when compared to non-sterile. The study clearly revealed that sterile VC is comparatively more suitable for the proliferation of biocontrol agents and biofertilizer organisms than non-sterile VC. But for field applications as soil amendment, it is not advisable to sterilize vermicompost due to the loss of enzymatic and microbial activities.

34. *In silico* identification of miRNAs in *Radopholus similis* and development of a comprehensive database of burrowing nematodes

Ms. K. Lakshmi Priya Darshini/Bharathiyar University/2009/
Guide: Dr. Santhosh J. Eapen

Radopholus similis is an endoparasitic migratory nematode belonging to the family Pratylenchidae which is more prevalent in the tropical and sub tropical region Expressed Sequence Tags or ESTs are small pieces of DNA sequence (usually 200 to 500 nucleotides long) that are generated by sequencing either one or both ends of an expressed gene. ESTs provide researchers with quick and inexpensive route for discovering new genes, for obtaining data on gene expression and regulation, and for constructing genome maps. EST resources are tissue specific and useful in studying the gene expression. Recently ESTs are used to find the microRNA detection to understand its role in plants, animals, nematodes and also viruses. MicroRNAs are the emerging topics in the field of genomics which constitute 20-22 nucleotides in length and has gene silencing as one of the functions. Available EST was analyzed to remove the redundancy. CAP3 was used for this purpose. In the present study the identification and characterization of the microRNA s of the nematode *Radopholus similus* by EST analysis is performed.

35. *In silico* screening of phytochemicals of five spices for their antimicrobial activity and docking studies using autodock targeting *Helicobacter pylori*

Ms. R. Sangeetha/Bharathiyar University/2009/Guide: Dr. Shamina Azeez

The present study has established two lead compounds 1,8-cineole and eugenol - effective in binding the virulence factor of *H. pylori*, VacA. But, lead optimization studies remain to be done to ascertain their efficacy. Additionally, *in vivo* studies and clinical trials would be required to experimentally prove and evaluate the potential of these compound as anti *H. pylori* agents, targeting VacA, and therefore to treat peptic ulcer. This study provides novel insights into the therapeutic effects of spice phytochemicals against *H. pylori*- infection, suggesting it as an alternative natural therapy and opens scope for further clinical trials to tight against peptic ulcer.

36. Identification of conserved orthologous gene segments in *Radopholus similis* using mitochondrial genome and available EST resources

Ms. Nima P. Lawrence/Mahatma Gandhi University/2009/
Guide: Dr. Santhosh J. Eapen

In the present study, we have identified conserved orthologous gene segments in *Radopholus similis* mitochondrial genomes and available EST resources. So far, whole genome sequencing program of nematode mitochondrial and other organelle genomes available are limited to study. ESTs of *Radopholus similis* were clustered and compared with non-redundant database using BLASTN. Similar sequences obtained were aligned with ClustalW to detect orthologous clusters. A total of 7 nematodes showed orthology to ESTs of *Radopholus similis*. Complete genomes of 16 nematodes were downloaded from Gen Bank National Centre for Biotechnology Information (NCB I). Conserved short regions were obtained using a Perl script, Murasaki. Os finder was used to find the orthologous segments shared between mitochondrial genomes of *R. similis* and other 15 nematodes. Orthologous segments were visualized with the help of GTK powered Murasaki Visualizer (GMV) programme. A phylogenetic tree was generated using VISTA online service. Extremely AT rich genome of the burrowing nematode *Radophulus similis*, which has the largest mitochondrial genome, was found to have orthologous segments from start position 4 to end position16791 with 15 nematodes. *Brugia malayi, Dirofilaria immitis, Onchocerca volvulus*, and *Xiphinema americanum* shares similar orthologous segment with that of *Radophlus similus*. The study revealed the conserved genes in mitochondrion and close phylogenetic relationship of nematodes belongs to different clades and different parasitism based on

the mitochondrial genome analysis. This study has many practical implications like reconstruction of ancestral genome of nematode and calculating evolutionary time.

37. Functional annotation and classification of ESTs of two entomopathogenic nematodes

Ms. Sruthi Mohandas/University of Calicut/2009/Guide: Dr. Santhosh J. Eapen

Insect parasitic (or entomopathogenic) nematodes, belonging to Heterorhabditidae and Steinernematidae, form unique models for the study of parasitism, pathogenicity, and symbiosis in general. These nematodes and their obligate bacterial symbionts form a highly species-specific and mutually beneficial relationship. *Heterorhabditids* associating with *Photorhabdus* and *Steinerematids* with *Xenorhabdus*, respectively. The potential of insect parasitic nematodes as biological control agents can be further improved with respect to IJ longevity, bacterial retention, tolerance to heat, ultraviolet radiation and desiccation, resistance to encapsulation in the hemocoel of some key insect pests, and trait stability. ESTs are small pieces of DNA sequence (usually 200 to 500 nucleotides long) that are generated by sequencing either one or both ends of an expressed gene. This large- scale expressed sequence tag analysis effort enables gene discovery and development of microsatellite markers. ESTs provide researchers with quick and inexpensive route for discovering new genes, for obtaining data on gene expression and regulation, and for, constructing genome maps. *Steinernema feltiae* and *Heterorhabditis* bacteriophora EST sequences were mined from dbEST. The EST sequences were preprocessed for contamination removal and trace quality. EST contamination removal programs, TrimEST, VecScreen were used. Further assembled using CAP3. EST annotation done by using BLASTX.

38. *In silico* screening of phytochemicals from major tree spices for their biological activities

Ms. Suja Sukumaran Nair/Mahatma Gandhi University/2008/
Guide: Dr. Santhosh J. Eapen

A total of 573 compounds were identified from the five spices selected for the study: *Cinnamomum verum, Syzygium aromaticum*, *Myristica fragrans*, *Garcinia cambogia*, and *Pimento dioica*. The *in silico* analysis of druggability and activity of the chemical compounds were studied and 15 compounds were found to be non-toxic compounds. These are proposed as potential drug candidates, with various activities such as, anti-inflammatory, antioxidants, chemopreventive etc. The present study has also helped to bring out several other interesting activities of the compounds present in

these spices. Some of them like epicatechin, alpha-humulene, alpha-phellandrene are found to possess toxic properties. These compounds could be used for specific disorders, and so structural alterations could be possible by introducing combinatorial chemistry approach in order to remove their toxic property. So, the current study motivates and initiates the screening of a diverse array of chemical compounds in other spices for their druggability. This approach may bring the success of phytochemicals in the treatment of many diseases and disorders with minimal side effects. It is clear that approaches used in the present study help in probing biological functions in greater depth. Such an approach, may throw light on new properties hitherto 'unknown for the compounds. However, from all of the spices that were screened, most of the compound s provide with analgesic, antipyretic, and anti-inflammatory activities which are promising areas demanding further investigations. In future, computational (*in silico*) methods will play an increasingly important role in drug discovery, which should be adapted, hence the failure rates faced in today's drug discovery project can be brought down.

39. Inhibition of *Radopholous similis* by extracellular proteases from nematode antagonistic bacteria

Ms. Anju Philip/Vellore Institute of Technology University/2008/
Guide: Dr. Santhosh J. Eapen

The molecular characterization of the nematode antagonistic bacteria was carried out by the ARDRA technique. The results have shown that that the strains BP17, IISR522, TC10 are different and have produced bands of different base pair sizes and the strains IISR853, IISR6 and BP35 were found to be similar with reference to the restriction with the two enzymes Msp-I and Alu-I. The effect of dipping black pepper rooted cuttings in the bacterial suspension for different durations was studied. The results have shown that the cuttings when dipped in the suspension for 15 minutes itself had resulted in x 10^5 cells per gram of the root tissue. The retrieval of bacteria from these tissues also did not show much variation in relation to the sampling intervals. The bacterial entry in to the tissues was further confirmed by obtaining 780 bp size products in the direct cell PCR using the species-specific primers. The ability of the strains to produce proteases was also studied and have found that among the *Bacillus* species BPI7 and among the *Pseudomonas* species IISR853 are the effective proteases producers. The crude protease extract was isolated from these strains and their nematicidal activity was also tested and has found that IISR853 has the maximum

naematicidal activity. The presence of proteases in the extract was confirmed using the plate assay and biochemical tests. The nematicidal proteases from these strains could be a better alternative for the chemical nematicides. More studies in the molecular level will allow us to obtain a better knowledge the molecular mechanisms for the production of these nematoxins and to develop novel approaches for the rational nematode management using the biologically produced nematicidal agents.

40. Comparative studies on two nematicidal strains of *Bacillus megaterium*

Ms. C. Swapna/Bharathiar University/2008/Guide: Dr. Santhosh J. Eapen

In present study the laboratory studies were taken up to compare two strains of *B.megaterium* having nematidical properties. Intially the growth condition such as pH and temperature were compared. It was found that the optimum PH ranged between 5to 9 for both BP17 and IISR522. However, the highest OD was noticed at pH5 for BP17 while it was 7 for IISR522. BP17 had a better multiplication record across a pH range of 5 to 9. Highly acidic or alkaline pH was detrimental to both bacterial strains. Similarly, the optium temperature for both strains was 25°C. In general BP17 has a better multiplication rate than IISR 522. Culture filtrates of both isolates were toxic to *R. similis* and caused > 60% mortality. However, BP17 was slightly more efficient when compared with IISR522. Similarly, the volatile metabolites produced by both isolates too were toxic to *R. similis* and the mortality was significiantly high in the case of IISR 522. Both the isolates had moderately caused repellence of *R. similis*. BP17 and IISR 522 have the ability to decrease the length of primary roots of tomato plants. There was significant difference in the root hair production and root architecture of tomato plants indicating the crucial role of endophytes in growth promotion. The present studies confirmed the efficacy of both isolates in suppressing *R. similis.* Besides, they play an active role in influencing the plant growth by modifying the root architecture. The information on optimum temperature and pH for the multiplication and growth of the *B. megaterium* will be useful in their mass multiplication and scaling up.

41. Optimization of PCR - RELP and RAPD techniques for phylogenetic analysis

Ms. Lima Ann Philipose/Mahatma Gandhi University/2007/Guide: Dr. T. E. Sheeja

A technique for amplifying DNA from different source could be achieved by PCR employing primers for the 16S rDNA genes and the non translated spacer (ITS) of rDNA. A method was developed for resolving and detecting the polymorphism based on sequence variations involving both non-denaturing and denaturing polyacrylamide gel electrophoresis and silver staining. Optimised procedure for

amplifying the DNA using random primers and also analysed the diversity in microbial community composition among the soil samples using UPGMA and cluster analysis. It was established that all the above mentioned techniques can be successfully used to profile samples from diverse environments for genetic diversity analysis.

42. Molecular differentiation of two *Steinernema* populations

Ms. N. V. Lasana/Bharathidasan University/2007/Guide: Dr. Santhosh J. Eapen

In this study, various molecular tools were employed to distinguish two *Steinernema* populations. The DNA of both isolates were first isolated by the phenol/ chloroform extraction protocol. The isolated DNA was quantified by agarose gel elechophoresis. In order to obtain reproducible PCR results, a dilution series was used to ascertain the amount of DNA and adjusted it to 50 ng by using a Biophotometer. Then the amplification of the ITS ribosomal (rRNA) in the isolates was done by using universal primer (ITS 1). The suitable temperature for the ITS rRNA from the isolates by using the universal primer set (ITS 1) was detected. In order to know whether the isolates are same or not, RFLP analysis of the amplified ITS rRNA was done. The banding patterns were different for the isolates. So, it was concluded that the two isolates are different. As RFLP gave information that the two isolates are different, DNA sequencing was done to get the sequence of the ITS rRNA which is eluted from the gel. The quality of ITS rRNA sequence data was not good enough to identify the isolates to the respective species.

43. Idenification of species specific signatures in Phytobacteria

Ms. Reshma Raghu/Bharathiyar University 2007/Guide: Dr. Santhosh J. Eapen

Signatures in bacteria can be defined as unique combinations of genes that indicate each bacterium's identity. The detection of signatures helps in discerning between and among bacterial strains. The region of 16S rRNA gene (1,500 bp in size) is ubiquitous in all eubacteria and has served as the principal target of bacterial identification protocols that are sequence-based. Each unique bacterial species has a distinctive 16S rRNA gene sequence profile (signature); hence, the signatures of unknown bacteria can then be compared to publicly available or commercial sequence databases to determine if the organism belongs to a particular known species. About 121 plant associated species sequences were analyzed and specific signatures for 50 species were obtained. The signatures identified were then used to design primers for the particular species. Based on this information a tool named "Sign-o-bacteria" was developed which encompassed all the names, signatures, their position and the corresponding primers of these plant associated species. Most approaches used to

identify DNA signatures are laboratory based and require a significant effort and time. This approach can provide results faster and more efficiently. The identification of a species-specific signature would be of great value to genome mapping, evolutionary studies and analyses of genome complexity.

44. Sequence analysis of *Badnavirus* genome for identification and classification

Ms.K. J. Urmila/Periyar University/2007/Guide: Dr. A. Ishwara Bhat

Badnavirus are bacilli form DNA viruses which have circular double stranded DNA. The aim of the study was the analysis of the genome of *Badnavirus* having complete genome for the development of primers which can be used as a criterion for the identification and classification of *Badnavirus*. The species of the genus *Badnavirus* having complete genome was retrieved from the Gen Bank. A total of about 30 strains having complete genome were obtained. There were 12 definitive species among these 30 members. The sequence of all the 30 strains were downloaded and converted into FASTA format. Multiple sequence alignment for complete genome, ORF- I, II, III and IGR was done separately by using the ClustalX. Percent identity was calculated using the BioEdit and the values were tabulated. The aligned sequences were used for viewing the phylogenetic tree using DAMBE software. This showed the genetic relationship among the different strains of the virus. From the aligned sequence of complete genome the more conserved regions was noted down, each having a maximum length of fifteen. With this data, a rough figure for the complete genome of *Badnavirus* was made with its conserved regions coloured. A total of II conserved regions were obtained out of which 10 regions were located in the ORF- III while one was located in the IGR. There were no conserved regions in ORF-I and II. T he conserved regions can be used for designing primers for *Badnavirus* which will be useful for identification and classification of new and existing *Badnavirus* infecting plant. In order to see its utility, a region of 794 bp flanking a portion of ORF- III and IG R (conserved region 10 - 11) was selected for its reliability for classifying Badnaviruses into species/strains. The multiple sequence alignment followed by the sequence identity matrix was performed for these 794 regions. For better comparison of complete genome with the 10 - 11 primer combination the values of the percent identity was presented. It was found that the percent identity obtained between different species/strains of Badnaviruses in the 794 region matched with the percent identity obtained with complete genome. So, from the study it was concluded that the amplification and sequencing of the 794 bp productspanning between the conserved regions 10 and 11 can be used as a criterion for identification and classification of *Badnavirus* up to species/strains level.

45. Amplification of ribosomal genes in *Radopholus similis* using universal and species - specific primers

Ms. K. V. Asha/Bharathidasan University/2006/Guide: Dr. Santhosh J. Eapen

Nematodes are small microscopic and most abundant multicellular organisms on earth. Two important nematodes such as *Radopholus similis* and *Meloidogyne incognita* are most damaging pests of black pepper. It cause "slow wilt" disease on black pepper, decline symptoms such as foliar yellowing, defoliation, loss in vigor and productivity. Several methods are used for the identification of nematodes. Mainly molecular marker techniques are used for the specific detection of nematodes. During the study the DNA of both *Radopholus similis* and *Meloidgne incognita* were first isolated. Then the amplification of ribosomal genes in *Radopholus similis* was done by using in universal and species-specific primers. The suitable temperature for amplification of *Radhopholus similis* species specific primer has been detected. The nematode *Meloidogyne incognita* was taken for a conformational analysis of the *Radopholus similis* specific primer. A species-specific primer is a primer which can only amplify a specific site present on the specific species. So, *Meloidogyne incognita* was taken as test organism whether it amplify, we can conclude that primer was not a species-specific primer. In the present study, the amplification was shown only in *Radopholus similis* species and concluded it as a species-specific primer.

46. Amplified ribosomal DNA restriction analysis of *Ralstonia solanacearum* causing bacterial wilt of plants

Ms. Soumya Madhavan/Bharathidasan University/2005/Guide: Dr. A. Kumar

Identity of *R. solanacearum* was confirmed by PCR using species specific primers, which produced amplicon with 281 bp using Rs specific primers. Genetic diversity of *R. solanacearum* isolates from different crops were analysed using Amplified Ribosomal DNA Restriction Analysis (ARDRA). 16S ribosomal gene, which is a conserved region and 16 -23'S intergenic regions, which is a hyper variable region were amplified using prokaryotic universal oligonucleotide primers. The size of the amplified 16S gene is 1493 and the size of the amplified intergenic region between 16-23S is 1682 and 1493. The amplification using PCR produced single discrete monomorphic band of size in the case of 16S gene. Polymorphic bands were obtained after restriction digested with enzymes Msp 1 or Taq 1. The restriction analysis clearly revealed the divergence of biovar 2 and Biovar 3 at 50% similarity coefficient. Biovar strains are highly related at genetic level with more than 90% similarity coefficient.

Ph.D. ABSTRACTS

BLACK PEPPER

1. Studies on *Pythium* species associated with yellowing of black pepper (*Piper nigrum* L.) (TH 200)

Ms. K.P. Subila/University of Calicut/ 2022/Guide: Dr. R. Suseela Bhai

Recently yellowing during post-monsoon reported and was characterized by declining symptoms like yellowing, wilting and in advanced stages of infection the plant suffers from defoliation and complete drying up. Pathogenic *Pythium* spp. were found in the rhizosphere soil and roots of damaged vines, according to a detailed analysis of the samples collected from various affected areas. *Pythium* spp. was found in 95% of the soil samples and 67% of the root samples collected. A survey was undertaken in different agro-climatic areas also clearly revealed the association of three species of *Pythium viz. P. deliense*, *P. cucubitacearum* and *P. catenulatum*. Three unique colony morphology patterns were noticed among the isolates, namely floral cottony, chrysanthemum, and stellate cottony patterns, which grew at a pH range of 4.5 to 10.0 and a temperature range of 15 -37°C. Torulated and globose sporangia were produced by the isolates. The three morphotypes infected black pepper plants, resulting in collar rotting and wilting. The epidemiology of the disease revealed that the pathogen, *P. deliense* could survive in soils with less moisture and high temperature than other oomycetes. Pathogenicity also showed that *P. deliense* is infective to all the eight released varieties *viz.* IISR Shakthi, IISR Malabar Excel, IISR Subhakara, IISR Girimunda, IISR Panchami, IISR Sreekara, IISR Thevam and IISR Pournami, six Panniyur varieties *viz*, 1,3,4,5,6 and 8 besides tomato and ginger. However, varieties Panniyur 2 and 7 are found resistant to *P. deliense*. *P. deliense* is a newly reported pathogen in the black pepper and is the first report of its kind from India. Field evaluation, as well as green house evaluation, supports this finding that *T. harzianum* is effective against *P. deliense*. Hence soil application of *T. harzianum* and actinomycete *S. albulus* during post-monsoon season thrice would be a better choice in reducing the intensity of yellowing. But as an integrated disease management mode, soil application of *T. harzianum* along with FYM and aerial spraying with Fenamidone MZ improved the soil qualities as well as suppressed the pathogen and there by increased the yield.

2. Characterization and identification of black pepper accessions (*Piper nigrum* L.) for stress tolerance and quality (TH 198)

Mr. K. M. Prakash/Kerala Agricultural University/2019/Guide: Dr. Jiji Joseph

Characterisation of the accessions as per the descriptor for black pepper developed by IPGRI, 1995 showed that majority of accessions had dimorphic branching habit, light purple shoot tip colour, many runner shoots, weak holding capacity, few adventitious roots, horizontal lateral branch habit, ovate leaf lamina, round leaf base shape and round fruit shape. Clustering based on qualitative characters grouped the black pepper accessions numbering fifty into 4 clusters. Multivariate hierarchial cluster analysis based on quantitative traits of vine, leaf, and spike characters grouped the accessions into two major clusters. The first cluster had three sub- clusters with 5, 6, and 35 accessions. The remaining four genotypes were grouped under the second cluster. The accessions were scored for high yield and low biotic susceptibility for foot rot infection and pollu beetle infestation as per IPGRI descriptor. Twenty accessions were selected based on superior yield and field tolerance to pollu beetle infestation and foot rot disease incidence. Screening of selected accessions for foot rot disease tolerance showed that none of the accessions were tolerant to foot rot. However, the accession 7259 was moderately resistant to foot rot. Ranking of accessions based on yield, foot rot tolerance and quality parameters showed that the accessions 7252, 7211, 7259 7222, 7249,7232 and 7211 are promising. Screening of selected accessions for drought tolerance showed that at field capacity all the accessions were on par in response with respect to all biochemical parameters. Comparison of responses of accessions to foliar nutrition on various biochemical parameters showed that there is a positive effect of foliar nutrition on reducing the impact of stress. Foliar nutrition was more effective on accessions having natural tolerance. The accessions 7215, 7240, P5 and 7211 were tolerating moisture stress better under both conditions. Comparison of visual observations at permanent wilting point showed that the foliar nutrition did not extend the PWP of the genotypes.

3. RNAi mediated resistance to cucumber mosaic virus (CMV) in black pepper (*Piper nigrum* L.) (TH 196)

Ms. K.A. Revathy/University of Calicut/2019/ Guide: Dr. A. Ishwara Bhat

Cucumber mosaic virus (CMV) ranks one among the top five predominant plant viruses and has emerged as a serious pathogen of black pepper during the past few decades in India and other black pepper producing countries across the world. The virus

nucleotide analyses performed in this study showed that the 2b gene is the most diverse of all the five genes and 3b gene is the highly conserved among the different subgroups of CMV. The percent identity analyses also inferred similar observations. Regarding the evolutionary forces acting on these genes, all the genes were undergoing negative selection except for the 1a gene that was nearing neutral selection. As a whole, out of the three RNAs of the present isolate, RNA2 was highly tolerant to amino acid changes and RNA1 was the least tolerant. Whether the deletion of three amino acids in the deduced 1a protein, compared to that of the other strains, has any significance on the function is inconclusive and, may be further investigated in detail. Once the complete gene sequences of different RNAs of the current isolate was ascertained, these were subjected to a computational tool 'dsCheck' to find out the most suitable region for dsRNA synthesis. Based on the earlier observations regarding the appropriate length of the transgene and the parameters of the software, a 400 bp region was selected from all the five genes (except the 336 bp 2b gene), as the most suitable regions for the hairpin construct preparation. The selected regions were subjected to sequence polymorphism study and again the results were consistent with complete genome sequence data, confirming the 3b gene as the highly conserved and the 2b gene as the least conserved among the different genes. Further, the potential siRNAs that will be coded from the selected regions were designed *in silico* with the help of another computational tool 'siDesign' and though potential siRNAs were designed from all the five genes, with maximum specificity and minimum off–targets, four common siRNAs were generated from the 3b gene and none from other genes. The common siRNAs designed from the 3b gene will target all the subgroup IB strains taken in the current investigation.

4. Characterization and development of molecular diagnostics for burrowing nematode infecting black pepper (TH 195)

Ms. P. B. Krishna/ University of Calicut/2019/Guide: Dr. Santhosh J. Eapen

Burrowing nematode, *Radopholus similis* (Cobb, 1893) is an obligate migratory endoparasite found worldwide in tropical and sub-tropical areas. It is a highly polyphagous plant parasitic nematode which infects about 365 plant species and is one of the 10 most damaging plant-parasitic nematodes worldwide. There are 30 species in the genus *Radopholus*, among them *R. similis*, is the only species of widespread economic importance. Slow decline disease of pepper, toppling disease of banana, spreading decline disease of citrus etc are some of the serious diseases caused by *R. similis*. The accurate identification and characterization of nematodes infecting a crop is

causes stunted disease in black pepper affecting the production and quality of the berries leading to the consequential drop in yield. The severity and high incidence of the disease make this the third major production constraint of black pepper. This *Cucumovirus* member, with a tripartite genome has the broadest host range of any known virus and is unmanageable by chemicals or traditional control methods. Among the different pathogen derived approaches used for engineering resistance against this most notorious virus, RNAi sounds to be highly promising. Hence, this study focused on developing transgenic black pepper plants using the hairpin construct of 3b and 2b genes of black pepper isolate of CMV. The study was accomplished through five prime objectives that included the complete genome sequencing of black pepper isolate of CMV, designing of common siRNAs targeting many subgroup IB strains of CMV, somatic embryo production from three different varieties of black pepper, *Agrobacterium* mediated transformation of the somatic embryos produced and testing of the regenerated putative transformants. Finally, a challenge inoculation method for CMV in black pepper was also developed to facilitate the screening of plants for resistance to this virus and its utility was tested in the transgenic plants carrying the CMV coat protein in the sense orientation, already available. The complete genome sequencing of CMV from black pepper was accomplished to get a thorough understanding of the genomic structure of the present isolate under study. Further, percent identity analyses, phylogenetic relationships and nucleotide diversity studies were performed that helped in analysing the level of conservation in each gene of the three RNAs, the relationship of the black pepper isolate with other subgroup I and II strains of CMV and the evolutionary forces driving these genes. An important finding of the study was a rare nine nucleotide deletion in the putative methyltransferase domain of 1a gene of RNA1 which was present in two other strains of CMV among the hundred 1a gene sequences available in the GenBank. The corresponding deletion of three amino acids was also observed in the multiple sequence alignment of the deduced amino acids of the 1a protein. Interestingly, the deletion was not found in the black pepper strain of CMV from China and not even in the CMV isolate from long pepper, a closely related species of black pepper. The percent identity analyses proved that all the genes of the current isolate shared highest sequence similarity to the subgroup IB strains at the nucleotide and amino acid level. Phylogenetic trees constructed, clearly marked the clustering of the black pepper isolate with the subgroup IB strains. These studies together classify the black pepper isolate of CMV as subgroup IB. Finally, the

a pre-requisite for designing effective control strategies. So, a combined morphological and molecular characterization study was undertaken for the accurate identification of *Radopholus* spp. isolates infecting black pepper and banana roots collected from different regions of Kerala and Karnataka. Further a real time PCR based diagnostic tool was developed for its detection. Several morphological characters prescribed for nematode taxonomy were studied in ten isolates of *Radopholus* spp. Collected from different parts of Kerala and Karnataka. Except for a few characters, most of the measurements fall within the range of measurements for *R. similis* reported by previous workers. These observations substantiate the hypothesis that the burrowing nematode isolates infesting black pepper in South India have the closest resemblance to *R. similis*. To support this hypothesis molecular characterization of these isolates was also carried out as well. For this, the internal transcribed spacer region (ITS1-5.8S-ITS2) region of the ribosomal DNA in tandem with the cytochrome c oxidase gene 1 (COX) and NADH dehydrogenase gene 1 (ND) regions of the mitochondrial genome sequence were used. The results obtained through phylogenetic analysis of ITS region showed a high level of similarity between the ten isolates and the ITS region of *R. similis* reported worldwide. The COX and ND regions also showed higher similarity to the *R. similis* complete mitochondrial genome sequence counterparts for COX and ND. Hence, the sequence analysis of these three regions, along with the previously conducted morphological characterization studies confirmed that all the 10 isolates used in this study belong to the species, *R. similis*. Besides phylogenetic study, a phylogeographical analysis was also conducted and the likely origin of Indian *R. similis* populations. In this analysis the Asian African populations assembled differently. The ten isolates used in this study grouped with the Asian isolates. However, the Asian group has a scattered presence among the rest of the world population, which indicates that the Asian population might have been the original species to spawn the rest of the *R. similis* population in other parts of the world. Developing a diagnostic assay for the accurate identification and quantification of plant parasitic nematode is critical for making plant management decisions. The traditional methods of diagnosis are not ideal as they are time consuming and require expertise in identifying *R. similis*. Loop-mediated isothermal amplification (LAMP) and several PCR assays have already been developed for detection of *R. similis*. However, these techniques are not suitable for quantifying nematodes in soil samples. Hence a qPCR assay was standardized for the detection and estimation of *R. similis* population in field samples. For this, species specific real-time PCR primer was

designed using the conserved region of the ITS sequences and the target DNA could be detected in suspensions containing as little as 100 fg of *R. similis* DNA. The real time PCR method developed for detection of *R. similis* in infected black pepper rhizhosphere soil was validated using field samples collected from different regions of Kerala. The technique developed is accurate and reliable and can be deployed for early detection and estimation of *R. similis* in soil and root samples which will pave way for the effective management of this serious nematode pest.

5. Microbial community dynamics and modulation of defence responses in black pepper by *Trichoderma harzianum* (TH 189)

Ms. P. Umadevi/University of Calicut/2018/Guide: Dr. M. Anandaraj

It is well known that the plant rhizosphere along with the associated microbial communities plays a vital role in the health of the plant and protection from soilborne pathogens. Linderman (1998) coined the term Mycorhizosphere as the roots colonized by arbuscular mycorrhiza exhibits a different community structure when compared to non-mycorrhizal roots. *Trichoderma* being an opportunistic endophyte (Harman et al., 2004) improves growth of the inoculated plants besides suppressing the diseases caused by soil borne pathogens especially *Phytophthora*. In black pepper this was elucidated by using metagenomic tools and the results showed the presence of differential microbial communities and thus justifying the use of the term "trichorhizosphere". The illumina hiseq sequenced soil metagenome assembled reads, when analyzed with double approach *viz*., stand alone and MG-RAST yielded coherent results in both taxonomy and functional categories. STAMP tool analysis of relative abundance on top ten bacteria and fungi showed statistically higher proportion of Acidobacteriaceae bacterium, *Candidatus koribacter versatilis* in *Trichoderma* inoculated sample, uncultured bacteria in control and *Fusarium oxysporum, Talaromyces stipitatus* in *Trichoderma* inoculated sample*, Rhizophagus irregularis, Pseudogymnoasus pannarum* (Human pathogenic fungi), *Oidiodendran* in control sample respectively. The relative abundance for the specific functional features showed the high abundance of heme and hemin uptake, iron acquisition, metabolism of aromatic compounds in *Trichoderma* treated soil metagenome and with the reduced abundance on pathogenicity is lands, phages and prophages than untreated soil (control). The population dynamics and functional richness of rhizosphere ecosystem in black pepper influenced by the treatment with *T. harzianum* provides evidence for the ecological importance of *T. harzianum* in the cultivation of black pepper. On the basis of

the present report and previous studies on effect of *T. harzianum* in the fitness of black pepper; it can be suggested that as mycorhizosphere, another micro ecological niche, *viz.,* 'trichorhizosphere' is also exists in altering the community dynamics of bacteria and soil fungi; and thus, the rhizosphere micro ecosystem developed by *T. harzianum* might contribute a pivotal role in imparting plant health, which is unlike the individual effect of *T. harzianum*. The methods employed in this study show a significant step toward possible application of metagenomics for the functional elucidation of *T. harzianum* - the valuable biocontrol, growth promoting fungus in the production system of black pepper. The rhizosphere and the trichorhizosphere metagenomes of black pepper elucidated in this study would become important factors in developing any IDM modules in the root ecosystem of black pepper. Root colonization of *T. harzianum*. The co colonisation and microscopy especially electron microscopic study demonstrated the localization, endophytic colonization and helper activity of *T. harzianum* MTCC 5179 in black pepper. This endophytic interaction of the fungus underwent several morphological changes during interaction with the root system of the host. Scanning electron microscopy showed the enlargement of hyphal tip as papillae at 12 HAI on the black pepper roots. The root clearing and bright filed microscopy showed fungal mycelium in the intercellular spaces at 24 HAI with hyphal tips as dark blue granules inside the cells. The intracellular chlamydospores were observed at 48 HAI. Enhanced AMF root colonization by the *T. harzianum* inoculation in black pepper form pot culture study indicates that *T. harzianum* acts as helper organism in the root ecosystem of black pepper for colonization by native AMF present in the soil on the plant. Black pepper -*T. harzianum* interaction the immune suppression by *T. harzianum* in black pepper during its colonization has been demonstrated by the label free proteomics. The expression pattern of proteins during *T. harzianum* interaction showed the down regulation of PR proteins to the greater degree indicating the absence of SA involvement at all the time intervals studied. The other phyto hormone ethylene biosynthesis is also down regulated. The up regulation and new protein induced group with PTI related receptors and R genes shows that the pattern recognition step itself the *Trichoderma* might be altering the plant activities favorable towards its own establishment. Some important down regulated proteins were found to be the marker proteins for the suppression of immunity in black pepper by *T. harzianum*. At 24 HAI the SOD, CAT, Glyceroldehde -3 Po4; at 48 HAI Rap guanine nucleotide exchange factor 2, Histone 2A; at 72 HAI, the germin like protein and

subtilisin marker proteins for plant defense; at 96HAI, the SAR marker ascorbate peroxidase and RAB GTPase which is immunity associated programmed cell death. Black pepper - *Phytophthora* interaction. The protein profile of black pepper- *P. capsici* showed much photosynthetic related protein down regulated suggesting that the photosynthesis is altered upon infection. The SAR reaction was initiated at 24 HAI than 12 HAI. Most of the proteins that were fully down regulated were with slightly increased expression in 24 HAI when compared to 12 HAI. Activation of ROS scavengers and the strong indicators of SAR, the PR proteins were not present in both the hour to a greater extent. This could be attributed to the susceptible genotype (Sreekara) of the present study. Our unpublished data on protein dynamics in the resistant genotype IISR Shakthi recorded up regulation of ROS scavengers and PR proteins at 12 and 24HAI.This shows the genotype specific resistance in black pepper for *Phytophthora*. Black pepper - *T. harzianum-Phytophthora*. Expression dynamics of proteins in tripartite interaction clearly showed the *T. harzianum* induced systemic resistance (T-ISR) the systemically modulated "defense readiness" in black pepper against *Phytophthora*. Twenty three defense related proteins and eighteen ROS scavenging proteins were identified as T-ISR proteins. The enrichment of strong ROS related activity suggests that the ROS mediated signaling as major component in T-ISR in black pepper and also the involvement of ET synthesis in the ISR development in black pepper against *Phytophthora*. The auxin mediated defense signaling component was absent in T-ISR in black pepper. The isoflavanoid pathway and lignin synthesis are also found to be important component of T-ISR in black pepper. The functional enrichment of protein showed the involvement of peroxisome from all condition of tripartite which suggest that the leaf peroxisome is acting as a major pathogen defense mechanism in black pepper (susceptible genotype) during early hours of defense which is induced by *T. harzianum*. The hypersensitive response or cell death is the qualitative resistance, while the the reduced susceptibility is considered as quantitative resistance. The disease resistance is due to additive effects of several resistance metabolites and proteins. The proteins identified in this study are considered as quantitative resistance candidates mediated by *T. harzianum* in black pepper. Our study is the first of its kind report on tripartite interaction using label free proteomics. We have elucidated the T-ISR in leaf of root primed black pepper plants upon *Phytophthora* infection. This study developed the entire peptide signatures from the proteins involved in black pepper + *T. harzianum*; black pepper + *Phytophthora* and black pepper + *T. harzianum* +

Phytophthora interactions. The peptides from black pepper + *T. harzianum*, can be used to understand the mode of suppression of immunity in this crop by this beneficial fungus using functional genomics studies. The peptide signatures of these important host defense proteins from black pepper + *Phytophthora* interaction could be the possible candidates which can be used to develop the protein-based QTL in screening and developing resistant varieties against *Phytophthora* in black pepper using genome editing technology in future. The T-ISR proteins from the tripartite interaction are the possible candidates for studying the defense signaling mechanism, designing the new molecules as inducers of defense and using it in field condition.

6. Diversity and bioactive potential of rhizospheric actinomycetes from black pepper (*Piper nigrum* L.) (TH 186)

Ms. Anusree Thampi/Mangalore University/2018/Guide: Dr. R. Suseela Bhai

The most over whelming disease of black pepper is the foot rot caused by oomycete pathogen *Phytophthora capsici* which leads to the sudden wilting of the plant. The Anthracnose diseases caused by the imperfect fungal pathogen *C. gloeosporioides* and basal rot caused by the mycelia sterilia - *Sclerotium rolfsii*, in the nursery are also equally important. The chemical control measures currently used for controlling these pathogens leads to hazardous toxic effect in human and other non target organisms in the environment. Biological control is the ultimate choice in the Integrated Disease Management in which the disease suppression and growth promotion of the plant is by microorganisms having antagonistic activity towards the pathogens. Actinobacteria are the choice of interest which is already established as biocontrol agent against many plant diseases in different crops. Soil samples were collected from different black pepper growing tracts of Kerala and Karnataka. Different locations covered for sample collection include Kasaragod, Kannur, Kozhikode, Wayanad, Thrissur, Ernakulam, Kottayam, Idukki and Malappuram districts in Kerala and Coorg district in Karnataka. A total of 123 soil samples were collected from these locations where black pepper is cultivated in a large and marginal scale. A total of 129 actinobacteria of different morphotypes were isolated from the collected soil samples. Colonies of actinomycetes obtained were purified and morphological characteristics such as appearance colour of the areal hyphae, growth of vegetative hyphae, production of diffusible pigments etc were recorded and documented based on the growth in ISP2 medium at 28ºC. The 129 isolates of actinobacteria were characterized and identified as belonging to the *Streptomyces,*

Actinoplanes, Micromonospora, Actinomyces, Nocardiopsis, and Actinopolyspora based on the morphological features. Out of 129 actinobacterial isolates, 100 isolates were identified as belonging to the genus *Streptomyces* having spore chain with coiling, spiral and looped structures. Ten isolates each were characterized as Actinoplanes group (spores in sporangia having spherical shape) and Micromonospora group (clusters of single conidia on substrate mycelium), two isolates as Nocardiopsis (aerial mycelium totally sporulated), three as Actinomyces (branching vegetative mycelium), one each isolate as Actinopolyspora (long chains of spores on aerial hyphae) and Dactylosporangium (Oligosporous, claviform sporangia on aerial mycelium). The isolates were given nomenclature as IISRBPAct where as IISR stands for institution (Indian Institute of Spices Research), BP for black pepper and Act for actinomycetes. All the isolates were assayed for their inhibitory activity against major pathogens of black pepper *viz*., *P. capsici, S. rolfsii* and *C. gloeopsorioides* by dual culture method. Among them 55% isolates showed more than 50% inhibition against *Phytophthora capsici*, 44% showed more than 50% inhibition towards *Sclerotium rolfsii* and 52% showed more than 50% inhibition towards *Colletotrichum gloeosporioides*. The isolate IISRBPAct1 showed more than 90% (92-94%) inhibition to all the tested pathogens followed by IISRBPAct25 and IISRBPAct42. These three isolates were selected for the further investigations. Spore surface ornamentation was observed through SEM. Under SEM IISRBPAct1appeared to be spiny, while IISRBPAct 25 and IISRBPAct42 were smooth (X 10,000 magnifications). On 16s rDNA sequencing, the three isolates were identified as *Streptomyces* spp. Further polytaxonomic studies revealed the close resemblance of IISRBPAct1 to *Streptomyces albulus*, IISRBPAct25 to *Streptomyces rimosus* and IISRBPAct42 to *Streptomyces olvacescleroticus*. The isolates were functionally characterised for growth promoting characters such as siderophore production, phosphate and zinc solubilisation and hydrolytic enzyme production. Siderophore production and zinc solubilization was found in all the three potential *Streptomyces* isolates while IISRBPAct42 exhibited phosphate solubilization also in Pikovskaya agar. IISRBPAct1 exhibited relatively high levels of amylase and lipase activity. IISRBPAct25 showed high cellulase and amylase activity while IISRBPAct42 showed high amylase, cellulase and protease activity. Green house experiments were also carried out for evaluating the growth promotive and biocontrol activity in the plant system. The promising isolates showed better agronomic performance of black pepper (*Piper nigrum* L.) in terms of growth parameters such as, height of the plant, fresh and

dry root and shoot biomass, number of nodes and laterals. IISRBPAct1 showed significant increase in fresh shoot weight, shoot height and number of nodes. Treatment with IISRBPAct42 showed extensive root system in black pepper and thus maximum fresh and dry root biomass was observed with IISRBPAct42. The biocontrol efficiency towards *P. capsici* and *S. rolfsii* was also proved by the potential isolates under *in planta* conditions. IISRBPAct25 showed maximum reduction of *P. capsici* infection (80.73%) followed by IISRBPAct1 which is as effective as fungicide metalaxyl- mancozeb (0.125%) for controlling *P. capsici* infection in black pepper. In the case of *S. rolfsii* infection, 98.10% reduction was noticed in treatment with IISRBPAct1 followed by IISRBPAct42 (83.77%). Also estimated the population dynamics of specific culturable rhizosphere microflora in *Streptomyces* inoculated soil. Phosphate solubilizing bacteria were found higher with IISRBPAct42, IISRBPAct25 and IISRBPAct1 which indicated that soil application with these potential strains did not hamper the beneficial microflora. Free living nitrogen fixers were also enhanced on application of IISRBPAct1 treated soil. The compatibility of promising *Streptomyces* isolates with other recommended beneficial microflora such as *Trichoderma* and *Pochonia* were also evaluated and found that IISRBPAct42 was more compatible with *Trichoderma* followed by IISRBP Act 25 and IISRBP Act 1. IISRBPAct 25 was more compatible with *Pochonia* followed by IISRBPAct42 and then IISRBPAct1. Effect of culture filtrates on different developmental stages of *P. capsici* and *S. rolfsii* was also evaluated under *in vitro* conditions. The inhibition of mycelial growth *P. capsici* was inversely proportional to the concentration of the culture filtrate. The crude extract of all the isolates completely inhibited the sporangial formation of *P. capsici*. Culture filtrates of IISRBPAct1 and IISRBPAct25 was found inhibitory to zoospore formation of *P. capsici*. Percentage inhibition of mycelial growth of *S. rolfsii* was also inversely proportional to the concentration. The culture filtrates were found inhibitory to the sclerotium formation by *S. rolfsii*. The extracellular secondary metabolites of the isolates were extracted by three organic solvents *viz*., ethyl acetate, chloroform and butanol. The concentrated extracts were dissolved in Dimethyl sulfoxide (DMSO) and bioassay was carried out for each metabolite to find the Minimum inhibitory concentration (MIC). The MIC of ethyl acetate of IISRBPAct1 was found to be 500 µg/mL against *P. capsici* and MIC of butanol extract of IISRBPAct25 was found to be 25000 µg/mL towards *S. rolfsii* to inhibit visible growth of the pathogen. The ethyl acetate extract of the isolate IISRBPAct1 showed highest activity against *Phytophthora*

capsici (73.5 % of inhibition) and butanol extracts of IISRBPAct25 showed highest activity against *Sclerotium rolfsii* (74.7% of inhibition), these two extracts were selected for further characterization and identification of the compounds responsible for the biological activity. The HPTLC finger printing analysis was carried out for the initial characterization of compounds. 15 compounds were estimated from ethyl acetate extract of IISRBPAct1 and 9 compounds with different Rf values were estimated from butanol extract of IISRBPAct25 at 254 nm. Under 366 nm, 10 different compounds were estimated from ethyl acetate extract of IISRBPAct1 and 4 compounds with different Rf values were estimated from butanol extract of IISRBPAct25. The detailed and precise identification of bioactive compounds were done by high resolution UPLC-(ESI) -QToF-MS analysis. A total of 51 compounds were identified from ethyl acetate extract of IISRBPAct1 and 11 compounds from butanol extract of IISRBPAct25. The major antibiotics identified were brefeldin A, dermadin, fusaric Acid, and salfredin B11. The antifungal compounds identified were brevianamide F, enniatin B, harzianopyridone 1, harzianopyridone 2, isonitrinic acid E, natamycin, trichodermin, and zeaenol. Interestingly a plant growth regulator harzianolide produced by *Trichoderma harzianum* was also identified from the ethyl acetate extract of IISRBPAct1. A fungitoxic sesquiterpene chokolic acid B was also identified. The antibacterial compounds identified were phytosphingosine, nectriapyrone, harzianopyridone 2, gliocladic acid 1 and brevianamide F. Phytosphingosine is an antibacterial lipid, harzianopyridone 2 and brevianamide F exhibits both antifungal and antibacterial activity. Besides all these compounds, mycotoxins such as roridin A, D, E, L, and enniatin D, were also identified from the ethyl acetate extract of IISRBPAct1. Natamycin and brevianamide F were also produced by IISRBPAct25 in butanol extract. Naphthoquinomycin B, an antibiotic, maculosin 6, an herbicide, verrucarin A, sesquiterpene toxins, (2E, 6E)-Farnesol, a natural pesticide for mites were also identified from the butanol extract of IISRBPAct25.The compounds such as (2E, 6E)-Farnesol, (2R)-2, 3-Dihydroxypropyl palmitate, Dihydrocoriandrin, Gamma-CEHC, N-[(2S, 3R, 4E)-1, 3-Dihydroxy-4-octadecen-2-yl] acetamide, Salfredin B11, Sphinganine, Levetiracetam and Tetradecenoylcarnitine were first report of its kind from *Streptomyces* spp. To the best of our knowledge, the compounds Levetiracetam, Tetradecenoylcarnitine and N- [(2S, 3R, 4E)-1, 3-Dihydroxy-4-octadecen-2-ol] acetamide were not yet reported from any microorganisms. The present investigation resulted in isolation and identification of efficient strains of actinobacteria in the genus

Streptomyces which was found effective against the major black pepper pathogens, *P. capsici* and *S. rolfsii*, besides, improving the overall growth of black pepper. The promising strains exhibited different antagonistic mechanisms like production of antibiotics, antifungal metabolites, siderophore and lytic enzymes rendered them as ideal biocontrol agents for black pepper. The *Streptomyces rimosus* has already been developed as a commercial preparation used to control *Phytophthora* spp., *Fusarium* spp., *Pythium* spp., *Alternaria brassicicola* etc. So the present study investigated the distribution, diversity of actinobacteria in the rhizosphere of black pepper and established its biocontrol potential and growth promotive traits by identifying the chemical moieties present in the secondary metabolites as well as by *in vitro* and *in planta* evaluation. By developing a formulation based on the indigenous strain of Streptomyces strains *viz.* IISRBPAct1 (*Streptomyces albulus*), IISRBPAct25 (*Streptomyce srimosus*) and IISRBPAct42 (*Streptomyces olvacescleroticus*), identified in the study either as individual or consortia form would be a better choice in the development of potential bioagents for black pepper.

7. Documentation and evaluation of fungal endophytes of black pepper (*Piper nigrum* L.) (TH 172)

Ms. K. Sreeja/Mangalore University/2018/Guide: Dr. M. Anandaraj

Endophytic fungi are the normal internal mycoflora of healthy plants and live and grow in the space available in plant tissues. Endophytes help their hosts in the fight against pathogens and in overcoming stress and by producing useful metabolites. In the present study, endophytic fungi were isolated from four major varieties of black pepper; identified using morphological as well as molecular methods; and screened for their antagonistic potential against *P. capsici* and *R. similis*. Mycoendophytes-free black pepper plants were produced through somatic embryogenesis and biohardening with endophytic fungi were carried out in a greenhouse. Secondary metabolites produced by endophytic fungi were extracted and evaluated against *P. capsici* and *R. similis*. The efficacy of endophytic fungi as biocontrol agents and their growth-promoting effect on black pepper were studied *in planta*.

8. Defense gene expression analysis in *Piper colubrinum* Link challenged with *Phytophthora capsici* Leonian emend. Alizadeh & Tsao. (TH 190)

Mr. I. P. Vijesh Kumar/Kannur University/2018/Guide: Dr. K. Johnson George

The present study focussed on identification of defense related genes in *Piper colubrinum* involved in the interaction with *Phytophthora capsici.*

Transcriptome sequence data analysis enabled the identification of a number of defense genes involved in imparting resistance against *Phytophthora*. Real time PCR analysis of several genes identified through transcriptome sequencing showed that they were up regulated during *P. colubrinum -P. capsici* interaction. Before conducting real time PCR experiments stably expressing reference genes for normalization of real time PCR data in *P. colubrinum* was identified using the tool Ref Finder. Out of the seven house keeping genes investigated for finding the most stable one amongst them, actin was identified as the most stable gene for quantifying defense gene expression in *P. colubrinum -P. capsici* phytopatho system. For innate immune response during biotic stress conditions in plants, pathogenesis related proteins (PR proteins) are very important. Pathogenesis-related protein genes like PR1, PR6 (cysteine protease inhibitor), PR9 (peroxidase), osmotin (PR5), beta-1, 3-glucanse (PR2), defensin (PR12), thaumatin like protein (PR5) were identified through transcriptome sequencing. Real time PCR analysis showed that the PR protein genes like PR1, PR6 (cysteine protease inhibitor), PR9 (peroxidase) were up regulated in *P. colubrinum* upon challenge inoculation with *P. capsici*. Real time PCR analysis of pathogenesis related (PR) protein genes like beta-1, 3-glucanase, osmotin, defensin and thaumatin-like protein in *P. colubrinum* challenge inoculated with two isolates of *Phytophthora*, varying in their virulence showed that inoculation with highly virulent strain of pathogen caused subdued expression of osmotin, defensin and thaumatin like protein. Genes involved in defense signalling like receptor-like protein kinase (RLK), mitogen activated protein kinase (MAPK), allene oxide synthase, subtilisin-like proteases and enhanced disease susceptibility protein (EDS1) were identified from the transcriptome sequence data. Real time PCR analysis of these genes showed up regulation during the interaction and the genes, receptor-like kinase, subtilisin-like protease and enhanced disease susceptibility protein (EDS1) were found to be induced at early hours (4 hpi). Allene oxide synthase showed marginal increase in expression at 24 hpi. The gene MAPK was showing higher levels of expression at 72 hpi. Genes associated with hyper sensitive response like glutathione peroxidase, metacaspase, lipoxygenase, superoxide dismutase, catalase and nitrate reductase were also identified in the interactive transcriptome. Metacaspase, lipoxygenase and catalase were induced during early hours of inoculation at 4 hpi. Nitrate reductase was induced early at 8 hpi. The highest expression for nitrate reductase was observed at 72 hpi. Glutathione peroxidase was showing higher folds of expression (40-50 folds) throughout the interaction time period

(4 hpi to 72 hpi) investigated. Several genes involved in the biosynthesis of secondary metabolites having antimicrobial properties were investigated. The genes forming part of the phenyl propanoid pathway, phenylalanine ammonia lyase (PAL), cinnamoyl CoA reductase, cinnamate-4 hydroxylase and chorismate mutase were identified and analysed through real time PCR. The genes like chalcone isomerase, polyphenol oxidase, cysteine synthase were also studied for their expression during the interaction with *Phytophthora*. The genes, phenylalanine ammonia lyase (PAL) and cinnamoyl CoA reductase showed early induction at 4 hpi and cinnamate-4 hydroxylase gene showed higher levels of induction at 8 hpi. The genes like chorismate mutase showed higher folds of expression at 16 hpi. Genes like chalcone isomerase and cysteine synthase showed high level of expression during early hours of inoculation at 4 hpi and 8 hpi respectively and polyphenol oxidase showed higer level of expression up to 125 folds at 16 hpi. Expression analysis of *Phytophthora* specific genes like glycoside hydrolase, NPP1, RXLR and pectate lyase *in planta* showed that they were up regulated during the infection time course. Glycoside hydrolase is a cell wall degrading enzyme present in *Phytophthora* which helps in the destruction of cell wall of host during attack. NPP1 is a protein present in the pathogen which causes hypersensitive response and cell death in the host. RXLR is an effector present in *Phytophthora* which helps the pathogen to suppress host immunity. Pectate lyase present in the pathogen plays a critical role in pectin degradation in the host cell when the pathogen attacks the host. The study also gave important information about how the various pathogen specific genes are expressed in plants during host pathogen interaction. In another set of experiment, amplification, cloning and *in silico* prediction of full length elicitin genes was done. Elicitin perception by host plant resulted in the induction of systemic acquired resistance (SAR) which helps to protect the plant from subsequent pathogen attacks. Expression analysis and docking study of beta 1, 3 glucanase gene from *P. colubrinum* and glucanase inhibitor protein gene (GIP) from *P. capsici* was conducted to understand plant-pathogen interaction at molecular level. The docking studies proved that the interaction between the two proteins has distinctive features of a co-evolving molecular arms race between the pathogen and the host proteins. Further, Nuclear Magnetic Resonance spectroscopy (NMR) and other studies can be undertaken to better understand the molecular mechanism behind the interaction between beta-1, 3-glucanase from the host and GIP protein from the pathogen. The present study has resulted in improving our understanding about the large number of defense related

genes that are activated upon pathogen challenge. High levels of expression exhibited by the defense genes in the experiments may be correlated with *Phtytophthora* resistance characteristics of *Piper colubrinum*. Further functional characterization of these defense genes will be desirable. Proteomics studies will be useful for further validation of the present findings. Many transcription factors identified in the transcriptome of *P. colubrinum* can be studied further in detail to get the full picture on the regulation of defense related genes during pathogen challenge. *Phytophthora capsici* being a highly dynamic and destructive pathogen evolving continuously, constant crop improvement strategies are essential to enhance disease resistance in black pepper as well as for sustainable management of foot rot disease. Host basal defense responses involving defense genes identified in the study can help in the development of strategies for imparting resistance against *Phytophthora capsici* using transgenic approaches. Stacking of desirable genes contributing towards *Phytophthora* resistance could also be explored. Most of the defense related genes identified in *P. colubrinum* are also expected to be present in black pepper and comparative analysis for variations in the coding as well as control sequences of these genes in tolerant and susceptible black pepper accessions should provide a platform for identification of QTL's associated with tolerance to *Phytophthora*. The conserved effectors of *Phytophthora* are believed to play important roles in virulence and therefore form attractive targets to disable pathogenesis. Gene silencing approaches involving the use of RNA interference (RNAi) and targeting different virulence and effector genes of this dreaded pathogen in planta may render the crops resistant to *Phytophthora.* Absence of whole genome sequence data information remains as a bottleneck for identification of sequence variations in important genes, especially in the promoter and other control sequences. Genome editing should also be possibly exploited if useful variations are determined in specific genes contributing towards *Phytophthora* resistance.

9. Deciphering responses of black pepper (*Piper nigrum* L.) to endophytic colonization by *Pseudomonas putida* BP25 (TH 178)

Ms. V. N. Agisha/Mangalore University/2017/Guid: Dr. Santhosh J. Eapen

Endophytic colonization of *Pseudomonas putida* BP25 was studied using a gfp tagged strain. The gfp tagged strain aided in tracking and estimating the bacterial population precisely. *P. putida* BP25 was found to attain a high population density in the rhizoplane and root internal tissues indicating its colonization ability. The movement of the bacteria from the root to stem through the internal tissues was

confirmed by a highly sensitive Bio-PCR. An accurate and reliable real-time PCR. Method was also developed for quantifying *P. putida* BP25 in black pepper tissues. Use of a gfp tagged strain enabled the visualization and localization of the bacteria in black pepper by fluorescent microscopy. These findings on the endophytic behaviour of the bacterium would be an important biocontrol trait for sustained crop protection. Studies on the biocontrol mechanisms of *P. putida* BP25 revealed several unique properties of the strain. *P. putida* BP25 was found to produce siderophores and indole acetic acid related to plant growth promotion. The ability of the bacteria to produce volatile organic compounds (VOCs) was also revealed. VOCs such as 2,5- dimethyl pyrazine, methyl pyrazine, 2-ethyl 3,6-dimethyl pyrazine, 2-ethyl 5- methyl pyrazine and dimethyl trisulfide displayed inhibitory activity against a broad spectrum of pathogens such as *Phytophthora capsici, Rhizoctonia solani, Colletotrichum gloeosporioides, Athelia rolfsii, Gibberella moniliformis* and *Radopholus similis*. Among the compounds, dimethyl trisulfide exhibited inhibitory activity at very low concentrations. *In planta* assays on black pepper cut hosts against *Phytophthora* rot further confirmed the efficacy of pyrazine compounds as potential crop protection chemicals. It was also found that dimethyl trisulfide can be exploited as a soil fumigant against soil borne pathogens. The inhibiting potential of these VOCs might provide an alternative strategy for eco- friendly disease management in agriculture. In the present study, biochemical analyses were done and increase in phenol content, lignin and, defense related enzymes such as peroxidase, catalase and phenylalanine ammonia lyase were observed. This can be correlated to the induction of resistance in black pepper associated with *P. putida* colonization. The molecular responses were studied by constructing a cDNA library using a PCR based suppression subtractive hybridization (SSH) method which enabled the identification of differentially expressed transcripts in black pepper roots upon colonization by *P. putida* BP25. The transcripts involved in defense responses included pathogenesis related proteins such as PR-4 and PR-1; reactive oxygen species (ROS) scavenging catalase, superoxide dismutase and metallothionein; stress induced glutathione S-transferase, cytochrome P450 reductase, heat shock protein (HSP) 70 and dnaJ protein; 5-enolpyruvylshikimate-3-phosphate synthase involved in phenylpropanoid metabolism; (RS)-norcoclaurine 6-O-methyltransferase involved in alkaloid biosynthesis etc. The upregulation of selected transcripts were also validated by quantitative real time PCR. This differential expression of transcripts associated with effective activation of plant immune responses may confer broad spectrum

resistance to plant pathogens. It can be concluded that the strain, *P. putida* BP25R possess several beneficial biocontrol traits which helps in potentiating the plant health. Hence, it can be exploited as an efficient biocontrol agent for sustainable crop protection.

10. Investigation on mechanisms of *Phytophthora* resistance in black pepper (TH 176)

Ms. V. V. Vandana/Kannur University/2017/Guide: Dr. R. Suseela Bhai

The present study was taken up to investigate the defense mechanisms of the black pepper line 04-P24 against *P. capsici*. Three strategies of defense responses were studied *viz*. structural mechanisms, biochemical mechanisms and molecular mechanisms, in comparison with a *P. capsici* susceptible black pepper line Sreekara. The role of cell wall modification and cell membrane integrity in *P. capsici* resistant 04-P24 was analyzed in terms of membrane damage induced by pathogenin gress into the root cells. The extend of membrane damage induced will beproportional to the leakage of root exudates components. The conductivity was found significantly high in the water phase of inoculated plants of Sreekara from 3 DAI onwards when compared to 04-P24. In 04-P24, conductivity increased gradually and significantly from 4th day of inoculation but it remained lower than that of Sreekara on all these days. In both lines highest conductivity was observed on 8 DAI and it was around 2.2 fold high in root exudates of Sreekara compared to 04-P24. Exudation of carbohydrate showed increasing trend from 1st day onwards in both lines. Maximum level of carbohydrate exudation was observed on 7 DAI in both line and it was around 1.4 fold high in Sreekara compared to that of 04-P24. In both lines highest exudation of amino acids was noticed on 1 DAI and thereafter it started decreasing. Exudation of amino acids by roots of Sreekara was 2.2 times more than that observed in 04-P24. Exudation of total phenols, polyphenols and OD phenols also increased gradually in both lines. The increase was significantly high in Sreekara. On 8 DAI, total phenol exudations was 2.8 fold high in Sreekara compared to 04-P24 under inoculated condition. Polyphenols and OD phenols in the water phase of inoculated Sreekara were around 2.4 and 1.9 fold high respectively in comparison with its inoculated resistant counterpart. Highest increase in these factors was noticed on 8 DAI. The findings of this study led to the conclusion that higher cell membrane integrity and lignification of cell walls of xylem vessels and adjacent parenchyma cells contribute to the major structural defense response of 04-P24 root to *Phytophthora* infection. Increased membrane conductivity, lignin content,

OD phenols and higher activity of peroxidase enzyme in the root tissue of the resistant black pepper line 04-P24 indicates the possible HR response at the site of entry of *P. capsici*. This resistant line can very well be used in crop improvement studies either by conventional breeding or by genetic engineering to develop disease resistance in black pepper varieties. The phenotypic traits of the plant show medium sized spike with full bearing and bold berries which can also be used for cultivation. Though the yield is not so high, it will give moderate and sustainable yield under normal cultivation practices. For crop improvement, this can be used as a resistant root stock for grafting with high yielding susceptible varieties. So the present finding established the fact that 04-P24 actually imparts root resistance due to its structural, biochemical and genetic makeup.

11. Elimination of *Piper* yellow mottle virus through somatic embryogenesis in black pepper (*Piper nigrum* L.) (TH 175)

Ms. Shina Sasi/Mangalore University/2017/Guide: Dr. A. Ishwara Bhat

Piper yellow mottle virus (PYMoV) is known to infect black pepper (*Piper nigrum*) in India and other parts of the world. Analyses of the sequence in the conserved reverse transcriptase (RT)/ribonuclease H (RNase H) coding region of the virus using *Badnavirus* specific primers from ten virus isolates of black pepper collected from different cultivars representing different geographical regions showed an identity of 87−100% at the nucleotide and 93−100% at the amino acid level with PYMoV indicating that they are isolates of PYMoV. Phylogenetic analyses showed close clustering of all PYMoV isolates that were well separated from other known badnaviruses. PCR, loop mediated isothermal amplification (LAMP) and real-time LAMP assays were developed to detect episomal PYMoV in black pepper plants. It was found that primers specific to ORF II region of PYMoV can detect episomal form of PYMoV. Real-time LAMP assay was found 105 times more sensitive than PCR and 103 times than LAMP assay. Polyclonal antiserum to PYMoV was produced using *in vitro* expressed coat protein of PYMoV and was used to for the detection of PYMoV in ELISA and IC-PCR. Cyclic somatic embryo were produced from matured seeds obtained from PYMoV infected black pepper plants of six varieties namely IISR Malabar Excel, IISR Shakthi, IISR Thevam, Panniyur-1, Sreekara and Subhakara. Sucrose concentration required for the production and proliferation of cyclic somatic embryo were optimized for each variety. Regeneration of cyclic somatic embryo into plantlets of all varieties was achieved in SH liquid medium with 3.5% sucrose, transferred into woody plant medium (WPM) for rooting and finally hardened. Genetic

fidelity testing of somatic embryo-derived plants of all varieties with corresponding mother plants using six simple sequence repeats markers, showed genetic uniformity. Testing of somatic embryo-derived plants for PYMoV by PCR and real-time LAMP assay showed virus elimination in 55−100% of plants. A protocol for meristem–tip culture of black pepper was developed which consisted of excising meristem from PYMoV infected black pepper plant and inoculation in regeneration medium with antibiotics (to remove endophytic bacterial contamination) followed by rooting and hardening in the green house. Testing of meristem-derived plants showed PYMoV elimination in 84% of plants. Somatic embryogenesis and meristem culture when combined with antiviral treatment such as ribavirin eliminated PYMoV in all plants.

12. Structural and functional characterization of *Phytophthora* resistance genes in *Piper colubrinum* (TH 181)

Ms. Neema Malik/Kannur University/2017/Guide: Dr. K. Johnson George

Black pepper is the most traded spice around the world. Black pepper production is seriously affected by a number of diseases, of which *Phytophthora* foot rot caused by *P. capsici* is the most devastating. A few *Phytophthora* tolerant varieties of black pepper have been identified by screening studies but none of them exhibits true resistance to the infection. Hence, *P. colubrinum,* a wild relative of pepper that is completely immune to *P. capsici* and is the sole source of *Phytophthora* resistance in the genus *Piper*. Plants are equipped with two lines of immune response; MTI and ETI, which functions to eliminate the invading microbes and pathogens. MTI is a non-specific immune response exhibited by all plants of a given species (non host resistance) whereas ETI is a highly specific immune response seen in some but not all plants of a given species (host resistance). ETI is mediated by the R genes that are triggered by the effector molecule from pathogen. The R gene mediated immune response is induced only if the plant is equipped with complementary R genes against the specific effectors. R genes from plant are classified into different classes based on the conserved motifs present, of which NBS-LRR type is the most abundant. Though *P. colubrinum* is a promising source of resistance against *P. capsici*, studies on *P. colubrinum* R genes and R gene mediated immunity is limited. In this context, the present study was undertaken to understand the abundance and diversity of R genes in *P. colubrinum* and to identify and characterize the key R genes involved in *P. capsici* resistance. The first step towards studying the R genes in *P. colubrinum* is to mine the set of R genes expressed in the plant upon *P. capsici* inoculation. For this we have used the

transcriptome data generated from leaves of *P. colubrinum* challenge inoculated with *P. capsici*. Reverse alignment (tblastn) of amino acid sequence using 42 R genes from other plants against the *P. colubrinum* transcriptome and a blastx analysis of the transcriptome identified 1289 non redundant candidate R genes. These were further clustered to 91 homology clusters and classified to four major R gene classes; NBS-LRR, LRR-TrD, LRR-TrD-KIN and enzymatic R gene. NBS-LRR was identified to be the most abundant class in *P. colubrinum* followed by enzymatic R genes. To determine the key R genes involved in *Phytophthora* resistance, time course expression of these R genes upon pathogen inoculation has to be understood. For this we selected twelve candidate R genes based on their FPKM value, from R gene pool of *P. colubrinum.* Quantitative real time PCR based on SYBR green chemistry was used for relative expression studies. The accuracy and reliability of a real time PCR study depends on the stability of reference gene used in the experiment. As the commonly used reference genes exhibits expression variation during different experimental conditions, the reference gene/genes with the most stable expression in any given experiment have to be identified. Out of the eight candidate reference genes evaluated, ACT, ATUB and EIF3A were selected as the three most stable reference genes during *P. colubrinum* - *P. capsici* interaction using Ref Finder tool the relative expression profiling of the twelve selected R genes were analysed in *P. colubrinum* leaves challenged with two virulent *P. capsici* strain; 05-06 and 98-93 at different time post inoculation (0.5 hpi to 24 hpi). The expression of these genes were also analysed in unchallenged leaves and tissue culture raised plantlets. The upregulation of R genes in the initial hours during *Phytophthora* inoculation was observed, suggesting that resistance in *P. colubrinum* can be due to the faster on set of R gene mediated immune response. R1-1644 and R12-4018 genes (NBS-LRR) class and R2-1990 (LRR-TrD class) showed the maximum relative expression in the *P. colubrinum* on interaction with either of the *Phytophthora* isolates. The expression of all the twelve R gene transcripts were also observed in unchallenged tissue culture plants and greenhouse grown plants suggesting that the expression of R gene may not require a pathogen trigger as are constitutively expressed in *P. colubrinum* at basal level. The R genes, R1-1644 and R12-4018 were selected for further characterization. RACE amplification employing cDNA synthesis by template switching principle was employed to identify the full-length sequence of the two R genes. Using RACE PCR technique 3682 bp and 3145 bp sequence of R1-1644 and R12-4018 were obtained respectively. Further analysis revealed the presence of STOP

codons in the R12-4018 cDNA sequence suggesting it to be a truncated or improperly spliced R gene. The R1-1644 sequences had an ORF of 2742 bp coding for a CC-NBS-LRR type R protein. The ORF corresponding to a 913 aa sequence was flanked by a 727 bp 5'UTR region and a 218 bp 3' UTR. The deduced protein sequence of R1-1644 (further reffered to as PcR1) had a molecular weight of 103.86 kDa and theoretical pI of 8.75. The presence of four putative ORF and polyadenylation sequence were identified in the 5' and 3' UTR respectively. The deduced amino acid sequence of R1-1644 aligned with CC-NBS-LRR type R genes from other plants and had the conserved sequences corresponding to Pre P loop, P loop/Kinase 1a, TVS, Kinase 2a, RNBS B, GLPL and RNBS D in NBS domain and seven potential LRR repeats in the LRR domain. The 3D structure of PcR1 was determined by i-TASSER tool. It predicted an apaf1 like structure for the NBS domain having an ATP binding pocket, a horse shoe shaped structure for the LRR domain with a peptide binding pocket and a coiled coil structure for the CC domain. The effector binding capability of the LRR structure was analysed by structure based molecular docking of the protein binding LRR domain to RXLR type Avr3a11 from *P. capsici*. The Avr3a11molecule docked to the protein binding cleft of the LRR structure suggesting the role of the domain in pathogen perception. The functional role of PcR1 in *P. colubrinum* immune response can be validated by post transcriptional gene silencing technique. For *Agrobacterium* mediated transformation studies, a good plant regeneration system is to be available. The tissue culture media for direct organogenesis from leaf explants was optimised and the genetic fidelity of the tissue culture raised plants were confirmed by ISSR and RAPD markers. Half strength MS media (with macro and maicronutrients at half strength) supplemented with BA 2 mg/L NAA 0.01 mg/L was found promote direct organogenesis from leaf explants of *P. colubrinum.* Post transcriptional gene silencing mediated by RNAi is routinely used for functional characterization of genes in plants. The ihp construct targetting the PcR1 gene was prepared by restriction ligation method with 2x35S promoter and nos terminator sequence flanking the intron containing Hairpin (ihp). The construct may be further mobilised to plant transformation vector of choice and used for functional validation of PcR1 gene by *Agrobacterium* mediated post transcriptional gene silencing technique. The method optimised for direct organogenesis and the ihp construction can be adopted for the silencing study of any functional genes involved in *Phytophthora* resistance in *P. colubrinum.*Thus the study conducted gave an insight into the R gene mediated immune response in *P. colubrinum.* The expression of R genes in the plant

without pathogen trigger suggests that the plant may be equipped with the R gene pool which is in continuous surveillance for pathogen entry. The immediate up regulation of the R genes in the *P. colubrinum* system on pathogen entry may help the plant to combat the pathogen through faster HR response. The full length sequence and 3D structure of PcR1, a promising CC-NBS-LRR gene involved in *P. capsici* resistance was predicted. The structure based molecular docking experiments also suggest the direct involvement of the LRR region in pathogen perception through effector binding. This functional R gene may contribute towards the *P. capsici* resistance in *P. colubrinum.* Hence the study identified a promising R gene, which may be exploited in developing resistant variety of black pepper by genetic engineering technique.

13. Molecular characterization and tagging of resistance genes to *Phytophthora capsici* in black pepper (*Piper nigrum* L.) populations (TH 170)

Ms. Cissin Jose/Mangalore University/2016/Guide: Dr. K. Nirmal Babu

Black pepper (*Piper nigrum* L.) is a perennial climbing vine grown for its berries that are extensively used as a spice and in medicine. The drastic drop in the black pepper production in all growing countries has been attributed mainly for pronounced death of vines by the dreaded disease foot rot caused by *Phytophthora capsici*. Black pepper is a perennial and this makes conventional breeding more laborious and time consuming. Molecular approaches are greatly increasing our ability to characterize and manipulate many genes responsible for various characters, especially disease resistance genes. Molecular markers allow the dissection of mono genes and quantitative resistance and thus enhancing the effective deployment of resistance genes to provide more stable resistance and provides the opportunity for transgenic disease control strategies. The present study aimed at association mapping analysis among black pepper genotypes for *Phytophthora* resistance. The diverse population of black pepper were phenotyped with a virulent isolate of *P. capsici*. Molecular markers (ISSR and SSR) were employed for evaluating the genetic diversity and the population structure was determined. Association of markers with target trait was analysed. The success of endeavours to breed for resistant cultivars of ten depends on the extent of genetic variation present in the pathogen population. Hence genetic diversity of *Phytophthora* isolates from black pepper was also analysed. Each aspect is detailed in the succeeding chapters. Fifty seven genotypes along with resistant and susceptible controls were screened by leaf, shoot and root inoculation techniques with a virulent isolate, IISR-05-06. The periodical observations of leaf and shoot lesion were analysed

by calculating the Area Under the Disease Progress Curve (AUDPC). AUDPC analysis coupled with hierarchical clustering grouped the association mapping population in to four classes based on leaf and shoot lesions. The number of days taken for mortality was adopted as the criteria for rating the plants in case of root inoculation. The genotypes displayed a continuous range of resistance - susceptibilty levels. This also indicates that resistance to *P. capsici* in black pepper is a polygenic and quantitative trait. Seven major clusters were identified among the population and no two isolates were found to be similar. It is inferred that the *Phytophthora* isolates from black pepper are at rapid pace of evolution with high level of diversity among isolates. The population structure was analysed by STRUCTURE program and three sub-populations were identified. Association analyses between the markers and the disease resistance were performed based on the general linear model by using TASSEL software. A single SSR marker PN D10 (200 bp) was found to be associated with *Phytophthora* resistance at $P<0.01$.

14. Studies on seed transmission and genome sequencing of *Piper* yellow mottle virus infecting black pepper (*Piper nigrum* L.) (TH 169)

Ms. K.P. Dheeshma/Kannur University/2016/Guide: Dr. A. Ishwara Bhat

Piper yellow mottle virus (PYMoV) is the *Badnavirus* infecting black pepper plants in India and other black pepper growing countries of the world. The stunted disease caused by PYMoV, sometimes in association with Cucumber mosaic virus (CMV) is the third important disease affecting black pepper in India. Besides black pepper, PYMoV also infects many other related species including betelvine and Indian long pepper. PYMoV is transmitted primarily through vegetative means (stem cuttings); while secondary spread occur horizontally through various species of mealybugs in the field and vertically through seeds. PYMoV has bacilliform shaped virions measuring ~30 x 130 -150 nm and a circular covalently closed double stranded DNA genome of about 7.5 kb size potentially encoding four open reading frames (ORFs). A few badnaviruses, which integrate their genome into host genome, are called as endogenous badnaviruses. The objectives of the present study included seed and pollen transmission of PYMoV, complete genome sequencing of PYMoV and studies on occurrence of endogenous PYMoV sequences in the black pepper genome. The different parts of black pepper berries like embryo, endosperm and perisperm from both PYMoV infected and healthy black pepper plants were separately tested for presence of the virus by Polymerase chain reaction (PCR). The PCR results confirmed presence of the virus in all the parts of black pepper berries tested including embryo and thus

confirming the true seed transmission of PYMoV in black pepper. Different sets of virus specific primers amplified specific regions of PYMoV genome in PCR with berries from varieties- Sreekara, IISR-Thevam and Panniyur 1. The study enabled detection of PYMoV for the first time in pollen grains and anthers collected from the virus infected black pepper plants. The pollen transmission studies were conducted to test whether virus transmission could take place through pollen. The berries and seedlings generated by cross pollinating infected pollen grains to stigma of healthy plants were positive for PYMoV when DNA was used as template. However same berries and seedlings showed negative results for PYMoV transcripts when tested by reverse transcription (RT)-PCR using primers specific to different ORFs of the virus. Thus further tests to determine the occurrence of PYMoV through electron microscopy (EM), enzyme-linked immunosorbent assay (ELISA), immunocapture PCR (IC-PCR) or rolling circle amplification (RCA) in the cross pollinated berries and seedlings are needed to confirm the pollen transmission of PYMoV in black pepper. Complete genome sequencing of PYMoV isolates from black pepper, betelvine and Indian long pepper was done to study the genetic diversity among the isolates and this is the first report of complete genome sequencing of PYMoV from betelvine and Indian long pepper. Genome length of PYMoV isolates from black pepper (PYMoV–PN– P1), betelvine (PYMoV–PB) and Indian long pepper (PYMoV–PL) were 7584, 7559 and 7580 bp respectively with four ORFs each in all the isolates. The length of ORFs of each of the isolates was- ORF 1 (408 nt and 135 aa), ORF 2 (465 nt and 154 aa), ORF 3 (5778 nt and 1925 aa) and ORF 4 (459 nt and 152 aa). The variability in the genome length of three isolates was only due to the variable length of intergenic region (IR). The PYMoV isolates from the present study showed an identity of 89–99% with the one already reported complete genome sequence of PYMoV from black pepper while, identity with other badnaviruses varied from 42–55%. Further, identities of 90–99% (in the nucleotide) and 94–99% (in the amino acid) in ORF 3 and 92–94% (in the nucleotide) and 94–98% (in the amino acid) in the reverse transcriptase (RT)/RNase H region were observed among PYMoV isolates. While, PYMoV isolates showed identities of only 44–58% and 31–54% in the ORF 3 and 59–67% and 14–59% in the RT/RNase H region with other badnaviruses. The above results clearly indicate that all three isolates sequenced in the present study are strains of PYMoV. Genome organisation of all three sequences followed similar pattern as reported for the reference PYMoV genome. The phylogenetic analysis based on complete genome, ORF 3 and RT/RNase H region of badnaviruses revealed close

clustering of all three PYMoV isolates with the already reported PYMoV isolate. The conserved amino acid motifs identified among the four isolates shared 99% identity. Conserved amino acid motifs identified from the ORFs of badnaviruses including PYMoV showed that ORF 3 is more conserved with a maximum of eight conserved sequences in the RT–LTR region. The 18 nucleotide consensus tRN A meth binding site in the IR of badnaviruses were highly conserved [5'TGGTATCAGAGCA(T/G) T(A/G) G(T/A) GT(A)T(G/A)–3'] among PYMoV isolates and other badnviruses with 100% identity among the first 12 nucleotides. Overall, the results of the present study clearly confirmed that the PYMoV isolates from black pepper, betelvine and Indian long pepper are strains of the PYMoV. Black pepper plants that tested positive in PCR for PYMoV, when tested by RT- PCR using different sets of primers targeted to different regions of the virus genome showed absence of PYMoV transcripts in a few plants indicating that they may harbour ePYMoV sequences. In order to confirm the occurrence of ePYMoV, these RT-PCR negative plant samples, which were positive for PYMoV in DNA PCR, were further tested by Southern hybridisation analysis. DNA isolated from the [PCR (+) and RT–PCR (+)] and [PCR (+) and RT–PCR (-)] plants of variety Panniyur 1 were digested using EcoRI restriction enzyme (which has a single restriction site on PYMoV genome), separated on agarose gel, blotted onto a nylon membrane and hybridised with DIG- labelled PYMoV specific probe. The results of Southern hybridisation showed multiple bands ranging from 250 bp to 20 kb in addition to the expected band of 7.5 kb (representing episomal PYMoV DNA) indicating the occurrence of ePYMoV. Similar kind of results were obtained when the DNA samples were restricted with different enzymes and blotted with PYMoV specific probes targeted to different regions (ORF 1, 2, 3, 4 and IR) of the virus thus confirming the occurrence of PYMoV in black pepper. The [PCR (+) and RT–PCR (+)] plants harbour PYMoV and ePYMoV whereas, the [PCR (+) and RT–PCR (–)] contained either ePYMoV alone or both PYMoV and ePYMoV. Other black pepper varieties tested (IISR-Thevam and Sreekara) also showed occurrence of ePYMoV. The number of hybridised fragments obtained was more when RT-LTR (in ORF 3) of PYMoV was used as a probe, which probably indicates that multiple copies of this region is integrated compared to other region. The study confirmed the presence ePYMoV sequences in black pepper genome for the first time and further studies are needed to analyse whether activation of ePYMoV sequences to infectious episomal form is taking place in black pepper.

15. *In silico* screening of phytochemicals in black pepper (*Piper nigrum* L.) and long pepper (*Piper longum* L.) for potential pharmacological activities and heir experimental validation (TH 167)

Mr. A. Riju/Kannur University/2016/Guide:Dr. Shamina Azeez

This study was intended to screen phytochemicals from two *Piper* species namely *Piper nigrum* L. and *Piper longum* L. for their drug likeness, favorable ADME, biological activity, and no toxicity, so as to process further as pharmacologically active lead compounds. The study used phytochemical databases and literatures as a starting point to collect phytochemical information and structural information of 163 compounds. Here we mainly relied on predictions which are useful decision support tools in biological research. This new methodology can be employed as a decision support tool in predicting toxicity within appropriate safety and risk analysis paradigms in phytochemicals, by evaluating carcinogenic risk in humans, so that priorities for further testing may be set. Using PASS prediction server, biological activity spectram of phytochemicals from black pepper and long pepper have been found. Predicted activities includes known biological activity/effect and novel. Particularly compounds such as alpha-bisabolol, cis-nerolidol, gamma-eudesmol and linalool have predicted as anthelmintic. GST (Glutathione S-transferase(s)) enzyme from *Wuchereria bancrofti* has been exploited as a target in Lymphatic filariasis therapeutics, the inhibition of which leads to a reduction in worm motility. The potential of predicted biological activity docking studies to predict the inhibitory property of phytochemicals (ligands) in *Piper nigrum* and *Piper longum* against *W. bancrofti* GST (PDB ID: 5D73), has been amply demonstrated in this work *In vitro.* studies have been carried out to verify the predicted activity using GST assay. All the compounds selected by molecular docking studies inhibited the GST enzyme, this observation sufficiently validates the *in-silico* study. The GST activity in the enzyme extract was measured according to the method of Habig et al. (1974). Anthelmintic activity as confirmed by *in vitro* GST inhibiting analysis, revealed that alpha- bisabolol, cis-nerolidol and linalool were toxic to the nematode when exposed. In brief, the current approach of combining *in silico* and *in vitro* studies have revealed novel biological activity - anthelminthic potential - of *Piper* secondary metabolites compounds. The study could provide additional inhibitors for the development of antifilarial drug development and new insights into the biological activity spectrum of potential secondary metabolites as elucidated by *in silico* studies. It cannot be emphasized enough that this approach is significantly economical but the computational model using existing experimental data for predicting biological activity

and ADME/Tox properties obviously consumes less time, less resources in the form of chemicals and experimental animals. The results of the present study demonstrated that the investigated compounds are biologically active molecules and will produce the physiological actions by multiple mechanisms after interacting with six most important drug classes such as GPCR ligands, nuclear receptor ligands, kinase inhibitor, ion channel, protease inhibitor and another enzymes inhibitor. It can be concluded that synthetic or semi synthetic analogs of these compounds can be designed and evaluated for their pharmacological activity as they are expected to be orally and therapeutically active. The result of the present study suggests that most biological activities of the major phytoconstituents are yet to be studied for their possible pleiotropic pharmacological potential for human health welfare. The PASSCOM database developed as part of this study will remain a reference point for researchers looking for biological activity of natural compounds.

16. Isolation and cloning of disease resistance gene candidates using degenerate primers from NBS region in black pepper and related *Piper* species (TH 166)

Ms. E. J. Suraby/Mangalore University/2016/Guide: Dr. K. Nirmal Babu

As an attempt towards mining R gene space of the black pepper genome, degenerate approach based on conserved motifs of known R genes were used to amplify resistance gene analogs in moderately resistant and susceptible genotypes of black pepper. In order to find cross species resistance gene analogs, wild *Piper* spp., *P. colubrinum* completely resistant to *Phytophthora* infections and *P. ornatum* were used. A total of 53 RGA from *Piper* including 33 from *Piper nigrum* (PnRGA), 10 from *P. colubrinum* (PcRGA) and 10 from *P. ornatum* (PoRGA) were isolated. The identified *Piper* RGAs were classified under non-TIR R gene class on the basis of tryptophan residue in the kinase 2a motif of NBS domain. Conserved domains characteristics of NBS-LRR R proteins were present along 53 *Piper* RGAs. For immediate functional validation, expression analysis after challenging with *Phytophthora* was employed and it is observed that *Piper nigrum* RGAs are differentially expressed in black pepper varieties and *P. colubrinum*. Basal level expression of *Piper* RGA and its early induction following *P. capsici* challenge were observed. Salicylic acid induced expression of PnRGA transcripts in black pepper genotypes. Foliar spray of salicylic acid is a method of choice towards protecting high yielding but foot rot susceptible black pepper genotypes. Further studies aiming at transgenic complementation or gene silencing are required to confirm the functionality of *Piper* RGA isolated in this study. A

cDNA library with expressed transcripts in moderately resistant variety, 04-P24-1 can be employed for the studies on molecular basis of black pepper- *P. capsici* interactions.

17. Molecular basis of pathogenic variability in *Phytophthora capsici* on black pepper (*Piper nigrum* L.) (TH 165)

Ms. K. B. Vinitha/Mangalore University/2016/Guide: Dr. M. Anandaraj

The genus *Phytophthora* shows considerable variation in virulence among the isolates of a species. The lack of knowledge on the virulent races of the pathogen and their infection mechanism are the gaps in developing a successful management strategy. Thus, this study was focussed on understanding the basis of the pathogenic variability in *P. capsici* infecting black pepper. Surveys conducted in the years 2009 and 2010, in 24 plantations in Karnataka and 65 in Kerala showed the incidence of *Phytophthora* disease in black pepper. Thirty-one new *Phytophthora* isolates were isolated from the samples collected. Majority of these isolates (63%) were isolated from soil, indicating the possibility for a large disease outbreak in future. Virulence studies of the isolates on the black pepper variety, Sreekara, susceptible to *Phytophthora* showed much variation among them. Based on this the isolates were grouped into non-virulent, less virulent, moderately virulent and highly virulent. Efforts were done to correlate virulence with fungicide sensitivity, morphology or geographical location of the isolate, but no correlation was found with any of these characters studied. To understand the molecular basis of this pathogenic variability, the differential expression in black pepper variety, 'Sreekara' infected with highly virulent (05-06) and less virulent (98-49) strains at different time intervals were studied. The *Phytophthora* isolates showed stage specific expression of various effectors and other proteins. The highly virulent isolate, 05-06 was found to be in biotrophic stage at 8 hpi and showed higher expression of various effectors. By 24 hpi, the isolate was in a necrotrophic stage. In the case of the less virulent isolate, 98-49, at 8 hpi, the isolate was in biotrophic stage and at 24 hpi it was in a stage of transition between biotrophic and necrotrophic stages. By 48 hpi it was in a complete necrotrohic stage. Thus, it is to be concluded that the over expression of various effector proteins and their tight temporal expression during the process of infection is the cause for the variation in virulence of the *P. capsici* isolates from black pepper.

18. Chemoprofiling and antioxidant potential of selected *Piper* species (TH 174)

Ms. D. Sruthi/University of Calicut/2016/Guide: Dr. T. John Zachariah

Piper is the most representative genus among Piperaceae family. The *piper* species have great economical, commercial and medicinal importance. However, many of the members of this genus are still under explored for their diverse therapeutic potential and chemical profile. Thus, *piper* species have assumed great significance in the field of biological research and hence, four medicinally valued piper species *viz*., *Piper nigrum* L., *Piper longum* L., *Piper chaba* Hunter. And *Piper colubrinum* Link. were selected for the proposed study. *P. nigrum* (Black Pepper) is the most important and most widely consumed spice in the world. Black pepper of commerce is the dried and matured berries of *P. nigrum*. It provides physiological benefits and prevent chronic ailment in addition to the fundamental nutrition. *P. longum* (Long Pepper) and *P. chaba* (Java long pepper) are mainly cultivated for its fruit and are the major components of traditional systems of medicines due to their diverse medicinal potential. *P. colubrinum* is an exotic *Piper* species known for its resistance to plant pathogens like *Phytophthora capsici*, *Radopholus similis* and *Meloidogyne incognita*. Detailed study is lacking with regard to chemical and pharmacological diversity among black pepper varieties. Likewise, systematic study for intrinsic quality of black pepper in relation to different locations has not been observed. Furthermore, information is scanty regarding the correlation between chemical constituents of black pepper berries. Even though high value compounds with significant importance in human life has been identified from *P. longum*, many of its phytochemicals and therapeutic potential are still unexplored. Likewise, *P. chaba* has not received a deserved pharmacological importance due to the dearth in the information regarding their detailed chemical profile and medicinal values. On the phytochemical and medicinal point of view, the studies on *P. colubrinum* are also scanty. Oxidative stress and related diseases, mainly cancer, are dangerous to human health. In the modern research, there is great inducement to discover antioxidant and anticancer compounds from plants due to their medicinal potential and fewer side-effects compared to synthetic drugs. However, many of the plants are still under explored with regard to their chemical profile, antioxidant and anticancer properties. *P. longum, P. chaba* and *P. colubrinum* are few among such plant species which require much more attention in these aspects.

19. Development of microsatellite markers for black pepper (*Piper nigrum* L.) and related species (TH 163)

Ms. K. Anupama/University of Calicut /2015/Guide: Dr. K. Nirmal Babu

Black pepper, the supreme among the spices is the most widely used spice in the world. The black pepper of commerce is the dried mature fruits of the tropical perennial climbing vine *Piper nigrum* L. In genus *Piper*, the role of phylogeny is particularly significant, as major break throughs in varietal evolution have been achieved through the conventional breeding. However, due to reduced floral structures, morphological characterization is only partially effective in estimations of diversity in *Piper*. Molecular markers being independent of environment conditions have come up as an effective tool for characterization of genetic material. DNA markers can augment phenotypic evaluation in estimation of the genetic variability between species more efficiently and they can be particularly useful in resolving complex phylogenetic problems. Among the DNA markers, microsatellites are the most frequently used molecular markers in genetic diversity analysis due to its multiallelic, codominant and high genome coverage. In this study three approaches were used for analysing inter relationships among *Piper* species, varieties and cultivars. They include SSR developed from genomic libraries enriched for SSRs (genomic SSRs), from the EST sequences deposited in the public domain (EST-SSRs) and SSRs reported earlier. Thirty nine black pepper genotypes including 23 cultivars and 16 released varieties from diverse geographical locations of Kerala and 21 *Piper* species including Western Ghats, North East and Exotic species maintained at IISR Experimental Farm, Peruvannamuzhi were used for genetic diversity analysis and cross-species amplification studies, respectively.

20. Development of biocontrol consortia for tissue cultured black pepper (*Piper nigrum* L.) plants (TH 161)

Mr. M.C. Sibi/Mangalore University/2013/Guide: Dr. M. Anandaraj

This thesis was done with an aim to develop a bio control consortium for tissue cultured black pepper plants for their better survival and enhanced growth during acclimatization and in the field. In recent years, the productivity of black pepper is severely declined due to the major root pathogens of black pepper namely *Phytophthora capsici, Radopholus similis* and *Meloidogyne incognita*. Biocontrol agents are widely used for the control of these diseases in the nursery and field. In this study, the bioagents, which have been proved as efficient in promoting the growth of

black pepper and suppressing the major root pathogens, were used. The microorganisms used in this study were nine PGPR strains, one antagonistic fungus, *Trichoderma harzianum* and a vesicular and arbuscular mycorrhiza, *Glomus fasciculatum*. In this particular study, various aspects such as characterization and identification of PGPR strains, optimization of biotic and abiotic conditions for hardening of micropropagated black pepper plants, nutrient uptake in black pepper microplants due to the inoculation with beneficial organisms at the time of *ex vitro* hardening compatibility studies among PGPR strains and a pot culture study to develop a biocontrol consortium for black pepper using PGPR strains and *T. harzianum* were carried out.

21. Biochemical and molecular characterization of black pepper (*Piper nigrum* L.) pericarp degrading bacteria (TH 160)

Mr. V. Vinod/Achariya Nagarjuna University/2011/Guide: Dr. A. Kumar

White pepper is one of the value-added products of black pepper (*Piper nigrum* L) produced by the decortications of ripened, matured green and even black pepper itself. An improved fermentation method using specific black pepper associated bacterium for producing high quality off-odour free white pepper from matured green pepper (*Piper nigrum* L) berries for 5-7 days. In the current study, forty five bacteria isolated from black pepper and black pepper associated environment for decorating black pepper pericarp was evaluated for their decortications efficacy. Among them eight were found to be efficient for conversion of black pepper into white pepper with > 60% conversion and matured green pepper to white pepper with 100% conversion. The fermentation technology yielded 22-26% recovery of white pepper. REP PCR (the three linearly combined fermentation patterns of BOX, ERIC and REP PCR) and ARDRA patterns revealed that the strains encompassed a high degree of genetic variability. The short listed strains were identified based on biochemical tools and comparison of 16s rDNA sequences as *microbacterium barkeri* IISRWP259 (MTCC5404), *Bacillus subtilis* IISRWP33 (MTCC5405), *Bacillus subtilis* IISRWP36 (MTCC5406), *Bacillus subtilis* IISRWP38 (MTCC5407), *Bacillus licheninforms* IISRWP43 (MTCC5408), *Klebsiella pneumonia* (IISR WP19), *Acinetobacter baumanii* (IISRWP35) and *Acinetobacter* (IISRWP26). The sequence has been submitted in NCBI GenBank. The effective five bacterial species were deposited with international depositary authority at MTCC in IMTECH, Chandigarh as long term patent deposit as per the guidelines of Budapest treaty for microbial deposition. The details of the microbes together with

source of their isolation have been informed to National Biodiversity Authority (NBA), Chennai. An agreement has been signed with NBA after obtaining approval for the use of biological material for value addition black pepper. A fermentation technology has been standardized for the production of white pepper from matured green pepper by exploiting these microbial resources. The creamy white pepper berries thus obtained from the fermentation and subsequent drying under sunlight for three days was found to have superior physical parameters such as colour, texture and appearance. Comparison of physical substances for the spicy quality of white pepper like oleoresin, piperine content. Interestingly the white pepper was free from off odour compound, especially skatole and superior in other biochemical contents. Microbiological standards in particular, play an important role in determining the quality of spices and their trade. The post fermentation drying of white pepper in oven at 55°C was highly inadequate for the total elimination of bacterial flora pre existing on the berries although such a heat treatment was enough to completely eliminate the fungi. However the residual microbial load found to be well below the acceptable level as suggested by American Spice Trade association (ASTA) and International Commission on Microbiological Specifications for Foods (ICMSF). PCR based methodology for specific detection of *Bacillus licheniformis* (MTCC 5408) was developed by exploiting the strain specific sequences found in REP-PCR based DNA Profiling. Enzymatic decortation of pepper is an effective method for the white pepper production. Pectinase is most effective for the degradation of the pepper pericarp. An exo-polygalacturonase produced by *Bacillus licheniformis* (MTCC 5408) have been purified by ammonium sulphate precipitation, ion exchange chromatography and gel filteration. The enzyme was optimally active at pH 8.0 and 60°C with an apparent molecular weight of 40 KDa. The kinetic properties and stability and activity of the enzyme at high temperature (60%) and pH (8.0) may also be a useful attribute to utilize when applied as an alternative commercial preparation for enzymatic decortations for the white pepper production. In conclusion an improved fermentation method was developed for decortications of green pepper for eventual white pepper production using specific bacterium which yielded high quality off odour free white pepper.

22. Development and formulation of effective biofertilizers for management of black pepper and cardamom (TH 149)

Mr. K.P. Sangeeth/Mangalore University/2011/Guide: Dr. R. Suseela Bhai

The main aim of the entire work was to develop a stable, eco friendly and easy to carry formulation with consortia of indigenous biofertilizers and the attempt is a new area as far as black pepper and cardamom nutrition is concerned. Study led to the development of a consortium of efficient indigenous biofertilizers holding N fixing, P and K solubilizing bacteria encapsulated in sodium alginate beads as a formulation specifically for black pepper and cardamom nursery and standardized the organic substrate which can release the organisms easily when introduced into the rhizosphere. Therefore, it is important to identify the effective indigenous strains of beneficial microorganisms for a cropping situation, based on their compatibility and combined efficacy, both *in vitro* and *in vivo* and employ consortia of microorganisms for efficient management and production to promote plant growth and soil health. Further research is required to study the performance of these efficient isolates in the form of consortia in combination with other inputs to improve growth of plants under various field conditions.

23. *Agrobacterium* mediated transformation of black pepper using sequences from cucumber mosaic virus and *Piper* yellow mottle virus (TH 146)

Ms. Jiby Mary Varghese/Mangalore University/2011/Guide:Dr. A. Ishwara Bhat

Present study was conducted with the objectives of standardization of an *Agrobacterium* mediated transformation protocol for black pepper and production of transgenic black pepper using the sequences of a portion of open reading frame III of *Piper* yellow mottle virus in sense and antisense orientation and Cucumber mosaic virus coat protein in sense orientation. Among different ex plants such as leaf, internode, petiole, embryo along with surrounding micropylar tissue and embryogenic mass, embryogenic mass was identified as a suitable explant for transformation of black pepper. Leaf, internode and petiole explants. Although produced callus, failed to regenerate in different media with varying levels of hormones tested. Although embryo along with surrounding micropylar tissue was able to produce primary somatic embryo and plant lets, in the presence of antibiotics (commonly used for *Agrobacterium* mediated transformation) their regeneration efficiency was reduced drastically. Embryogenic mass was established by culturing scooped out embryo along with surrounding micropylar tissue on SH medium containing 3% sucrose under darkness. Primary somatic embryos were seen after 90 days. The brownish yellow tissue at the

root pole of the primary embryo proliferated into a small mass of tissue from which several secondary embryos merged. Embryogenic mass was maintained by sub culturing in to fresh medium of composition at monthly interval. Embryogenic mass was converted in to fully developed plantlets by transferring proliferated embryogenic mass to basal SI-I (liquid) medium with 3% sucrose followed by transfer to woody plant medium with 3% source 0.8% agar and charcoal. About 300 – 400 plantlets were obtained from embryogenic mass within 60 days. Genetic fidelity of embryogenic mass derived plants was tested using random amplified polymorphic DNA (RAPD) markers. A total of 150 scorable able bands were analysed by 23 RAPD primers with an average of 6.52 band per primer. All the 150 markers were monomorphic across all the plants tested indicating that there was no genetic variation among the progeny and the parent plant from which they were originated. Effect of commonly used antibiotics in *Agrobacterium* mediated transformation on embryogenic mass was studied. Out of two bactericidal antibiotics, cefotaxime and carbenicillin tested at different concentrations, cefotaxime at 100 g/mL enhanced somatic embryo prefractionation and was also able to control *Agrobacterium* over growth and hence this concentration of cefotaxime was used in all further experiments. In the case of selective antibiotic kanamycin, 200 ~g/mL gave complete selection of non transformed plants both at embryogenic mass and at plantlet stage. But presuming that such a high concentration of kanamycin at the initial stage itself may cause death of transformed tissue, the following strategy for selection of transform ants was adopted. Embryogenic mass after co cultivation with *Agrobacterium* in the regeneration medium were subjected to selection in three stages with increasing concentration of antibiotic: initially at 25 g/mL for 3-5 weeks followed by 50 g/mL for next 30 days. At plantlet stage selection was carried out at 100 g/mL of kanamycin the fully developed putative transformed plantlets with roots were hardened and established under greenhouse conditions. For confirmation of transgenicity of putative transformant's PCR, dot blot and southern hybridization was conducted. In the case of PYMoV sense construct, 100% and in the case of PYMoV antisense construct, 79.5% of plants tested were positive in pe R. Dot blot test showed 80 and 86% of plants as positive in sense and antisense construct of PYMoV respectively, Similarly Southern analysis showed 20 and 57% of plants as positive in sense and antisense constructs respectively. For plants with CMV sense construct 95, 53.7 and 100% of tested plants were positive in PCR, dot blot and southern hybridization respectively. All the hardened plants in the greenhouse exhibited normal growth and morphology indicating that the integration of viral sequences did not affect normal development and morphology.

24. Studies on endophytic colonization of bacteria in black pepper (*Piper nigrum* L.) roots against *Phytophthora capsici* and *Radopholus similis* (TH 139)

Mr. R. Aravind/Mangalore University/2009/Guide: Dr. A. Kumar

The salient achievements of the thesis project on Studies on endophytic colonization of bacteria in black pepper (*Piper nigrum* L.) roots against *Phytophthora capsici* and *Radapholus similis* are furnished below Endophytic bacterial strains isolated from root, stem and leaves of apparently healthy black pepper vine were characterized and identified upto the species level based on morphological and biochemical keys. A total nine genera were found, among them *Bacillus* spp. followed by *Pseudomonas* spp. dominated the collection. The diversity of bacterial communities was further confirmed by Amplified Restriction Digestion of Ribosomal DNA Analysis (ARDRA) as well as Repetitive Extragenic Palindromic DNA -PCR (REP-PCR). Multiple screening assays for identification of antagonists culminated in six endophytes such as IISRBP 17, IISRBP 2S, IISRBP 35, IISRBP 71, IISRBP 104 and IISRTC 10 based on suppression of *P. capsici* & *R. similis* and their in black pepper. These isolates shared very high 16S rDNA sequence homology (99%) with *Bacillus megaterium* (IISRBP 17). *Pseudomonas Putida* (IISRBP 25), *Pseudomonas aeruginosa* (IISRBP 35), *Bacillus pumilus* (IISRBP 71), *Bacillus cereus* (IISRBP 104) and *Curtabacterium luteum* (IISRTC 10). 16S rDNA sequence based primers which were found to yield amplicon sizes 708 bp, 426 bp, S74 bp and 350 bp for *B. megaterium*, *P. putida*, *P. aeruginosa* and *C. luteum*, respectively was designed for detection of isolates in black pepper tissues. Similar pattern was observed in greenhouse grown rooted cuttings of black pepper. *Pseudomonas aeruginosa* colonized the root and aerial plant parts such as stem and leaf whereas the other endophyte *P. putida, B. megaterium*, and *C. luteum* was found only in root and stem. *Pseudomonas aeruginosa* could be tracked in stem and leaf in one to two weeks whereas other endophytes could be traced in the aerial parts in two to three weeks. In general, the population level was more in roots than stem and leaf tissues. These promising antagonistic endophytic bacteria were exploited to produce disease-free rooted cuttings of black pepper by pre-plant root and stem bacterization with endophytic bacteria. The pre-plant root bacterization with *P. aeruginosa*, *P. putida* and *B. megaterium* suppressed *P. capsici* in rooted cuttings of black pepper by 60% whereas *C. luteum* and *B. megaterium* reduced over 70% of the nematode population in soil. Besides protecting the plants from the pathogens, the bacteria were found to enhance the growth of rooted cuttings. The promising results obtained with the short-listed four endophytic

bacteria in black pepper stem and rooted cuttings provide a strong basis to further develop these bacterial strains as potential bioagents to manage foot rot and slow decline diseases of black pepper under field conditions. Further studies are warranted to understand the mode of action of these bacteria. The present study is the first report on exploitation of endophytic bacteria against *P. capsici* and *R. similis*, two major soil-borne pathogens of black pepper. An observation on suppression of *R. similis* by *C. luteum* is being reported for the first time.

25. Studies on bacterial antagonists of plant parasitic nematodes of black pepper (*Piper nigrum* L.) (TH 130)

Ms. B. Beena/University of Calicut/2008/

Guide: Dr. K.V. Ramana, Co-Guide: Dr. M. Anandaraj

Altogether 190 soil and root samples were collected from black pepper and nematode antagonistic plants like *Chromolena odorata* (Eupatorium), *Stryichnos nuxvomica* (nux-vomica), *Pimenta dioica* (allspice), *Piper colubrinum* (wild species) and from highly nematode susceptible plant *Coleus blumeri* (coleus) and processed using standard techniques. Isolation of bacteria was carried out from the soil and root samples and various life stages of root knot nematodes. The efficiency of the bacterial strains in suppressing nematodes was evaluated by conducting a number of *in vitro* experiments. Suppressive action of the bacterial isolates on *M. incognita* and *R. similis* was assessed in these experiments. Bioefficacy of 108 bacterial isolates for suppressing *M. incognita* was evaluated *in vitro* by different methods. In the buffer method, the mortality of nematodes was comparatively less. Out of 108 bacterial isolates tested, only II bacterial isolates caused nematode mortality more than fifty per cent, maximum being the treatment with bacterial isolate Is. No. 110 (87.83 %) followed by is. No. 113 (84.63%) and Is. 119 (66.38%). The direct action of bacterial cells was also evaluated by adding nematodes directly to the bacterial cell suspension in peptone water. more than 90 per cent of bacterial isolates caused very high mortality (>90 %) to nematodes in peptone water. The mortality ranged from 32.05% (Is. No. 114) to 98.03 % (Is. 109) and maximum nematode suppression was obtained by the treatment of bacterial isolate is no. 109 (98.03%) followed by C25 (97.98%). Cell free culture filtrates (CFCF) of 107 bacterial isolates were also evaluated by assaying the mortality of root-knot nematode juveniles and the mortality was very high in culture filtrates too. A total of 216 bacterial isolates were evaluated in green house including the 108 bacterial isolates tested *in vitro,* in a series of 11 experiments using tomato as the test plant in heat treated soil.

Based on the efficiency to suppress nematodes and to enhance growth of tomato, 25 bacterial isolates were short listed and further evaluated for their mode of action on nematodes. An attempt was made mainly for the identification of *Pseudomonas* and *Bacillus* strains based on the morphological and biochemical reactions. Out of the 319 bacteria isolated, 77 were tentatively identified as *Pseudomonas* spp. 55 as *Bacillus* spp. and 187 bacterial isolates could not be identified. Out of the 70 bacterial isolates directly isolated from soil, 25 belonged to *Pseudomonas* spp. and seven were *Bacillus* spp. Sixteen bacteria isolated through the baiting of alginate prills yielded a total of 16 bacteria, with 6 and 2 *Pseudomonas* spp. and *Bacillus* spp., respectively. Out of 181 bacteria from roots of black pepper, coleus and nematode antagonistic plants, 37 were *Pseudomonas* spp. and 35 were *Bacillus* spp. Twenty five short listed bacterial isolates were evaluated for the root knot nematode egg hatching suppression and all the isolates significantly suppressed the hatching of *M. incognita* eggs though none of them caused 100 per cent hatching suppression. There was no sign of egg parasitism by any of the bacterial strains. BI4 (87.89%) and B15 (91.66%) recorded maximum hatching suppression. The most effective strains of bacteria in nematode suppression in this study are Is.113 (*Bacillus megaterium*), Is. 119 (*Comamonas testosterone*), Is. 136 (*Alcaligens*) Ap4, (*Pseudomonas aeruginosa*) and Apl6 (*Pseudomonas* sp). These isolates are proven to be effective in both in the greenhouse and laboratory studies and also posses multifarious mode of action on other pathogens of black pepper. They differed in their mode of action towards nematodes. The *P. aeruginosa* isolate (Ap4) in the *in vitro* studies resulted in 93.60%, 34.10% and 95.15 % mortality of *Meloidogyne* in peptone broth, phosphate buffer and in culture filtrate respectively. This isolate resulted in the maximum mortality of *R. similis* (80.43 %) among the 46 isolates tested. In the greenhouse studies with tomato as the test plant this isolate was very effective both in nematode suppression and growth promotion. Ap4 suppressed hatching of nematode eggs (82.38%). It effectively solubilized phosphate and controlled *Phytophthora.* Though, ammonia was produced, Ap4 does not produced HCN or H_2S. It was found to be producing fluoresin and pyocyanin pigments. It produced protease enzyme and liquefied gelatin. This isolate was having multiple antibiotic resistance and was found to be suppressive only to nalidixic acid and polymyxin among 14 antibiotics tested. It was also found effective against root knot and burrowing nematode infesting black pepper, effective in growth promotion of black pepper and was capable of even rejuvenating black pepper plants severely infected with root knot nematode. This

isolate is equally effective inducing resistance to nematodes and it can easily be formulated.

26. Molecular characterization and seed transmission of the *Badnavirus* infecting black pepper (*Piper nigrum* L.) in India (TH 133)

Mr. P.S. Hareesh/Acharya Nagarjuna University/2008/Guide: Dr. A. Ishwara Bhat

The present investigations on the *Badnavirus* infecting black pepper in India involved identification and determination of taxonomic identity of the *Badnavirus* infecting black pepper, its molecular characterization, development of PCR based diagnosis and seed transmission in black pepper. Based on the identity of nucleotide and deduced aminoacid sequences of a portion of ORFI (700 bp) and ORF III (600 bp), the *Badnavirus* infecting black pepper in India was identified as a strain of *Piper* yellow mottle virus (PYMoV). The virus showed >96% identity at the ORF I with PYMoV reported from Sri Lanka. For the first time portion of ORF III (600 bp) of PYMoV was cloned and sequenced. The sequence variability studies which involved the comparison of the 700 bp portion of ORF I and 600 bp portion of ORF III of the virus from four different isolates showed that PYMoV isolates from India does not possess much sequence variability. However one of the isolate (Wayanad) showed a distinct nature with respect to nucleotide and amino acid sequence of ORF l, indicating existence of variability. On the other hand, all the four isolates showed highly conserved sequences with respect to ORF III region. In general ORF III sequence was found to be more conserved than ORF I. In the phylogram developed based on nucleotide and deduced amino acid sequence of both ORF I and ORF III, all the Indian isolates formed a single cluster, showing their closeness and common origin. Two PCR based detection methodologies were developed for the detection of PYMoV in black pepper. In one method the total DNA from infected plant was used as template in PCR. The primer pair SCBV Rl and BADNA 1R consistently gave an amplicon of 700 bp from the infected plants. When 230 black pepper plants collected from nurseries and field were subjected to PCR, 84 plants showed positive for PYMoV infection that included both symtptomatic and asymptomatic plants. In the immunocapture PCR, PYMoV IgG was used to trap PYMoV particles in the PCR tube. The optimum concentration of IgG for successful amplification of PYMoV was determined as 200 ng. All the three primer pairs SCBV R1 & BADNA 1R, Badna 3F2 & Badna 3Rl and, AlB 35 & AlB 36 were successful in IC-PCR. The PYMoV was detected in leaf samples and berries through IC-PCR. Seed transmission of PYMoV in black pepper was demonstrated for the first

time through grow out test and PCR in four varieties of black pepper (IISR-Sreekara, IISR-Subhakara, IISR-Shakthi and Panniyur-1). The study showed that PYMoV is transmitted through the virions pre-existing in the germ line cells. The conclusions based on DNA-PCR and IC- PeR experiments suggested that transmission can also occur through integrated sequences of PYMoV. In the studies conducted for the detection of PYMoV integrants, the combination of DNA-peR and IC-peR identified a few plants from the varieties IISR-Sreekara and Panniyur-l having possible integrants. However, when these plants were subjected to Southern hybridization and FISH using PYMoV ORF I and ORF III specific probes, no hybridization could be seen indicating lack of integration. The immediate line of work that needs to be taken up is the whole genome sequencing of PYMoV which will help to understand its relationship with other badnaviruses. The peR based diagnostic protocols developed should be extended to virus indexing of black pepper plants. The immunocapture protocol will have great significance if PYMoV integrants occur in black pepper genome. The present study indicated the probability of integration of PYMoV sequence in black pepper genome. However a detailed investigation on nuclear integration of PYMoV in black pepper genome need to be taken up with more number of samples. This will be useful in answering the queries such as if these endogenous viral sequences are present in the black pepper genome, to which category-activatable or non-activatable they belong, whether they impart episomal infection, their role in gene silencing/anti-silencing in black pepper etc. Understanding of these endogeneous para retroviruses (EPRV) activation and console by the host could have important implications for plant breeding strategies to prevent viral disease caused by EPRVs in newly generated cultivars.

27. Studies on characterization and variability of *Phytophthora* species pathogenic to black pepper (*Piper nigrum* L) (TH 154)

Ms. P. Vijaya/University of Calicut/2008/Guide: Dr. Y.R. Sarma

The present investigation has brought out clearly that *P. capsici* is the predominant pathogen (85% isolates) of black pepper. In addition, *P. parasitica* (6.6% isolates), *P. meadii* (5% isolates) and *P. palmivora* (3.3% isolates) were also identified as pathogens of black pepper pointing to the possible threat in future by these three species of *Phytophthora,* in addition to *P. capsici. Phytophthora* species were isolated adopting selective medium from various infected plant parts like leaf, stem, collar, spike, root and rhizosphere soil of the affected plants. 60 isolates were used for characterization of which 32 were from leaf, 7 from stem, 9 from root, one each from

collar and berry and 10 from soil. They were obtained from black pepper plantations from Kerala, Karnataka a Tamil Nadu and Andhra Pradesh. The percentage of other species was meagre while considering *P. capsici* which is predominant. Most of the *P. capsici* isolates were producing chlamydospores. The entire Group I, and Group IV and seven isolates of the Group III produced chlamydospores. Two of the *P. meadii* isolates (98-86 and 98-90) produced chlamydospores and 98-192 did not produce chlamydospores. All *P. palmivora* and *P. parasitica* isolates also produced chlamydospores. The chlamydospore diameter was also variable. Studies on mating type of *P. capsici* showed that Al (88.4%) as the most predominant followed by A2 (1.739/0) and (4.62%) were sterile isolates. Out of the 60 isolates 51 belong to *P. capsici,* 3 belong to *P. meadii,* 2 to *P. paimivora* and 4 to *P. parasitica.* Among the three *P. meadii* isolates two were sterile and one AI. Two of the *P. palmivora* isolates were A2. Among the four *P. parasitica* three isolates were Al and one sterile. *P. capsici* was found pathogenic to arecanut, rubber, black pepper, betel vine, small cardamom, cocoa and coconut. Thus the cross infectivity studies proved the wide host range of *P. capsici. Phytophthora* species from other plantation crops *viz,* rubber, arecanut & coconut isolates were not pathogenic to black pepper. Cocoa, cardamom, and betel vine isolates infected black pepper. Besides this, some of the *Phytophthora* isolates from other plantation crops *viz. P. meadii* from rubber, cardamom and cocoa, *P. palmivora* isolates form cocoa and coconut known to be predominantly A2, point to the possibilities of mixing up of *Phytophthora* spp under high density multi species cropping systems there by leading to possibility of development of new races biotypes pathogenic to a broad spectrum of hosts. The present study opened up new opportunities and avenues both for disease management & breeding programmes in black pepper. The present investigations calls for the extension of the present studies with larger population of *P. capsici* isolates 0 from black pepper, and also for inclusion of more number of land races of black pepper in the differential hosts, to confirm the present racial picture and also how the racial picture correlates with molecular detail any.

28. Identification and cloning of chitinase gene from *Piper colubrinum* link. (TH 182)
Mr. R. Sandeep Varma/Mangalore University/2007/Guide: Dr. V.A. Parthasarathy

The present study, the isolated RNA was found to be pure without DNA contamination and could be successfully used in reverse transcription experiments. DNA isolation was also standardized for *P. colubrinum.* The protocol was a modification of the original DNA isolation protocol of Doyle and Doyle (1990).

Approximately 120-160 mg/g of DNA was obtained which could be successfully amplified using polymerase chain reaction. A total of 34 primers (degenerate) were designed based on conserved/semi conserved regions of chitinase gene identified in other plants. These primers were synthesized and screened for amplifying chitinase specific cDNA/DNA from *P. colubrinum.* RT-PCR was standardized in *P. colubrinum* using oligo dT/chitinase specific primers in the first strand synthesis followed by chitinase specific forward and reverse primers in the second strand synthesis.

29. Taxonomic and genetic characterization of black pepper and related species (TH 124)

Mr. K.V. Saji/University of Calicut/2006/Guide: Dr. K.S. Manilal

The distribution and diversity of south indian species of black pepper was studied and ecological niches of each of the species identified. Four species *viz*. *P. siienlva/leyensis*, *P. hapnium, P. bababudani, P. barberi* are endangered and need special conservation efforts to protect these species from extinction. Study on population density indicated that sexual reproduction coupled with vegetative propagation is the most important factor for population size and diversity within the species and absence of sexual reproduction coupled with less efficient vegetative propagation makes the species endangered. The Shannon diversity index indicated the regions comprising Palghat and Wayanad districts of Kerala; Nilgiris District of Tamil Nadu and Kodagu District of Karnataka indicate the area of gamma diversity of *Piper* species and needs to be protected to conserve all the species of South Indian *Piper* except *P. hapnium*. Digital herbaria of each of the species were prepared and inter-relations studied using both morphological and molecular characterization. This study in general agreed with our earlier understanding except that *P. wightii* is distinct and not related to *P. nigrum*. Among the *Piper* species *P. barberi* is the most divergent. Molecular characterization indicated no clear differences between *P. attenuatum* and *P. argyrophyllum* and also between *P*. *trichostachyon* and *P. galeatum* questioning their distinct species status. The study also supported the origin of *P. sugandhi* as a hybrid between *P. nigrum* and *P. galealum*/*P. irichoslachyon* indicating the presence of intermediate forms between these two species. A new improvised key was suggested for identifying South Indian *Piper*. Morphological and molecular characterization of 33 cultivars revealed good genetic variability between the cultivars indicating their origin as seed progenies followed by selection and vegetative propagation. Molecular characterization also supported intra-cultivar variability and the presence of

intermediate forms between the cultivars. The present study coupled-with earlier observations by various workers indicate that vegetative mutation, segregating selfed progenies, occasional crossed progenies and progenies of controlled crosses account for the existing variability in black pepper cultivars and the progenies of controlled crosses being most divergent followed by open pollinated progenies, segregating selfed progenies and vegetative mutants. It is suggested that crosses or hybridization between divergent genotypes can enhance the genetic variability within black pepper and hence can be used in crop improvement programmes. This study further indicates the possibility that in south Indian *Piper* the species isolation is not yet complete, with the occurrence of many intermediate forms and the possibility that many species are still crossable.

CARDAMOM

1. Development of microsatellite markers for small cardamom (*Elettaria cardamomum* Maton) (TH 168)

Ms. Anu Cyriac/Mangalore University/2016/Guide: Dr. K. Nirmal Babu

Small cardamom (*Elettaria cardamomum* Maton) of the family Zingiberaceae is an important spice and occupies a unique position in the national as well as international spice market. However, understanding of this crop at molecular level is very limited and little genomic research has been done. The present study was aimed to develop a repository of reproducible SSR markers to study genetic diversity and taxonomic inter relationships in small cardamom. For these three approaches were followed *viz*., i) selective hybridization enrichment method ii) development of SSR markers from EST databases iii) transferability of previously reported SSR markers to cardamom. The *denovo* isolation of microsatellites from small cardamom resulted in the identification of 140 microsatellite repeats from 270 clones. Primers were designed for 58 microsatellites and 44 amplified products of expected size in cardamom. Among them six SSR markers were polymorphic and detected a total of 22 alleles among the 20 cardamom genotypes with an average of 3.6 alleles per locus. The allelic variants ranged from 2-7alleles. The PIC value for the markers ranged from 0.14 to 0.38 with an average of 0.28. The mining of SSR sequences in the dbEST database (NCBI) of *Zingiber* resulted in 188 EST SSR primers from the contigs. A set of 22 primers were shortlisted for transferability to cardamom and utilized with 79 SSR primers from

different related genera (Rice*, Zingiber, Curcuma* and *Amomum*). In total 30 SSR primers from different related genera was produced consistent amplification in cardamom out of which three markers (one ginger EST SSR primer and two large cardamom primers) were polymorphic. A total of fifteen alleles were detected with a range of 2-7 alleles per SSR marker. The average number of alleles per SSR marker was five. Four SSR markers generated unique banding patterns for different cardamom genotypes. The nine polymorphic SSR markers differentiated the 20 genotypes into two major clusters at 53% similarity based on their genetic characters and the genotypes showed a correlation with their geographic origin. This is the first report of development of microsatellite markers in small cardamom. This study is an initiative towards molecular profiling of more unexploited accessions of small cardamom that can provide insights into patterns of genetic diversity.

2. Characterization and development of diagnostics for viruses infecting small cardamom (*Elettaria cardamomum* Maton) (TH 162)

Mr. Siljo Abraham/Mangalore University/2013/Guide: Dr. A. Ishwara Bhat

In this study, a survey was conducted in major cardamom growing areas of south India for recording the incidence of viral disease. During the survey, cardamom plants exhibiting different viral symptoms were collected and used for characterization of associated causal viruses and development of diagnostics for quick and sensitive detection of viruses in plants. The survey indicated that the incidence of mosaic disease was 0-85%. In Karnataka, disease incidence ranged from 2- 85% whereas in Kerala it ranged from 0-12%. The incidence of the disease was highest in Madikeri taluk of Karnataka while no incidence were recorded in Peermade, Chittoor taluks of Kerala and all the locations except Kodaikanal taluk in Tamil Nadu. In order to study the variability in the coat protein gene of CdMV, six geographically distinct isolates of the virus were selected and the coat protein (CP) gene of the virus from each isolate was amplified using degenerate primers. The amplified product was cloned and sequenced. The coat protein (CP) gene consisted of 816-822 nucleotides, potentially coding for 272-274 amino acid residues in different isolates. Karnataka and Vythiri, Meppadi, Pampadumpara and Myladumpara of Kerala were used to standardize RT-PCR based detection of CdMV and the method validated using 30 cardamom isolates (mentioned above) including 16 symptomatic and 14 asymptomatic plants collected from different regions. RT-PCR could successfully detect 15 symptomatic and 7 symptom less plants from different geographical regions of Kerala and Karnataka. Comparative study to

determine the detection limit between RT -qPCR and the conventional RT-PCR assays in detecting CdMV in infected samples showed that RT qPCR is 1000 times more sensitive and time saving than conventional RT -PCR. The RT qPCR method was validated using 30 field samples. The study also reported the association of Banana bract mosaic virus (BBrMV) with cardamom plants affected with chlorotic streak disease. In a survey for mosaic disease in cardamom plantations, a new kind of viral disease showing chlorotic streak on veins was observed. Based on the type of symptom, the disease was named ' chlorotic streak. The plants infected with chlorotic streak tested negative for CdMV. Survey on the occurrence and distribution of the chlorotic streak disease showed an incidence ranging from 0-15% in different cardamom-growing regions. The incidence of the disease was higher in plantations where either banana was grown nearby or banana had been the previous crop. In the present study, a RT-PCR assay was developed for the detection of BBrMV in infected samples. Primer pair was designed to the conserved region among the isolates. RT-PCR method was standardised using five known infected samples from Sirsi, Madikeri and Mudigere Districts of Karnataka and Idukki and Wayanad Districts of Kerala and validated using 20 field samples (including 11 symptomatic and 9 asymptomatic plants) collected from different regions of Kerala. Comparative study to determine the detection limit between RT -qPCR and the conventional RT-PCR assays in detecting BBrMV in infected samples showed that RTqPCR is 1000 times more sensitive than conventional RT-PCR. The RT-qPCR method was validated using 20 field samples. Sensitivity of detection of this virus by RT- LAMP was studied using serial dilutions of total RNA and compared with conventional and RT-qPCR. Result showed that detection limit of RT-LAMP was up to hundred times higher than conventional RT -PCR and on par with RT- qPCR using SYBR Green.

GINGER

1. Role of elicitors in evading pathogens causing rot diseases in ginger (*Zingiber officinale* Rosc.) (TH 201)

Ms. Alka Nasser/University of Calicut/2022/Guide: R. Suseela Bhai

Changes in cellular stress markers, defence enzymes, and antioxidant enzymes were used to investigate the direct influence of KSi on ginger plants. Even in

pathogen infected plants, stress factors such as MDA, proline, and H^2O^2 concentrations exhibited a decrease in cellular concentration after the administration of KSi. The use of KSi also increased the synthesis of defence and anti-oxidant enzymes such as PPO, PAL, POD, and SOD. The application of silicate fertilisation also elevated the concentration of total phenol, which is a major pathogenic inhibitor. The induction of biochemical defence activation by silicate addition was demonstrated by the activation of defence enzymes, anti-oxidant enzymes, and phenol. PPO, PAL, and POD are important enzymes. PPO, PAL, and POD are crucial players in the formation of phenol and lignin, which are potent inhibitors of necrotrophic pathogens. Microscopic alterations in plant tissues, like silicon deposition and cell wall reinforcement patterns such as lignification and suberization, were seen after the KSi treatment. Various fluorescence staining techniques were used to examine the intricacies of silicate deposition and cell wall fortification. The silicate deposition was initially localised on the root tissue's outer epidermal layers, then advancing towards the cortex and inner vascular parts. The cell wall strengthening patterns were identified as lignification and suberization surrounding the pseudostem's vascular bundles and root casparian thickenings. The hypothesis of mechanical defence activation by silicate treatment, which is a barrier defence mechanism against killing necrotrophs, was corroborated by these findings of cell wall strengthening. The addition of KSi to the soil improved microbial count and soil nutrient levels, as well as soil enzymes such as alkaline phosphatase and dehydrogenase. The use of silicate also enhanced the soil's mean MBC. These are measures of soil health whose improvement has a favourable impact on the soil environment.

2. Diversity and host preference of *Pythium* species infecting major Zingiberaceous spices (TH 197)

Ms. P.K. Bijitha/Kannur University/2019/Guide:Dr. R. Suseela Bhai

India is popularly known as the 'The Land of Spices', has been blessed with a wide spectrum of agro-climatic conditions favorable for the growth of various spices. The important commercial spice crops *viz*., cardamom, ginger and turmeric belong to the family Zingiberaceae. The crops are vulnerable to many diseases of both soil and air borne nature including bacterial and viral diseases. However, the major constraints limiting the production of three crops are rhizome rot diseases incited by *Pythium* spp. It occurs in several parts of India wherever these crops are grown. Though all the three

crops belong to the same family Zingiberaceae and all are rhizomatous crops, there is host specificity for species of *Pythium* infecting these crops; cardamom rhizome rot is reported to be caused by *P. vexans* whereas ginger rhizome rot is caused by *P. myriotylum* and *P. aphanidermatum* and turmeric rhizome rot by *P. aphanidermatum* and *P. myriotylum*. *P. vexans* did not infect ginger or turmeric and *vice versa.* Hence there is a curiosity in studying the diversity and host preference of *Pythium* species infecting these crops. Similarly, for developing disease management strategies, correct identification of the species is very much important because the sensitivity of the species varies towards the fungicides and pathogens associated. Though, some of the fungicides are recommended for the crops, the present-day farmer perception and environmental hazards are compelling to search for alternative eco friendly disease management strategies. So based on this background, detailed surveys were conducted in hot spot areas for collection and isolation of *Pythium* spp. from various cardamom, ginger and turmeric growing tracts of three South Indian states. A total of 119 *Pythium* isolates were collected of which 36 were cardamom isolates identified as *P. vexans.* Out of 48 isolates from ginger 40 were identified as *P. myriotylum* and 8 as *P. aphanidermatum*. Similarly, out of 35 *Pythium* isolates from turmeric, 18 were identified as *P. aphanidermatum* and rest as *P. myriotylum*. The isolates were characterized based on phenotypic and morphological features and identified by molecular methods. Analysis of soil samples from respective locations revealed a positive correlation between the soil pH and the percentage disease incidence. In pathogenecity assays, all the collected *Pythium* spp. from the three crops reproduced typical symptoms of rhizome rot in each crop and virulence assays showed differential reaction between isolates from each crop. The study confirmed the association of *P. vexans* (100%) in cardamom and predominance of *P.myriotylum* and *P. aphanidermatum* with ginger and turmeric respectively. However *P. myriotylum* and *P. aphanidermatum* were also isolated from turmeric and ginger. As far as the growth of the isolates in different media are concerned, potato dextrose agar and potato carrot agar were found as the best media for the growth and sporulation of *Pythium* spp. whereas V8 vegetable juice agar supports very much for oospore production. All the isolates differed slightly in temperature optima for the growth. The optimum temperature for the growth of all the *P. vexans* were found to be 20- 35°C while the best temperature for the growth was found as 28°C. The optimum temperature for *P. myriotylum* was found to be 25-35°C, where the isolates grew best at 30°C. In case of *P. aphanidermatum*, the optimum

range was 30°C and 40°C, but a sparse growth was observed even at 45°C also. The host specificity of *P. vexans* can be attributed to various factors of which temperature is one of the main factor involved in host preference. *P. vexans* being a weak pathogen can survive only at low temperature profile for its growth and perpetuation and hence it prefers to adapt to the cool climate crop like cardamom. It may not be able to survive at higher temperature where crops like ginger and turmeric being grown. *P. aphanidermatum* and *P. myriotylum* are broad host range species favored by very warm conditions. This may be one of the reasons for the absence *P. aphanidermatum/P. myriotylum* in association with cardamom which is adapted to cool climate where it grows only in the high ranges at an altitude of 700 and 1500 m above MSL. From the biochemical analysis of the host tissue for the induction of defense related enzymes and total phenolics after challenging with three species of *Pythium*, it was observed that the PAL activity was very much upregulated in cardamom, whereas the same activity was very low in ginger and turmeric. But the activities of enzymes like LOX, PO and PPO and the total phenolics were very low in cardamom when compared to ginger and turmeric. *P. vexans* being a weak pathogen, the host specificity to cardamom can be attributed to this low level of phenolics as well as defense enzymes like LOX, PO and PPO, whereas all these activities were very high in other two crops up on challenging with the pathogens. Usually *Pythium* spp. are attracted towards growing roots of the host plants due to the release phenolic compounds that act as growth stimulants to *Pythium* spp. and these compounds predisposes the host to infection. The *Pythium* spores germinate and penetrate feeder roots directly on coming in contact with such rhizosphere. The non-infectivity of other species of *Pythium* on cardamom can be attributed to the poor release of phenolics from the roots of cardamom. *P. vexans* being an intermediate or weak pathogen, it prefers cardamom due its low release of phenolics. Among the seven new generation fungicides tested against the 119 *Pythium* isolates from three crops, metalaxyl 4% + mancozeb 64% and metalaxyl 8% + mancozeb 64% were found to be the best with >80% inhibition even at the lowest doze tested. The recommended concentration was highly inhibitory to all the isolates. The next best fungicide was found to be iprovalicarb 5.5% + propineb 61.25% (66.75 WP). In this study, a potential environmentally safe bioagent for combating rhizome rot was identified. *In vitro* and in planta studies revealed that the isolate IISRCLRB5 (*Burkholderia cepacia*) is very inhibitory showing more than 70% inhibition when inoculated simultaneously with *Pythium* spp. and complete inhibition on sequential

inoculation. *B. cepacia* strain IISRCLRB5 is found to be nitrogen fixing, phosphate solubilizing and potassium utilizing and also produces hydrolytic enzymes and plant growth promoting metabolites. Our findings confirm that *B.cepacia* strain IISRCLRB5 is a promising biocontrol candidate which can control rhizome rot diseases.

3. Molecular and chemo profiling of ginger (*Zingiber officinale* Rosc.) genotypes (TH 192)

Ms. H. J. Akshitha/University of Horticultural Science Bagalkot/2018/
Guide: Dr. K. Umesha, Co-Guide: Dr. D. Prasath

Studies on molecular and chemo profiling of ginger (*Zingiber officinale* Rosc.) genotypes were carried out during 2016-17 and 2017-18 at ICAR-Indian Institute of Spices Research, Kozhikode and ICAR-IISR, Experimental Farm, Peruvannamuzhi, to know the morphological, biochemical and molecular diversity among 28 ginger genotypes, 1 *Curcuma* sp. and 1 *Kaempferia* sp. Among the 28 genotypes studied, highest projected yield was recorded in genotype Maran (17.71 t/ha) followed by Acc. 247 (16.33 t/ha) and Himachal (16.06 t/ha). Growth and yield parameters *viz*., number of tillers per clump, total number of leaves, yield per plant, essential oil, oleoresin and crude fibre content exhibited high heritability coupled with high genetic advance as per cent mean which can be the reliable selection parameters for ginger crop improvement. Grouping of genotypes based on DUS descriptors showed narrow variability for most of the morphological characters, whereas rhizome characters exhibited remarkable variability. Chemical profiling showed that, red ginger had the highest oleoresin and essential oil percentage of 12.18 % and 6.00 %, respectively. Among other ginger genotypes, Arunachal Pradesh local (8.55 %), Rio de Janeiro (7.77 %) and Acc. 65 (7.10 %) revealed high oleoresin content and genotype Arunachal Pradesh local had higher oil content (3.00 %). Zingiberene was the major component present in the essential oil of ginger genotypes and the highest content was observed in cultivar 'Maran'. Molecular profiling of ginger genotypes by RAPD and SSR primers revealed that, the markers were efficient in clustering the other species *viz*., mango ginger and black ginger but, in case of ginger genotypes irrespective of place of collection or origin, genotypes were grouped into different clusters. Grouping was not on the basis of any morphological, yield or biochemical characters. Identification of SNPs using comparative transcriptome was carried out that can be further utilized to identify the genotype specific markers.

4. Molecular and biochemical characterization of bacterial wilt resistance in mango ginger (*Curcuma amada* Roxb.) (TH 184)

Ms. R. Karthika/Mangalore University/2018/Guide: Dr. D. Prasath

The thesis work is aimed at deciphering the molecular and biochemical factors involved in bacterial wilt resistance in mango ginger. The study identified RGAs expressed as a result of pathogen inoculation, detected the conserved domains, classified them based on specific motif signatures, predicted their subcellular location and carried out gene expression studies of the selected sets of RGAs. Nine candidate genes namely Ethylene Response Factor (ERF), HMG-CoA Synthase (HMGS), HMG-CoA Reductive (HMGR), ABC transporter, WRKY8 Transcription Factor, β-(1, 3)-glucanase, Callose synthase, Heat Shock Protein (HSP) and MLo14 were studied at different time intervals in leaf and rhizome tissues of mango ginger and ginger post inoculation with *R. solanacearum* using real-time PCR. The study throws light on the dynamic changes in incompatible and compatible interactions in the regulation of different infection related genes over a time course in a tissue specific manner. The genes identified herein will provide a basis for further characterization and functional verification studies and will aid in understanding the regulatory mechanism of mango ginger against *R. solanacearum*. The study evaluates the changes in the levels of defence related enzymes *viz.,* peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL), lipoxygenase (LOX) and total phenols in ginger and mango ginger after encountering *R. solanacearum*. The interaction of both resistant and susceptible plants with the pathogen resulted in varying levels of induction of defence related enzymes in tissue specific manner to restrain penetration of the pathogen. The mango ginger rhizome powder was sequentially extracted with hexane, chloroform and methanol in the order of their increasing polarity. The essential oils were extracted by hydrodistillation. The solvent extracts and essential oils exhibited inhibitory activities against the growth of *R. solanacearum.*

5. Studies on biovar specific diagnostic for *Ralstonia solanacearum* (Smith) infecting ginger (*Zingiber officinale* Rosc) and evolution of apoplastic microbes for biocontrol (TH 173)

Ms. T.P. Prameela/Mangalore University/2016/Guide: Dr. R. Suseela Bhai

In the present study 42 *Ralstonia solanacearum* isolates were collected from different bacterial wilt affected ginger and small cardamom plants from different endemic areas during 2009-2015. The isolates represented geographically separated locations such as Kerala, Karnataka and Sikkim in India. The isolates were

characterized for biovars and found to be of race 4 biovar 3 strains. Pathogenicity tests proved that there is variation in the infectivity of *R. solanacearum* isolates on ginger as evidenced by the days taken to express the typical wilt symptoms. The highly virulent isolates caused wilting in 6-13 days of inoculation while the less virulent strains caused wilting in 15-25 days. Cross infectivity studies clearly revealed that ginger is infected only by the race 4 biovar 3 strains of *R. solanacearum;* the race 1 biovar 3 strains infecting the solanaceous crops could not infect ginger. However, the race 4 biovar 3 strains of ginger can infect solanaceous crops also. Twenty one *R. solanacearum* isolates representing major crops (ginger, small cardamom, tomato, potato, chromolaena (*Chromolaena odorata*), chilli, paprika, and eggplant) and geographical locations in India were selected for comparative genetic diversity analysis using different molecular tools. Multiplex-PCR based phylotyping has done to understand the geographical origin ofthe *R. solanacearum* isolates and the study revealed the predominance of phylotype 1 among the isolates showing the Asian origin. Only one isolate from potato was found to be in phylotype II indicating their American origin. These short listed isolates were evaluated against bacterial wilt under green house conditions using seed priming and soil drenching method. *Bacillus licheniformis* (IISRGAB 107) was found to be very promising in inhibiting *R. solanacearum* by showing a disease reduction up to 67% over pathogen challenged control. The increased population of introduced bacteria in the rhizosphere soil, roots, rhizomes and apoplastic fluid revealed the colonization of the targeted bacteria when compared to control showing the efficiency of bacterization of soil and seeds. To summarize, the present study leads to the development of a race specific diagnostic for a quick, early and on farm detection of race 4 biovar 3 strain of *R. solanacearum* infecting ginger and also identified one potential biocontrol agent *viz*, *Bacillus licheniformis* from the apoplastic fluid of ginger which can be used for the biological control of bacterial wilt of ginger. The study also leads to the understanding of the diversity of race 4 strains of *R. solanacearum* and also the diversity of culturable microorganisms in the apoplastic fluid of ginger.

6. Biochemical investigations and post-harvest management of aflatoxin contamination in black pepper, ginger and turmeric (TH 143)

Ms. S. Sindhu/Mangalore University/2011/Guide: Dr. B. Chembakam

The critical factor that contributes to the post harvest management is the moisture content, which should be maintained below 10%. Moisture content of black

pepper and turmeric samples collected from farmers were in the range of 10-13% and 8.2-13.1 % respectively whereas among traders, black pepper, ginger and turmeric were as high as 9.0-21.9%, 10-27%, 10-19% respectively. Among co-operatives, black pepper and turmeric samples collected had moisture content of 10.08-10.9% and 10.6-11.7% respectively. Moisture content in turmeric samples collected from co-operatives and processors was in the range 10.64- 11.70% and 8.2 1-10.17%. Essential oil in major spices was found to be lower among the farmer's samples: black pepper -2.8-3 .6% and turmeric -1.6-5.6%. In traders samples from all places essential oil in black pepper was 2.8-3.6%, in ginger 0.8-2.4% and in turmeric 1.0-5.6%. Among Co-operatives, essential oil in black pepper and turmeric samples was 2.8-3.2% and 2.0-4.0 % respectively. Black pepper samples collected from processors and exporters had 2.4-3.2% essential oil. Oleoresin content in black pepper samples collected from farmers was 6.9-9.7% and in turmeric 2.9-15.5%. Oleoresin was lower in black pepper (3 .6-10.1 %), ginger (2.4-6.4%) and turmeric samples (2.6-13.2%) in the traders samples from all places. Oleoresin content in black pepper samples collected from cooperatives, processors and exporters was 7.6-9.8%, 7.7%, 5.7-6.15% respectively, whereas in turmeric sample s collected from co-operatives and processors it was 2.9-7.7% and 3 .7%. The fungal and bacterial sp. isolated from these samples were *Aspergillus flavus*, *A. parasiticus*, *A. niger*, *Pencillium* spp., *Mucor* spp. and certain isolates of non-pathogenic saprophytic bacteria such as *Pseudomonas* sp., *Bacillius* sp., *Streptococcus* sp., *Serratia* sp. and *Klebsiella*. The bacteria were tested for their efficiency as biocontrol agents.

7. Studies on mechanization of field level post harvest operations in ginger (*Zingiber officinale*) with reference to washing, peeling and drying (TH 135)

Ms. E. Jayashree/Tamil Nadu Agricultural University/2009/

Guide: Dr. R. Visvanathan

Ginger (*Zingiber officinale*) is one of the most widely used species of the family Zingiberaceae. It is valued for the dry ginger spice, preserved crystallized ginger and as fresh vegetable. India is the largest ginger producing country in the world with an annual production of 3,30,350 tonnes from an area of 85,900 ha. during the year 2004. Among the Indian states, Nagaland is the largest fresh ginger producing state followed by Meghalaya, Kerala, Arunachal Pradesh, Orissa and Mizoram. Ginger when used as vegetable is harvested from sixth month onwards while for preparing dry ginger, the produce is harvested after eight months of planting when the leaves of the plant turn

yellow and starts drying. The harvested clumps of ginger are cleaned manually to remove the dried roots and soil clods. The clumps are then broken to sufficiently large size rhizomes suitable for preparing dry ginger. After cleaning, the rhizomes are subjected to peeling, drying and polishing operations. Technologies and equipment's for mechanization of post harvest handling of ginger at field level like washing, peeling and drying are limited. Since these operations are labour intensive and time consuming, development of suitable devices will reduce the drudgery of these operations. Hence the proposed research work was undertaken with the objectives to study the mechanical washing of ginger, develop a suitable mechanical peeler and to optimize the drying methods to obtain quality dried product. The engineering properties of ginger are essential for the design of peelers and driers and hence the physical, mechanical and thermal properties of ginger were determined. The average moisture content of fresh and dry ginger rhizomes was 81.70 and 8.85 per cent, respectively. The mean value of cylindricity for fresh and dry ginger rhizome was 0.46 and 0.48 per cent, respectively. The average bulk density, true density and porosity of fresh ginger rhizome was 471.49 kg m^{-3}, 1107.01 kg/m^3 and 66.80 per cent and the corresponding values for dry ginger were 460.09 kg/m^3, 1013.22 kg nfl and 54.09 per cent, respectively. The angle of repose was 34.6° and 39.5° for fresh and dry ginger rhizomes, respectively. The coefficient of friction of fresh ginger rhizomes against plywood, stainless steel, aluminium, galvanized iron and mild steel surfaces were 0.53, 0.57,0.68,0.72 and 0.74, respectively. The mechanical properties of fresh and stored ginger rhizomes (under ambient conditions for two months) were studied. The penetration force, rupture force and cutting force of peel and meat increased during storage. The thermal properties of fresh and dry ginger such as specific heat, thermal conductivity and thermal diffusivity were studied. Mechanical washing of ginger was performed using Punjab Agricultural University (PAU) model vegetable washer (60 kg). The conditions of the mechanical washer for microbial washing efficiency above 80 per cent were considered as the optimum conditions operating conditions. Hence, at washing speed of 55 rpm for 5 min, microbial washing efficiency of 92 per cent was achieved and hence considered as the optimum conditions of washing. At the optimum washing condition, the mechanical washing efficiency and bruise index were 97.8 per cent and 7.5 respectively. The colour values 'L', 'a' and 'b' of ginger were 46.34, 7.44 and 18.71, respectively. The chemical pee ling of ginger was performed by boiling ginger. In the dye solution (NaOH) at temperatures, 60, 70, 80 and 90°C and concentrations of sodium hydroxide, 2.5, 5.0, 7

.5, 10, and 12.5 per cent for minimum contact duration to achieve ' very good peeling' (peeling > 98 '). Ginger, after dye treatment became darker with the increase in intensity of redness and decrease in yellowness of colour value. Commercially dry ginger having lighter colour is valued as a spice and hence the process of dye peeling was not encouraging. Mechanical peeling of ginger, by abrasion against a hard abrasive surface was considered. Six models of mechanical peelers were fabricated and evaluated for their performances. The peeling efficiencies of square mesh drum peeler, diamond cut mesh drum peeler, concentric diamond cut mesh drum peeler, large diameter peeler, semi mechanical brush peeler and concentric drum brush type peeler at maximum loading capacity were found to vary from 58.2 to 65.4 per cent. The material loss in the above ginger peelers varied between 2 to 7.1 per cent. Experiments on drying of ginger were conducted for evaluating the quality of dried ginger obtained under different drying methods (open sun drying, solar tunnel drying and tray drying at 50, 55, 60 and 65°C) for varying slice lengths (5, 10, 15, 20, 30, 110, 50 mm drying whole rhizomes) and for varying levels of pretreatment with potassium meta bi sulphate (KMS) (1, 2, 3, and 4 per cent). The effect of slicing and drying methods on the essential oil content, oleoresin content, volatile constituents of essential oil and non volatile constituents of oleoresin were determined. It was found that both slicing and drying methods had significant influence on the biochemical quality of dry ginger. Maximum retention of essential oil and oleoresin was obtained from sun dried and solar tunnel dried samples. Pretreatment with KMS did not have any significant effect on the quality of dried ginger except for the colour value. Drying characteristic of ginger under sun drying showed that the moisture content, moisture ratio and drying rate decreased continuously as drying progressed. The time required to dry ginger from an initial moisture content of around 594.01 per cent (d.b.) to a final moisture content of around 9.89 per cent (d.b.) was eight days at average ambient temperature of 35.7°C. Thin layer drying behaviour of ginger under sun drying was best described by diffusion approximation model. The effective moisture diffusivity for sun drying of ginger was calculated as 1.9 1 x 10^{7} m^{2}/S . Test sample of 40 kg was taken for dehydration studies in a multi rack type solar tunnel drier. The variation in moisture content, moisture ratio and drying rate was observed as the tray levels varied inside the solar tunnel drier. Ginger placed at the top most level dried faster as the produce was in contact with the direct solar insolation entering the solar tunnel drier. Towards the end of drying on the eighth day, the moisture content of ginger for the tray levels first, second, third, forth and fifth from the top to bottom were 10.34, 7.39, 10.3, 9.8 and 10.6 per cent (d.b.), respectively. The time required to dry

ginger in a solar tunnel drier from initial moisture content 594.0 per cent (d.b.) to a final moisture content of around 9.69 per cent (d.b.) was eight days. Mathematical modeling of thin layer drying in a solar tunnel drier showed that the diffusion approximation model described the 'over all' drying of ginger in a solar tunnel drier. The 'over all' effective moisture diffusivity of ginger in solar tunnel drier was 1.82 x 10-7 m^2S^{-1}. There was temperature gradient inside the tray drier which caused differences in drying at different levels. Drying was performed during the day time for 7 h per day and sufficient time was provided for moisture equilibrium with in the product. Trays were rearranged from fifth day and drying was completed in 8 days. Towards the end of drying, the moisture content of ginger for tray levels first, second, third, fourth, fifth and sixth from the top to bottom were 7.34, 8.57, 7.80, 8.75, 9.62 and 9.45 per cent (d.b.) respectively. Modeling of thin layer drying showed that diffusion approximation model best described the drying process. The 'over all' effective moisture diffusivity of ginger in mechanical drier was 1.59 x 10^{-7} m^2 S^{-1}. The HUF, effective heat efficiency and thermal efficiency of the tray drier decreased with the progress in time. The quality evaluation studies showed that biochemical quality parameters like essential oil and oleoresin of sun and solar tunnel dried ginger were higher than the quality parameters of mechanically dried ginger. But in terms of microbial quality, the microbial load of solar tunnel dried ginger was very less (1.33 x 10^6 cfu/g/L) when compared to sun (4.66 x 10^6 cfu/g/L) and mechanically dried ginger (5.33 x 10^6 cfu/g/L). The cost economics for mechanical washing, peeling and drying in solar tunnel drier was worked out. The average cost of mechanical washing was Rs.0.37 per kg.

8. Molecular and biochemical characterization of ginger (*Zingiber officinale* Rosc.) germplasm (TH-137)

Mr. K. Jaleel/Acharya Nagarjuna University/2008/Guide: Dr. B. Sasikumar

Molecular characterization of 46 ginger accessions showed that genetic difference among the ginger accessions are much less except one exotic ginger 'Kintoki' and one cultivar collected from Meghalaya, 'Pink ginger'. Primitive types gingers such as 'Sabarimala', 'Ellakkallan', 'Kakkakkalan' and ' Kozhikkalan' showed their unique genetic identity in all the dendrograms. Most of the accession collected from more or less close geographical regions clustered together in the dendrograms. Biochemical characterization revealed the oleoresin and essential oil level are high in all the primitive types. Less fiber content found in the exotic ginger 'Kintoki' and cultivar 'Nadia' suggesting the possibility of exploiting these accessions for making end

products needing less fiber. GC/MS analysis of oil showed 'zingiberin', citral and z-citral are the major compounds found in the ginger accessions. Dendrogram based on GC-MS analysis did not show much similarity with dendrogram constructed based on molecular markers. For bioprospecting of the pharmacologically important constituents gingerol, shogaol and zingiberin, the cultivar 'Angamali' is found ideal. Knowledge about the distribution of genetic variation among ginger land races plays a crucial role in their conservation. The most crucial point emerged from the study is that geographical variation is dependent of genetic variation in ginger. Hence collection strategy has to be reoriented.

9. Investigation on direct *in vitro* shoot regeneration from aerial stem explant of ginger (*Zingiber offioinale* Rose.) and its field evaluation (TH 126)

Ms. A.K. Lincy/University of Calicut/2007/Guide: Dr. B. Sasikumar

The present study is an attempt in this direction through developing successful and reproducible protocols for direct regeneration of plantlet from aerial stem, vegetative bud explants as well as indirect organogenesis from callus and somatic embryos including the basic aspects of ontogeny of direct regeneration and somatic embryogenesis, genetic fidelity of *in vitro* regenerated plants and field and quality evaluation vis-a-vis the control. Altogether 31 hormone combinations, two types of explants (aerial stem and vegetative bud) along with callus cultures of the two varieties *viz*. var. Varada and Jamaica were used in the study over a period of 3 years. In case of direct regeneration of plantlets from *in planta* aerial stem, significant effects of varieties, hormones and variety X hormone interaction were observed. Maximum shoot and root regeneration was observed in the cultures containing TDZ and IBA (1:1; 1:0.1 mg/L) in both the varieties. Cultures containing. Alone showed poor shoot and root formation. The var. Varada produced more number of shoots as compared to the var. Jamaica. However, the var. Jamaica showed maximum number of roots as compared to the other. Out of the three explant sources tried (basal, middle and top portions of *in vitro* aerial stem), basal portion containing axillary meristem followed by middleportion containing apical meristem produced maximum number of shoots and roots inboth the varieties. The number of shoots and roots varied significantly with varieties and levels of hormones. The interaction between variety X hormones was significantonly for shoot development. *In vitro* aerial stem produced multiple shoots in all combinations of BAP and NAA but maximum shoot and root regeneration was observed in BAP and NAA (2:1 mg/L) in the var. Jamaica and in BAP and NAA (2:1, 5:1 mg/L) in the var. Varada.

Cultures containing BAP alone showed poor shoot and root development. The var. Jamaica produced more number of shoots and roots compared to the var. Varada. Protocol for direct regeneration of plantiets from vegetative bud was also developed. Significant effect of hormones on shoot and root development was observed in this case. The effects of varieties and varieties X hormone interaction were non-significant. Vegetative buds produced multiple shoots in all the combinations of BAP and NAA but maximum shoots and roots were observed in BAP and NAA (1:1 mg/L) in both the varieties. The var. Varada produced more number of shoots and roots as compared with the var. Jamaica. Callus induction and callus regeneration studies were also carried out. In the present study, both *in planta* and *in vitro* aerial stem explants were used for callus induction. Percentage of explants call used varied with type of explants, varieties and levels of hormones. In callusing response, the var. Varada was better than the var. Jamaica. Similarly, *in vitro* aerial stem explants produced more callus as compared to *in planta* aerial stem in both the varieties. However, both the explants showed good callusing with 2,4-D (2 mg/L) in both the varieties. The results of qualitative analysis revealed that the dry recovery varied significantly with varieties and explants. The variety X explant interaction was also found significant in this case. The essential oil and the fibre content were varied significantly with varieties and explants, while, oleoresin and starch content were significantly influenced by explants used for plant propagation. However, the total carbohydrate content varied significantly with varieties only. The var. Jamaica showed high dry recovery and volatile oil content, while the var. Varada was superior for oleoresin, starch and total carbohydrate contents. The rhizomes of callus regenerated plants exhibited high dry recovery in both the varieties. The oil and oleoresin contents were high in the rhizomes of aerial stem regenerated plants in both the varieties coupled with low dry recovery. The present study spanning over a period of 3 years revealed aerial stem of ginger can be a better source of explant for micro propagation of true to type, disease free plantlets with good yield and quality when compared to plants derived from other means of micropropagation in ginger. A novel protocol for direct and indirect somatic embryo induction was also developed. The study also revealed the anatomical structure of aerial stem of ginger as well as the ontogeny of shoots and roots from the aerial stem for the first time. The origin and development of somatic embryos were also discussed This viable protocol need to be scaled up for adopting as a commercial propagation method in ginger.

10. Studies on somaclonal variations in Zingiberaceous crops (TH 127)

Ms. V. Sumitha/University of Calicut/2007/Guide: Dr. K. Nirmal Babu

Most of the plant species in the family Zingiberaceae are solely or predominantly vegetative propagated plants and sexual reproduction is completely absent or rare in some species like ginger and turmeric respectively. This leads to narrow genetic diversity in intra and inter species populations. In some other plant species like cardamom, though sexual reproduction is very efficient, the monotypic nature of the species also reflects narrowness of the genetic base. Sexual reproduction is essential for intra and interspecies gene flow and in addition to variability that occurs due to recombination. However, in these crops with rare or no sexual reproduction there seems to be high degree of variability observed. For example, huge number of variable cultivars are available in completely vegetatively propagated plants like ginger and turmeric. It is particularly impossible to have so many variants occurring in populations without sexual reproduction. Obviously factors other than recombination, like natural mutations must be playing a major role in generating this variability. These natural mutations, whose rate of occurrence may be comparatively higher in these crops, are fixed in populations by the predominantly vegetative propagation and are unconsciously selected for their preferential performance. This indirectly indicate natural mutation leading to genetic mosaics among the vegetatively propagated propagules, must have contributed significantly in the development of variations among the vegetatively propagated taxa of Zingiberaceae especially in ginger and turmeric. These natural variations can be effectively harvested using *in vitro* culture technology. Thus the resultant somaclonal variation can effectively be used to compensate the lack of variability through sexual reproduction and recombination III crops like ginger. Many useful somaclonal variants with increased yield, quality and resistance to biotic and abiotic stresses have been identified in crops like ginger, turmeric and cardamom. Occurrence of somaclonal variation during culturing is a frequent and consistent event. Somaclonal variations not only can be distinguished by their morphological traits but also by their biochemical, physiological and genetic characteristics. Somaclonal variation was found to be an important source of variation in many crop plants though in many cases these variations were found to have progressively lost in subsequent generations. Some success has been realized in selecting for qualitative traits such as resistance to anti metabolites, herbicides, toxins and abiotic stresses. This has more relevance in vegetatively propagated

Zingeberaceous plants more so in crops like ginger and turmeric where the sexual reproduction is either absent or rare.

11. Potentiating bioefficacy of biocontrol agents and development of integrate disease management of rhizome rot (*Pythium aphanidermatum*) of ginger (*Zingiber officinale*) (TH 122)

Ms. N. Beena/ University of Calicut/2006/Guide: Dr. Y.R. Sarma

In the present investigation, efforts were made to enhance the bioeffieacy of the biocontrol agents used in the management of rhizome rot and integrating them with chemical control and other biocontrol agents to develop a blue print for further integrated disease management strategies. A survey was conducted in five major ginger growing areas of Kerala *viz.* Ernakulam, Kottayam, Kozhikode, Idukki and Wayanad districts. About 150 fields were surveyed and healthy as well as diseased samples were collected from the fields visited. One hundred and seventy seven (177) biocontrol agents which included 68 bacterial and 109 isolates of *Trichoderma* species were isolated by plating the healthy root samples on different culture media. The bio efficacy of the organisms isolated were tested rapidly by the dual culture plate technique. Of the 109 isolates of *Trichoderma* only 57 were found promising in their potential to suppress the pathogen. Of these 19 were identified as *Trichoderma virens,* four as *Trichoderma aureoviride* and 34 were found to *Trichoderma harzianum.* A comparison of the developed strains with the wild type *T. harzianum* indicated that the albino mutant, M, -25 excelled all other strains in all the parameters like increased rhizosphere competence, competitive saprophytic ability, increased enzyme activity and yield. This clearly showed the superiority of mutants in terms of an enhanced bioefficacy. The leads obtained in the present investigation justifies the biopotential of the strains developed by mutagenesis. There is a need to field evaluate these mutants for their disease suppressive potential. The results obtained in the present study clearly opened up the avenues for strainal improvement. It highlighted the role played by the mutagens in enhancing the biopotential of the antagonists. This opens up new vistas in disease management of rhizome rot as the strainal improvement, like varietal improvement could be opted for as one of the disease management strategiy.

TURMERIC

1. Diversity and characterization of curcumin biosynthetic genes and transcription factors from *Curcuma* spp (TH 199)

Ms. P. Prashina Mol/University of Calicut/2022/Guide: Dr. T.E. Sheeja

Transcriptome sequencing could be judiciously used as a cost-effective method for developing genomic information in non-model species. RNA-seq data generated in our study enable identification of DEGs involved in specific pathway of curcumin, paralogs of genes involved in the pathway as well as identified molecular biomarkers for curcumin. A higher percentage similarity of transcriptome was observed with related member of Zingiberaceae. The functional annotation indicated maximum genes grouped under ATP binding, translation and integral component of membrane allowing identification of novel genes involved in curcumin biosynthesis. A large number of PPP genes directly related to curcumin showed differential expression in high and low curcumin lines and correlated well with curcumin levels. These genes were shortlisted by us as associated to curcumin biosynthesis. This, study has focused on only two important genes PAL and OMT due to limitations in time and resources. The candidate isoform protein molecules involved in the pathway were identified by co-expression analysis that correlated gene expression to curcumin content under different field experiments. Invariably all the identified candidate genes correlated with curcumin under all conditions. These genes were cloned fully, structure of gene and protein analysed by *in silico*, and docking studies, to further support the co-expression analyses. The study enabled optimization of several techniques like SMARTER PCR, RACE PCR, co expression models etc. which may be extended to other candidate genes and crops.

2. Genetic variation, genotype-environment interaction and molecular geneticd diversity analysis for yield and curcuminoids in turmeric (*Curcuma longa* L.) (TH194)

Ms. S. Aarthi/Tamil Nadu Agricultural University/2018/
Guide: Dr. J. Suresh, Co- Guide: Dr. D. Prasath

The aim of the present investigation was to assess the morphological variation, extent of variability, heritability, correlation structure, stability analysis for yield and quality components, variation in curcumin, essential oil constituents over

location and molecular diversity in selected turmeric genotypes. The genotypes characterized as per DUS guidelines for qualitative and quantitative characters revealed considerable variation for coma bract colour, rhizome colour and rhizome characters. The specific characters apart from DUS character like collar girth, leaf lamina pigmentation, emerging shoot pigmentation were identified and can be used as a morphological marker for genotype identification. The comparison of mean performance of 15 genotypes for 27 traits using critical differences revealed existence of high level of variability. The genotypes, BSR 2, Acc. 849 and Rajendra Sonia registered the highest yield across the environments. Certain genotypes performed good in particular environment and even found superior. IISR Prathiba, Varna, Rajendra Sonia and Megha Turmeric 1 performed best in Appangala location; IISR Pragati and Rajendra Sonia were best at Peruvannamuzhi location. At Coimbatore location, BSR 2 and CO 2 performed well in terms of yield. Based on per se performance for high dry recovery Suvarna, SC 61, Acc. 849, Varna, Narendra Haldi 98, Megha Turmeric 1 and Rajapuri performed better. SLP 389/1, IISR Prathiba, IISR Pragati, Rajendra Sonia, CO 2, Duggirala Red, Megha Turmeric 1 recorded significantly high oleoresin with more than 10%. Essential oil was found significantly high in Suvarna, SLP 389/1, Varna and Megha Turemric 1. The genotypes, IISR Prathiba recorded the highest curcumin and were on par with SLP 389/1, IISR Pragati, Duggirala Red, Rajendra Sonia, BSR 2, CO 2 and Punjab Haldi 1 based on pooled mean data. High GCV combined with high PCV was observed in collar girth, length of mother rhizome, number of mother rhizome, weight of mother rhizome, weight of primary rhizome, number of secondary rhizomes, primary rhizome inter-nodal length, BDMC, DMC, curcumin and yield. High heritability coupled with high GAM was recorded in plant height, number of shoots, leaf petiole length, leaf length, collar girth, length of mother rhizome, girth of mother rhizome, primary rhizome inter-nodal length, dry recovery, oleoresin, BDMC, DMC and curcumin. The simple correlation coefficients showed that yield was significantly associated with collar girth and rhizome characters *viz*., weight of mother rhizome, number of primary rhizomes, weight of primary rhizome and number of secondary rhizomes. The path coefficient analysis at phenotypic level revealed that, positive direct effect was high for length of mother rhizome, followed by, number of mother rhizomes, weight of primary rhizome and curcumin content. Based on AMMI stability analysis, IISR Prathiba, Narendra Haldi 98, Rajendra Sonia, Acc. 849, BSR 2 and CO 2 found stable with less interaction and also recorded high mean yield across environments.

Stable genotypes for curcumin were BSR 2, CO 2, IISR Prathiba and Duggirala Red with values nearing to zero which indicated that they possess comparatively less interaction and were less sensitive to environmental interactive forces. The GCMS analysis for essential oil constituents of 15 genotypes revealed that, the major volatile compounds obtained were zingiberene, β sesquiphellandrene, ar-turmerone and curlone (β-turmerone). IISR Prathiba and Duggirala Red recorded highar-turmerone + turmerone. Zingiberene was recorded high in Acc. 849 and Narendra Haldi 98. Curlone was recorded high in SC 61 and Rajapuri. The minor compounds identified based on GCMS were α-phellandrene, 1,8-cineole, α-terpinolene, α-santalene, α-bergamotene, caryophyllene, β-santalene, β-farnesene, α-humelene, α-curcumene and β-bisabolene. α-santalene was recorded high in Megha Turmeric 1 and Suvarna. α-humelene was recorded high in Acc. 849 followed by Narendra Haldi 98 compared to other genotypes. RAPD and SSR markers were used to elucidate the diversity among 15 turmeric genotypes comprising land races, released varieties and promising genotypes. The Polymorphic Information Content (PIC) value as a relative measure of polymorphism level among the polymorphic primer ranged from 0.23 (OPK 7) to 0.98 (OPG 11) in RAPD markers and 0.13 (CUMISAT 34) to 0.78 (CUMISAT 28) among SSR markers. Among the all primers studied, OPL 12 and CUMISAT 33 were able to distinguish Acc. 849 and Narendra Haldi 98 from other genotypes. CLEST 7 and CUMISAT 19 distinguished IISR Prathiba. OPB 8 and CUMISAT 10 distinguished SLP 389/1 from other genotypes. Based on this similarity index, dendrogram for 15 genotypes was constructed and grouped into seven clusters at 0.62 similarity coefficient. The clustering pattern based on molecular characterization revealed the diversity of the genotypes. The finding concluded that the genotypes studied showed wide variability for many traits. A close examination of phenotypically stable genotypes for various traits revealed that the genotypes IISR Prathiba, CO 2 and BSR 2 were found to be stable for both curcumin and yield over the environment. Such genotypes may be used in the trait specific advanced breeding programme.

3. Diversity and characterisation of a few *Curcuma* genetic resources (TH 193)

Ms. P. K. Sajitha/University of Calicut/2017/Guide:Dr. B. Sasikumar

Zingiberaceae is an important family in the plant kingdom and many members of the family are used in fresh and processed form for medicinal, ornamental, aromatic and aesthetic purposes. *Curcuma* is an important genus of family Zingiberaceae, composed of 70-80 species of annual or perennial herbs. The genus

consists of economically and medicinally important species, characterized by volatile oils and oleoresins, well known for their broad range of pharmacological and culinary uses. Genus *Curcuma* is reported to display diversity in habitat, morphology, biochemical and ethno-medicinal use. Intraspecific variation for various taxonomically important characters and biochemical traits has already been reported. The intraspecific variation observed in the *Curcuma* species was believed to be influenced by both genetic and non-genetic factors. Diversity studies are often carried out in *Curcuma* species to assess relationship and genetic variability among germplasm in order to conserve the genetic resources and for crop improvement. The classical approaches for analysing genetic diversity includes morphology, comparative anatomy, physiology, embryology etc. that have been often complemented with molecular marker-based studies. *Curcuma* species such as *C. amada*, *C. aromatica, C. caesia* and *C. xanthorrhiza* are reported to have multifaceted properties including ethno-botanical values in traditional systems of medicines, cosmetics and culinary uses. These species are rich source of bioactive secondary metabolites which are responsible for its pharmacological uses. Previous studies have revealed intraspecific variation in morphological characters and biochemical traits in these underutilized economically important species. The present study was an attempt to evaluate the genetic diversity present among the four economically important *Curcuma* species (*C. amada, C.aromatica*, *C. caesia* and *C. xanthorrhiza*) using morphological, biochemical and molecular parameters with special reference to intraspecific diversity. To understand the diversity in different population, accessions from the four species were grouped into different population and studied. In order to assess the effect of maturity on growth, yield and quality traits, a phonological study in *C. amada* and *C. aromatica* at three growth stages has also been carried out. Besides biochemical characterization of primary and secondary metabolites, qualitative analysis of starch from four *Curcuma* species has also been carried out with special reference to starch yield and their structural characteristics and some physicochemical properties to bring out the potential use of *Curcuma* species as an alternative starch source.

4. Transcriptome and proteome analysis of the curcuminoid biosynthetic pathway in turmeric (*Curcuma longa* L.) (TH 191)

Ms. K. Deepa/University of Calicut/ 2017/Guide: Dr. T.E. Sheeja

Turmeric (*Curcuma longa* L.) is highly valued as a spice and known for its medicinal properties from time immemorial. Although most of the therapeutic

properties of turmeric are attributed to the presence of non-flavonoid polyphenol called curcumin, studies on biosynthesis of curcumin and factors affecting its biosynthesis is still in its infancy. The biosynthesis of curcumin takes place via phenyl propanoid pathway. In turmeric, curcumin is present in very low quantity and its accumulation was reported to be influenced by both genotypic as well as environmental factors, which is a major constraint for the spice industry. In order to increase the yield and stability of curcumin accumulation, there is a need to have an in-depth knowledge of both structural as well as regulatory genes of curcumin biosynthesis pathway. In this context, the present work was undertaken to isolate and characterize the key genes/enzymes involved in curcumin biosynthesis. The turmeric rhizome being a recalcitrant tissue and in our present study extraction of functional total RNA is a prerequisite for gene expression and transcriptome analysis, four RNA isolation protocols reported from recalcitrant tissues were evaluated to extract RNA from leaf and rhizome tissues. All the protocols worked well for leaf tissues but, either completely failed or yielded low quality/quantity RNA in case of rhizome tissues. Hence a rapid SDS-acid phenol: chloroform-based protocol was optimized to extract RNA from rhizomes of turmeric. The optimized protocol was compatible in extracting RNA not only from rhizomes but also from leaf, pseudostem and root tissues of turmeric. The protocol worked well in isolating total RNA from turmeric tissues stored in RNA later and from rhizomes of related Zingiberaceae members. The extracted RNA was well-suited for gene expression analysis, ds-cDNA library construction, next generation sequencing and low molecular weight RNA isolation. To develop a resource enriched with full length cDNA from four-month-old turmeric seedling, a normalized ds cDNA library was synthesized from pooled turmeric tissues. Concurrently, rhizome specific transcriptomes developed from turmeric with high curcumin content and wild turmeric (*C. aromatica*) of zero curcumin content on Illumina HiSeq 2000 platform were compared to identify the transcripts related to curcumin biosynthesis. All the reported candidate genes of curcumin biosynthetic pathway were mined from both the transcriptomes. Surprisingly, none of the reported genes of150 curcuminoid biosynthetic pathway showed significant variation in expression even though the transcriptomes were developed from *Curcuma* species with contrasting curcumin content. However, the transcriptomes were rich in differentially expressed novel polyketide synthases and transcription factors. Twelve PKS transcripts up-regulated in turmeric and transcription factors belonging to thirty nine transcription factor members including 406 transcripts orthologous to R2R3

MYBs were identified from turmeric transcriptome. Since the identification of reference gene is mandatory for accurate gene expression analysis, six reference genes were analyzed for stable gene expression in different tissues of turmeric under three developmental stages. EF1α and UBIQUITIN were selected as stable reference genes for normalizing the expression of target genes in the present study. The expression of ten reported genes of curcumin biosynthesis pathway namely; PAL, C4H, 4CL, C3H, HCT, COMT, DCS, CURS1, CURS2 and CURS3 and eleven differentially expressed transcripts mined from transcriptome analysis including polyketide synthases and transcription factors were analyzed in two turmeric genotypes with contrasting curcumin content from three different experimental conditions. The expression of C4H, 4CL, DCS, CURS1, CURS3, ClPKS a-d and WRKY were up-regulated in high curcumin genotype (IISR Prathibha) and correlated with curcumin content. Moreover, the expression of C4H and ClPKS a-d were significantly correlated with curcumin content. An MYB transcript orthologous to transcriptional repressor (At MYB4) was down-regulated in IISR Prathibha when analyzed from curcumin favourable condition (Kozhikode-field) and was up-regulated in stress conditions (Kozhikode-green house and Coimbatore-field), where the curcumin accumulation was lower, suggesting the probable role of MYB transcript as a transcriptional repressor. Polyketide synthases (PKSs), being the key enzymes of curcumin biosynthesis, full length coding sequence of key PKSs in curcumin biosynthetic pathway, namely DCS, CURS1 and CURS3 were amplified from genomic DNA. Sequence analysis showed that CURS1 and CURS3 have an intron of 121 bp and 88 bp respectively. DCS had two introns of 113 bp and 85 bp. Since ClPKSa transcript that correlated with curcumin content and showed maximum fold change in gene expression studies is a partial fragment (326 bp), the elucidation of its full length cDNA sequence is indispensible for post-genomics studies. A protocol for amplification of full length cDNA based on inverse PCR utilizing normalized full length cDNA library synthesized from pooled tissues of turmeric was 151 optimized for this purpose. The efficiency of protocol was analyzed by amplifying the full length cDNA of CURS3. The full length coding sequence along with UTRs of CURS3 was amplified. High degree of conservation in the coding sequence of CURS3 was observed when the sequences from three high curcumin genotypes, three low curcumin genotypes and two other *Curcuma* species with zero curcumin content; *C. aromatica* and *C. caesia* were compared. Notably, variation in the 3'UTR sequence is observed when the sequence of CURS3 was analyzed between *C. longa* and *C.*

aromatica. The amplification of full length cDNA of ClPKSa based on the optimized inverse PCR protocol identified that the transcripts ClPKS b-d shared similarity with full length sequence, suggesting that they can be either fragments of the full length sequence named as ClPKS11 or its isoforms. The ORF of novel PKS, ClPKS11 is 1176 bp, bordered by 5'UTR of 81 bp and 3'UTR of 262 bp corresponding to a deduced protein sequence of 391 amino acids with predicted molecular mass of 42.9 kDa and pI of 6.11. The factors affecting translation efficiency namely, uORF in 5'UTR and polyadenylation sequences in 3'UTR were also identified from both ClPKS11 and CURS3 sequences. The amino acid sequence of ClPKS11 shared only ≤ 80% identity with reported PKSs but with conserved catalytic triad (Cys164-His303-Asn336), a feature of plant PKSs and clustered with non- CHS type III PKSs. Interestingly, ClPKS11 showed uniqueness in amino acid residues lining cyclization pocket, substrate binding cavity, CoA binding tunnel and in terms of geometry shapers; suggesting its novelty in mechanism of action. ClPKS11 was predicted to be localized in cytoplasmic matrix with alpha helix structure as dominant structural component and has a predicted cavity volume of 1690 Å3. The reported substrates of turmeric PKSs namely; feruloyl CoA, p-coumaroyl CoA, feruloylydiketide CoA and p-coumaroyldiketide CoA interacted with predicted model structure of ClPKS11 with higher binding energies. Only one amino acid residue; Arg64 which is novel in ClPKS11 interacted with all these substrates, suggesting it may have a role in substrate binding and imparting novelty of ClPKS11. As observed for CURS3, the sequences of ClPKS11 are conserved in the coding region in *Curcuma* spp but showed variations in the intron sequence, which varied from 88-101 bp. Pre-mRNA analysis of ClPKS11 showed the absence of complete post transcriptional silencing activity as no pre- mRNA amplification was observed while mature mRNA was amplified from both turmeric genotypes of contrasting curcumin content with higher expression in high curcumin genotype. A sequence of 495 bp upstream of coding region of ClPKS11 was amplified from genomic DNA to identify the promoter sequence using restricted genomic DNA- inverse PCR method. TATA box and CAAT box promoter regions, transcription start site and cis-regulatory binding regions including those of transcription factors involved in secondary metabolism namely WRKY and MYB; especially the binding site of transcriptional repressor MYB4 were predicted from this upstream region. A phenol based protein isolation protocol was standardized and was found to be ideal for the study of differentially expressed proteins from a low curcumin and high curcumin turmeric

rhizomes using 2-dimensional gel electrophoresis. Based on the study 42 differentially expressed proteins were visually detected. The present method was ideal for the separation of the proteins in the 2nd dimension as the gels were free of any streaks and had a clear background. It is evident from the present study that biosynthesis of curcumin is a complex metabolic pathway and only a few PKSs were studied in detail till date. It is observed that Illumina sequencing can be applied as a rapid method for *de novo* transcriptome analysis of non model plants lacking genomic information. The transcriptome data generated in *Curcuma* spp. will accelerate research on the gene expression and functional genomics of turmeric. The identification and characterization of appropriate isoforms of curcumin biosynthetic pathway genes including phenylpropanoid biosynthetic genes, O-methyltransferases, reductases and hydroxylases is a prerequisite for unravelling the basic mechanism of curcumin biosynthesis. A novel polyketide synthase, ClPKS11 correlating with curcumin content was identified and its structural and *in silico* functional characterization was studied. The isoform analysis of ClPKS11 including the UTR and promoter regions in different tissues of turmeric and different genotypes with differential curcumin content and its functional characterization may pin point the role for this novel candidate in curcumin biosynthesis. This methodology may be adopted to identify, clone, characterise and determine the functional properties of other novel genes and transcripts identified by NGS. It is evident from the study that the accumulation of curcumin is influenced by genetic, environmental and developmental factors; suggesting the existence of a regulatory mechanism involved in manipulating the expression of structural genes of curcumin biosynthetic pathway. The present study could detect the presence of MYB binding sites in the upstream region of ClPKS11; suggesting MYBs may regulate the expression of structural genes. The transcriptomes identified several MYBs in response to stress as well as differential curcumin content. These transcription factors identified from turmeric transcriptome requires in-depth gene expression studies and coexpression analysis to identify the key players in this complicated regulatory pathway. The identification of proteins correlating with curcumin content by utilizing different model systems of contrasting curcumin content and using a gel free proteomics approach may deepen our understanding of the role of novel enzymes/transcription factors in this pathway. Thus the identification and characterization of key structural and regulatory genes in curcumin biosynthesis pathway and how the regulatory elements respond to internal and external factors and the mechanism by which they regulate structural genes needs to be investigated in future.

5. Exploring the potential of *Curcuma caesia* as an antitumor agent (TH 177)

Ms. Reenu Joseph/Kannur University/2017/Guide: Dr. Shamina Azeez

Rhizomes of the plant are aromatic with intense camphoraceous odor and are applied externally to sprains and bruises. Fresh and dried forms of rhizomes and leaves of *C. caesia* are used in traditional medicinal systems for centuries as a folklore remedy. Numerous *in vitro* and *in vivo* studies have demonstrated the pharmacological properties of *C. caesia*. The medicinal attributes of *C. caesia* is due to the presence of its phytochemical constituents. Much work has been conducted on the antioxidant activities of crude extracts of *C. caesia* and few reports on the cytotoxicity of different extracts of *C. caesia* rhizome revealed that the plant possessed significant cytotoxicity. Most of these studies were based on hot (Soxhlet) single solvent extraction either in fresh or dried rhizomes. Most researchers use Soxhlet apparatus to ensure near complete extraction, but this is not suitable for extraction of thermolabile compounds, as prolonged heating may lead to degradation of these compounds. Also to ensure the complete extraction of compounds of diverse chemistries, exhaustive serial extraction using solvents of increasing polarity is recommended. Taking this into consideration, the present study was undertaken to characterize *C. caesia* using biochemical and molecular tools; as also, to study the antioxidant and cytotoxicity effects of *C. caesia* sequential extracts and identification of the major bioactive principles in the accessions and promising extracts. Results from analysis of primary metabolites in *C. caesia* showed high content of primary metabolites namely starch and protein in rhizomes of *C. caesia* accessions. Acc. 751 significantly recorded maximum starch content (618.8 mg/g) which was at par with all accessions except Acc. 1135 (542.8 mg/g). Acc. 1171 recorded the highest total protein content (121.9 mg/g). The content of total soluble sugars and reducing sugars varied from 18.7 to 36.8 and 7.5-10.0 mg/g respectively. The highest content of soluble and reducing sugars was observed in Acc. 1135 (36.8 and 10.0 mg/g respectively). These metabolites are essential for the normal growth and development of the plant; proximate analysis provides valuable information and help to assess the quality of the sample. Secondary metabolites are useful in the long term defense of plants, and give plants their characteristics such as odor and color. The yield of essential oil varied between 2.2 and 3.0% in dry sample, highest being recorded in Acc. 751 (3.0%) which was at par with all other accessions except Acc. 292 which recorded the lowest percentage of essential oil, 2.2%. The essential oil of the black turmeric accessions primarily had bluish violet color with camphorous aroma which

changed to brown color on storage. GC-MS analysis of the essential oil compounds led to the identification of 29 components, constituting 70.67% of the oil. Of the peaks identified, sesquiterpenoids were the major constituents and the oil was found to be rich in epicurzerenone and 1,8-cineole which varied from 23.5 to 27.9% and 13.1 to 14.9% respectively. Acc. 1135 was the only accession with *ar*-turmerone and α-turmerone and that too in very low concentrations (0.6%). The oleoresin content ranged from 6.1 (Acc. 1171) to 7.3% (Acc. 1154). *C. caesia* is placed among the non-curcumin species of *Curcuma*, containing very less curcumin, and this is in agreement with the results from the present study where total curcuminoid ranged between 0.02-0.03 percent. High quality genomic DNA with high yield is a crucial pre-requisite step for molecular studies. Good quality DNA with high yield was isolated from fresh leaves and the yield ranged from 103-147 µg/g of tissue. Acc. 1006 gave the maximum yield of DNA (147.0 µg/g) followed by Acc. 1171 (142.0 µg/g). The minimum yield was recorded in Acc. 292 (103.0 µg/g). The most abundant phenolic acid in Acc. 292, Acc. 751, Acc. 1154 and Acc. 1171 was syringic acid. But the major phenolic acid in Acc. 1006 and Acc. 1135 was vanillic acid with an exception in fresh rhizomes of Acc. 1006 where ferulic acid was predominant. Similarly, the prevalent flavonoid in the accessions of *C. caesia* was catechin followed by naringenin and hesperetin. Gallic acid, protocatechuic acid, syringic acid, vanillic acid, ferulic acid, catechin, luteolin, naringenin, and hesperetin identified as the potent phenolics in *C. caesia* are potent anticancer agents for skin, colon, and lung cancers. These phenolics are present in significant amounts in *C. caesia* and their presence can be correlated with the anticancer properties exhibited by this species. Accordingly, *C. caesia* could be regarded as potential anticancer agent because of the presence of phenolics like hesperetin, luteolin and gallic acid and possible source of new therapeutics. The information from the present study will help in conducting further studies on determination of the efficacy of bioactive constituents by *in vivo* studies and demonstration of their safety and effectiveness in clinical trials.

6. Cloning and characterization of miRNAs from *Curcuma longa* (TH 171)

Ms. R. Santhi/Kannur University/2016/Guide: Dr. T. E. Sheeja

Turmeric (*Curcuma longa* L.) of the family Zingiberaceae is a rhizomatous perennial originated in Indo-Malayan region. Plant is mainly cultivated in India and other areas of Southeast Asia. Turmeric powder derived from the underground swollen stem, rhizome is widely used as a medicinal spice and food preservative, and as a cosmetic and flavoring agent. The most important and active secondary metabolite of

turmeric responsible for biological activities and yellow colour is curcumin. Use of curcumin in clinical trials against Alzheimer's disease is also reported. Therapeutic properties of curcumin including anti-carcinogenic, antioxidant, analgesic, antioxidant and anti-inflammatory activities have also been reported. Turmeric is also used as herbal medicine against skin cancer, wound healing, rheumatoid arthritis, liver disease and urinary tract infections. Traditional medicinal systems like Ayurveda, Unani and Siddha suggested the use of turmeric against a number of diseases. Turmeric oil also possesses anti fungal and anti-inflammatory activity. In our country, turmeric occupies a significant position, forming a central part of ceremonies, cuisine and rituals. India is reported as the largest producer, consumer and exporter of turmeric in the world. In India, turmeric production is hurdled by abiotic and biotic stress conditions, which are reported as growth and yield limiting factors. Most severe biotic stress, turmeric soft rot caused by *Pythium* is considered as a major threat to the cultivation of turmeric. The intricate agricultural characters like plant growth and yield are under the control of many alleles in different genes and complex molecular networks. Transgenic plant production on the basis of protein coding genes can only influence monogenic traits. Such simple monogenic traits do not need manipulation of complex molecular pathways. Stress factors have been regulated by more than one signaling pathways. Plants cope to adverse environmental conditions through various physiological and biochemical process. This is mainly achieved by reprogramming the expression of genes at the level of transcription, post-transcription and translation. Turmeric plant is conventionally propagated vegetatively through mother or finger rhizomes with one or two buds. In some locations it is reported to set seeds very rarely. Morphological variability was also observed in good amount in many cultivated types of turmeric due to vegetative mutations or environmental effects. Asexual propagation and high stigmatic incompatibility prevent conventional breeding in turmeric and therefore biotechnological tools gain importance. Recent studies suggest that process of gene reprogramming is mainly controlled by newly discovered molecules called small RNAs. Small RNAs are known to regulate their target mRNA and direct the modification of DNA and m histones. Based on the mode of biogenesis and function small RNAs are broadly classified into microRNAs (miRNAs) and small interfering or siRNAs. miRNAs have been reported as the best characterized class of small RNAs miRNAs are endogenous, 18-24 nt class of small regulatory RNAs that regulate post transcriptional expression of genes either by target mRNA cleavage or translational

repression. Through its negative regulatory mechanism miRNAs play important roles in plant growth and development, metabolism, hormone signaling, nutrient homeostasis, small RNA metabolism, maintenance of genome integrity and biotic and abiotic stress responses. The main class of miRNA targets includes transcription factors and other proteins playing key roles in plant development and signal transduction. Hence miRNA characterization has been reported as a dynamism area of research. In order to gain complete understanding of small RNA based regulatory mechanism in turmeric, identification of entire set of miRNAs is essential. In plants miRNAs have been identified from various plant species including model organisms like Arabidopsis and rice. There was no systematic report on identification and characterization of miRNAs from turmeric. Hence the present study aimed discovery, target prediction and validation of miRNAs from turmeric. The study also analyzed the expression profile of miRNAs in response to drought. miRNAs identified from the present study would enhance the database of miRNAs from turmeric in addition to offering basic and important information for additional functional characterization of miRNAs related to different aspects like plant growth and stress response. Detailed understanding of regulatory mechanism of genes related to different agronomic characters will also help to use novel gene resources like miRNAs for crop improvement in the near future.

7. Etiology and disease management of rhizome rot in turmeric (*Curcuma longa* L.) (TH 148)

Mr. K. Anoop/Mangalore University/2011/Guide: Dr. R. Suseela Bhai

A detailed survey on rhizome rot disease of turmeric in major turmeric growing tracts of south India showed a wide diversity in the cultivation practices. During the survey, a total of 37 different locations in 12 districts of South India were covered. Differences were noted on cropping system, practices of crop rotation and crop protection strategies. Generally, two system of cultivation was followed *viz*., bed system under rainfed condition (Kerala) ridges and furrow system under irrigated conditions (Andhra Pradhesh, Karnataka and Tamil Nadu). All the farmers included in the survey followed crop rotation and used the traditionally cultivated crops. The farmers use both organic inorganic fertilizers and plant protect ion measures. But one of the most important practices of crop protection, i.e. seed rhizome treatment is not found to be followed by any of the farmer included in this survey. According to the information collected, the disease incidence often coincided with the monsoon season of the respective locations.

8. Development of micro satellite markers in *Curcuma longa* L. and its cross-species amplification (TH 145)

Mr. Siju Senan/Mangalore University/2011/Guide:Dr. A. Ishwara Bhat

The cross-species amplification potential of turmeric SSR markers were tested in 13 *Curcuma* species. All the 17 EST -SSR markers were completely transferable to related *Curcuma* species. Out of 40 genomic SSR markers, 37 were completely transferable and polymorphic. Further cloning and sequencing of alleles generated by a randomLy chosen genomic SSR marker (CuMiSat- 17) confirmed the cross species transfer and presence of microsatellites in related *Curcuma* species. Fifty two SSR markers (17 EST-SSR markers + 35 genomic SSR) were selected for characterizing *Curcuma* species based on their complete transferability and clear banding patterns. The grouping was done using EST-SSR markers alone, genomic SSRs alone and in combinations. The dendrogram based on EST-SSRs showed slight variation from that of genomic SSRs and the combined analysis. Cluster analysis done using the pooled SSR markers revealed 100% similarity between *C.malabarica* and *C. zedoaria.* These two species generated identical finger prints with all the SSR markers generated in the study. Genetic uniformity revealed among turmeric accessions collected from diverse geographical locations suggests a revisit to the germplasm collecting strategy based on vernacular identity. Out of the eight clones, a 207 bp cDNA encoding 69 amino acids showed sequence similarity with the class I chitinase from a coniferous plant. The second clone encoded a 313 bp cDNA encoding 93 amino acids and was found to be significantly similar to several class I and class II chitinases from various plants. The clone showed a maximum of 66% similarity with class I and II chitinase from bell pepper, potato, garden pea, tobacco and *Medicago.* This is the first study on the isolation and characterization of chitinase in the genus *Piper.* Based on the above results, the following future line of work is suggested. The immediate objective is to isolate the full length chitinase gene using suitable molecular biology techniques. Once the full length chitinase gene is isolated, they can be transformed into desirable plants for enhancing the defense response against devastating pathogens.

9. Molecular, biochemical and morphological characterization of selected *Curcuma* accessions (TH 129)

Mr. S. Syamkumar/University of Calicut/2008/Guide: Dr. B. Sasikumar

The study on morphological, molecular and biochemical characterisation of fifteen *Curcuma* species, thirty-six turmeric varieties/cultivars were conducted. All the

grouping patterns (morphological, molecular and biochemical) revealed that the entities recognized now as *C. zedoaria* and *C. malabarica* in India may be synonyms as these two species showed very high similarity between them. Similarly, the separate species status given to *C. raktakanta* and *C. montana* also warrants revisiting as these entities also clustered together in all the dendrograms. The entity recognized as *C. zedoaria* in India and Japan may be one and the same and distinct from the Chinese population of *C. zedoaria* as revealed by the 18S rRNA studies. Three turmeric cultivars namely 'Amalapuram', 'Amrithapani' and 'Armoor', all from Andhra Pradesh known by vernacular identity, showd very high similarity among them. This implies that the cultivars recognized as 'Amalapuram', 'Amrithapani' and 'Armoor' may not be genetically distinct. For bioprospecting of pharmacologically important constituents/ molecules the species such as *C. aromatica* (essential oil), *C. longa* var. 'Prathibha' (oleoresin), *C. longa* Kedaram' (curcumin I), *C. longa*. 'Prathibha' (curcumin II), C. *longa*. 'Basal' Along' (curcumin III), and *C. sylvatica* for turmerin are found ideal.

NUTMEG

1. Biochemical variability in nutmeg (*Myristica fragrans*) and related taxa (TH 123)
Ms. K. M. Maya/University of Calicut/2005/Guide: Dr. T. John Zachariah

Nutmeg and mace are used for extracting essential oil by steam distillation. The essential oil is colourless or pale yellow coloured with the characteristic spicy odour. Major constituents are sabinene, [3-pinene, dipentene, p-cymene, d-linalool, terpinen-4-ol, terpineol, geraniol, safrole, eugenol, isoeugenol, myristicin, myristic acid, esters of myristic acid and other fatty acids. Myristicin in the oils of nutmeg and mace is believed to be responsible for the toxicity of nutmeg. Oleoresin consists of both volatile and nonvolatile components. Levels of total carbohydrate, starch, reducing sugars and leaf-protein are all on par in cultivated and wild taxa. Fat content in nutmeg (*M. fragrans*) is about 40%. Only M. *prainii* had the nearest fat content compared to *M. fragrans.* In case of the mace samples, *M. fragrans* and *M. malabarica* had high fat compared to *M. prainii.* Cultivated and wild taxa had the same fat in the rind in all the cases. The rind of *M. beddomeii, M. malabarica* and *M. prainii* had crude fibre content similar to that of *M. fragrans* (21 %). Essential oil is high in the leaf of *M. fragrans*when compared to the wild taxa. Among the wild taxa, only *M. beddomeii* has significant

content of oil (0.125%). Gas Chromatographic-Mass Spectral (GC-MS) study of leaf oils of *M. fragrans* and *M. beddomeii* revealed some important findings. *M. beddomeii* is very rich in β caryophyllene (40%). *M. fragrans* leaf oil contains 2.6% of ~ caryophyllene, 30.85% of myristicin and 1.32% of elemicin. M. *beddomeii* lacks myristicin, elemicin, a- and *β* pinene. Phenylalanine was found to be the dominant amino acid in all the different species except *Knema andamanica,* which is very rich in threonine and alanine. Its alanine content is very high compared to other wild plants. From the study, it can be concluded that, biochemically, the cultivated and wild taxa are distinctly different. The dissimilarity is very profound in essential oil content, its profile, fat, minerals, amino acid profile, etc. The study also concludes the uniqueness of male and female lines having uniform oil and amino acid profile.

CINNAMON

1. Phylogenetic and commodity authentication studies in *Cinnamomum* pp. and *Myristica* spp. (TH 187)

Ms. V. P. Swetha/University of Calicut/2017/Guide: Dr. B. Sasikumar

Spices are high value compounds traded in the form of whole spices, powders, essential oils or their extractives that assume importance in the international trade and are extensively used in food, medical and cosmetic industries both within the country and outside. The high cost coupled with the low volume of spices have resulted in its adulteration with closely related inferior plant based adulterants that may pose health hazards to consumers in addition to the various synthetic adulterants. Identification of genuine commodity from the adulterant becomes difficult once the commodity loses its morphological diagnostic features on powdering, drying and storage. Hence adulteration detection has now become a matter of primary concern. Testing for the presence of plant based adulterants were initially carried out using physical and chemical approaches that were later replaced by molecular marker techniques like DNA barcoding owing to their sensitivity and specificity. Cinnamon (*C. verum*) and nutmeg (*M. fragrans*) belongs to genus *Cinnamomum* and *Myristica*, respectively, which are characterised by more than 100 species. They are two important aromatic tree spices that have high economic significance. *C. verum* is reported to be adulterated with its closely related species, *C. aromaticum* and *C. malabatrum* while *M.*

fragrans is reported to be adulterated with *M. malabarica*. The present study was an attempt to detect the presence of adulterants in traded samples of cinnamon bark and nutmeg mace using the barcoding genes *viz*. rbcL, matK, psbA-trnH and ITS. Attempts were also made to elucidate the relationship of *C. verum* and *M. fragrans* with other species of genus *Cinnamomum* and *Myristica*, respectively.

GARCINA

1. A comparison on morphology, biochemistry and molecular markers of Indian *Garcinia* spp. in relation to geographical variation (TH 159)

Mr. O. P. Nandakishore/Mangalore University/2014/

Guide: Dr. Utpala Parthasarathy

Genus *Garcinia*, belonging to family Clusiaceae, is an underutilized crop mostly grown (in wild. Lack of awareness of the economic and nutraceutical utility of the crop and habitat destruction has already resulted in the extinction of several species in India. Hence, In the present study, nutritional properties of the fruits and the physico-chemical properties of seed kernel butter and tree bark exudates were studied. Further, as the species are reported 10 be difficult to identify and taxonomy purely based on morphological characters, an attempt was done to identify suitable molecular markers to help in identification of the crop and to study genetic relationships. The habitat aspects were analysed to understand the optimum growth conditions for the species and to predict the suitable domains of *Garcinia* in India using GIS tools. In this study, *Garcinia* species were collected from the two geographically separated Distinct ecosystems, namely Western Ghats and N.E Himalayas. Though the climates of both ecosystems were different, GIS studies indicated the weather patterns of *Garcinia* adobes to be similar. Further, the optimum climatic parameters namely temperature, rainfall, altitude and the soil types were predicted and using the growth parameters required for *Garcinia,* it's domains In India were predicted. Richness and diversity indices were estimated and the geographical features of maximum diverse grids were analyzed. *Garcinia* species showed a great variation in the plant morphology, with significant variation In canopy features, leaf shape and fruit morphology. Biochemical analysis indicated that organic acids were the major compounds present In the fruits, followed by carbohydrates and proteins. HCA and malic acid were the predominant acids. Vitamin and mineral contents

of *Garcinia* were similar to several tropical fruits. Further, the antioxidant activities of the fruits were also studied. Seed kernel contained a high quality fat source, known as *Garcinia*-butter which was solid at room temperature like ghee and hydrogenated fats, while the chemical properties and the levels of unsaturated fatty acids were similar to that of common plant oils like olive and sunflower. Barks produced yellow exudates, which were solidified to hard opaque brown substance. Exudates were resinous gum type and Xanthones were the major compounds present in the exudates. To study the molecular variation among the species, (19) ISSR and (12) RAPD markers were used. The molecular profile characters indicate that the ISSR primers selected for the study were suitable in determining the diversity among the species.

2. Biochemical, molecular and spatial (GIS) variability in *Garcinia* species with special reference to *Garcinia gummigutta* (L.) Robsn. and *Garcinia indica* Choisy (TH 147)

Mr. G. R. Asish/ Mangalore University/2011/Guide: Dr. T. John Zachariah

Garcinia is one of the potential under exploited multipurpose crops and recently gained a lot of attention as a popular means of weight lose because of the presence of (-) hydroxy citric acid in its fruit. Out of the 35 species found in India, 17 are endemic out of which 7 are available in Western Ghats. The increase in the level of endemism from 50% to 60% is an important indication of the shrinking population of these species in Western Ghats region. Expanding market size and product range of *Garcinia* have been visualized in the near future, hence issues related to chemical composition, methods for quantification, diversity and distribution among *Garcinia* spp. assume a greater importance. There are distinct morphological variations exist among the species of *Garcinia* in Western Ghats *viz. G. gummigutta, G. indica, G. tinctoria* and *G. cowa.* It is essential to study the biochemical and molecular characters of these species to get an overall idea about the crop. Domestication and improvement of the species will be easy if the detailed profile is known. Two main species of *Garcinia* such as *G. gummigutta, G. indica* were noticed in large number during the survey. The species *G. tinctoria* was found only in Coorg areas of Karnataka. At the time of collection only one hugeestate of wild *G. gummigutta* was noticed in Thirunelveli (8° 59' Lat, 77° 18'Long) located in southern part of Western Ghats region. The prepared collection map based on longitude and latitude of collection sites indicates that the density of *Garcinia* spp. is more in the central region (14°58' Lat to12°03' Lat) of Western Ghats than the rest of the area. Conventional method for the quantification of (-)

HCA from *Garcinia* extract is titration with phenolphthalein indicator and this method of assay of (-) HCA in *Garcinia* extracts has the limitation of interference by other organic acids present in the samples. However, the percentage of (-) HCA from the leaves, fresh and dry rinds of *Garcinia* spp. were quantified exactly by the present HPLC method using sodium sulphate as mobile phase and found comparatively high in *G. gummigutta* and almost nil in *G. tinctoria.* It is also possible to record the exact percentage of (-) HCA in commercial samples by this HPLC method and can trace about any possible adulterations of market sample if any. A dendrogram (SPSS) was also prepared based on the leaf and fruit (-) HCA percentage to understand the distribution of (-) HCA with different geographical area. It was not found any locational influence but the species wise variation was specific in this dendrogram. In order to study the inter species and intra species genetic relation, DNA isolation protocols are needed to be optimized in *Garcinia* species. Till no attempts have been made to apply the molecular techniques to screen the intra and inter specific variability in *Garcinia* spp in India so far. This study is the first report of molecular characterization in *Garcinia* spp available in Western Ghats. Optimum DNA isolation was possible with 4% CTAS (100Mm Tris, 30 mM EDTA 1.4 M NaCl) followed by 1.5% PVP and 0.3% mercaptoethanol in leaves, but in fresh fruit rinds it was with 2% CT AS (100 Mm Tris, 30 mM EDTA 1.4 M NaCl) by 1.5% PVP and 0.3%. NTSys-pc-UPGMA dendrograms of *G. gummigutta* and *G.indica* were prepared separately and these dendrograms were compared with the BIOCLIM models of altitude, rainfall and temperature maps (20 km x 20 km grid) of G. *gummigutta* and *G.indica* prepared with the help of DIVA-GIS to study the impact of environmental factors associated with molecular grouping. The molecular distribution among *G. gummigutta* and *G. indica* was found similar. The accessions collected from its natural origin were grouped together. Those accessions collected from other parts of Western Ghats were grouped separately. It was clear from the GIS analysis that, though rain fall and temperature do not have much influence on molecular variation, the altitude plays a key role in genetic of genus *Garcinia* in Western Ghats.

3. Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.) (TH 136)

Mr. P.S. Manoj/Kerala Agricultural University/2011/Guide: Dr. Sarah T. George

Mangosteen (*Garcinia mangostana* L.) is identified as one of the fruit crops deserving priority attention with a potential for increased acceptability. It is recognized as the ' queen of tropical fruits' due to its instant visual and taste appeal and has recently

been popularized for its medicinal benefits. It yields profusely and fits very well as a component in the homesteads of Kerala. Its slow growth and long gestation period limit its commercialization. Almost all trees are female and variability is meagre. The investigation on "Stimulation of growth and induction of variability in mangosteen (*Garcinia mangostana* L.)" was undertaken in the Department of Pomology and Floriculture, College of Horticulture, during 2006-2009 with the objective of developing techniques for accelerating seedling growth in the nursery, reducing gestation period and inducing variability through mutation and polyploidy. The effect of different growing media and growth regulators on enhancing seedling growth in the nursery revealed that vemicompost medium was the most superior in terms of all the growth and physiological parameters, foliar nutrient content and uptake of nutrients followed by coir pith, poultry manure and well rotten cow dung. Use of vemicompost medium without any additional growth regulators was sufficient to accelerate seedling growth in the nursery. When coir pith compost, poultry manure or well rotten cow dung were used as media, growth regulators had specific effect. Along with coir pith compost, IAA (Indole acetic acid) 300 ppm, SA (salicylic acid) 200 ppm and SA 300 ppm were ideal while IAA 150 ppm, IBA 150 ppm and SA 300 ppm were superior in poultry manure medium. Along with normal potting mixture, IBA (indole butric acid) 450 ppm, SA 200 ppm and IBA 300 ppm showed superiority. With the use of suitable media and growth regulators, seedling growth can be accelerated in the nursery and transplanting stage can be attained much earlier. Use of different growth promoting substances in normal potting mixture showed that application of nutrient solution 30: 1: 1 (NPK) -0.50 % and *Azospirillum* sp. (10 g per plant) were the superior treatments with respect to all the growth parameters. In two year old grafted plants in the main field, GA (gibberellic acid) 200 ppm + benzyl adenine (BA) 100 ppm was the best with respect to majority of growth para meters followed by GA 100 ppm + BA 100 ppm and GA 100 ppm. These growth regulators can successfully be used in early stages for promoting growth. In five year old juvenile orchard trees also GA, BA combinations namely GA 200 ppm + BA 100 ppm, GA 100 ppm + BA 100 ppm and GA 100 ppm + BA 200 ppm were the best treatments in accelerating the growth. For the induction of flowering, paclobutrazol 2.0 g a.i. per tree, GA 200 ppm + BA 100 ppm and GA 200 ppm + BA 200 ppm were superior and equally effective. For improving yield and yield attributes, paclobutrazol 2.0 g a.i. per tree was the most superior followed by GA 100 ppm + BA 100 ppm and GA 200 ppm. In incidence of gamboge was minimum in paclobutrazol treatments compared to GA, BA combinations. Among the various rootstocks tried, mangosteen was most

compatible with its own rootstock whereas all other rootstocks showed varying degrees of incompatibility. On comparing the growth of softwood grafts with seedlings, seedlings showed faster rate of growth. Seedling growth could also be promoted by the use of nurse stocks. Seeds exposed to 5 Gy to 50 Gy gamma radiation showed wide variation in gemination. Beyond 30 Gy, seeds failed to germinate. Seedlings from 30 Gy dose showed stunted growth indicating a possible genetic variation. Irradiation of scions with 5 Gy to 50 Gy hadan adverse effect on days required for sprouting of scions, percentage of sprouting and final graft success. All the irradiated scions showed stunted growth even after one year. Two seedlings treated with colchicine at 3.0 and 3.5 per cent in the apical bud showed vigorous growth and distinct variation in I growth characteristics. Five mangosteen strains with distinct morphological variation selected from irradiated and colchicine treated seedlings were subjected to RAPD analysis. Clustering of five strains based on dendrogram separated the strains into two groups. Clustering pattern of the strains indicated that seed irradiation with 25 Gy and 30 Gy gamma rays and bud application of colchicine 3.5 per cent were effective in inducing variation in genomic DNA of mangosteen.

VANILLA

1. **Development of transgenic vanilla (*Vanilla planifolia* Andrews) resistant to cucumber mosaic virus (TH 142)**

Mr. S.T. Retheesh/Mangalore University/2010/Guide: Dr. A. Ishwara Bhat

The present study was conducted with the objectives of elimination of viruses from naturally infected vanilla through meristem culture and production of transgenic vanilla expressing antisense RNA of *Cucumber mosaic virus* (CMV) coat protein (CP) gene. Vanilla (*Vanilla planifolia* Andrews) infected by *Cucumber mosaic virus* (CMV) and *Cymbidium mosaic virus* (CymMV) was freed from combined infection through meristem culture. Apical meristems measuring from 0.1 to0.25 mm were isolated and cultured in MS medium supplemented with 0.45 ~Mthidiazuron for 40-45 days to initiate the growth. Following the enlargement ofmeristem, it was transferred to MS medium supplemented with 4.43 ~M 6-benzyl adenine (BA) and 2.68 ~M a-naphthalene acetic acid (NAA) for regeneration. The regenerated plantlets were planted in cups filled with sterilepotting mixture and hardened in insect-free glass house. Elimination of viruses was confirmed by reverse transcription PCR (RT-PCR)

with virus specific primer pairs. The frequency of CMV elimination was 79.41% while that of CymMV was 82.35% when ascertained individually. Simultaneous elimination of both the viruses recorded a frequency of 75%. In the present study, coat protein (CP) gene of CMV infecting vanilla cloned in pPCR ScriptAmpSK (+) was released by double digestion with *BamHI* and SacI and ligated to the binary vector pBI121 in antisense orientation under CaMV35S promoter. The recombinant binary vector (pBI121CMVCP) was used to transform competent cells of *E. coli* strain DH5α by heat-shock method. After verification of insert orientation in pBI121CMVCP by PCR and restriction digestion, it was transformed into *Agrobacterium tumefaciens* strain EHA105 by freeze-thaw method. Of the 96 putative transgenic plants tested by PCR, 46 were randomly selected for Southern hybridization to confirm incorporation of T-DNA into genomic DNA and to determine the number of T-DNA insertion events per genome. Ten μg of genomic DNA was restriction-digested using NacI and size fractionated in 0.7% agarose gel by electrophoresis, subsequently transferred to a nylon membrane by capillary method. CMV CP was amplified in PCR, DIG labelled by random priming and used as probe. Out of 28 Southern-positive plants, 18 were randomly selected and screened for the transcript of transgene by northern hybridization. Total RNA was isolated from 300 mg leaf tissue and 20 μg was subjected to formaldehyde-agarose gel electrophoresis followed by transfer to Hybond N+ nylon membrane by capillary method. Expression of transgene was confirmed in northern blot where 16 of the 18 tested individuals showed the presence of transcript. The non-transformed control plant did not show presence of CMV CP antisense transcript as no band was detected in the northern blot. This further reveals that the transgene is stably integrated in the genome and is actively transcribed at least in 16 of the 18 plants tested.

2. Identification, molecular characterisation and development of diagnostics for the viruses associated with vanilla (*Vanilla planifolia* Andrews) (TH 132)

Mr. V. Bhadra Murthy/Mangalore University/2008/Guide: Dr. A. Ishwara Bhat

In this study 30 vanilla plants exhibiting different viral symptoms were collected from different geographical regions of Karnataka and Kerala and maintained through vegetative propagation in an insect-proof glass house. The present investigation revealed four viruses associated with vanilla in India. The viruses include BCMV, BYMV, CMV and CymMV. A vanilla plant exhibiting stem necrosis, mosaic, leaf chlorosis, and drying of aerial adventitious roots collected from Calicut, Kerala, was found to be infected with BCMV. Electron microscopy revealed flexuous

filamentous pallicles characteristic of potyviruses. Partial biological characterisation based on mechanical inoculation of the present vanilla isolate on to 19 plant species from five families showed that the virus infected *Chenopodium amaranticolor, Nicotina benthamiana, Vigna unguiculate* (cv. C-52, C-152, Kanakamani and Lola) and *Vanilla planifolia.* Hosts like *C. arietinum, G. max, C. ensiformis, Phaseolus vulagris, P. lunatus, C. gladiata, C. cajan, V. faba, V. unguiculata* (cv.CO-6) from Fabaceae did not take up infection. When total RNA isolated from the infected vanilla isolate was subjected to RT-PCR, primer pair AIB90/AIB91 which is specific for BCMV amplified at - 850 bp region of the virus. The resultant product was cloned and sequenced. The sequence contained 849 nucleotides in which the first 27 nucleotides correspond to the 3' end of NIb region while the remaining 822 nucleotides to the coat protein gene. The nucleotide and deduced amino acid sequence of the nucleotides to the coat protein gene was used in sequence analysis. The nucleotide and deduced amino acid sequence identities of the present isolate with different strains of BCMV ranged from 87-96% and 87-98% while it was 91 % and 93-94% with isolate/strains of BCMV from India. Maximum nucleotide identity of 96% was shared by isolate/strains 'Blk1' from Taiwan, 'Blk2' from Vietnam and 'FI' from USA while maximum deduced amino acid sequence identity of 98% was with' Blk1 ' which is a Black eye cowpea isolate of BCMV from Taiwan and 'CAMV', BCMV, infecting Yard long bean from Thailand. The highest identity of the present BCMV isolate showed closeness to cowpea isolates of BCMV. A vanilla sample exhibiting severe yellow mosaic on leaves with green islands and leaf deformation, collected from a vanilla plantation in Madikeri, Karnataka was found to be associated with BYMY. Primer pair A1B88/A IB89 specific for BYMY amplified a 950 bp region of the viral genome was cloned and sequenced. The sequence contained 951 bp of which the first 174 nucleotides correspond to the 3' end of Nib region while remaining 777 nucleotides correspond to the coat protein gene. The sequence containing 777 nucleotides that potentially codes for 259 amino acids was selected to carry out sequence analysis. Percent nucleotide and deduced amino acid identities of the partial coat protein sequence of the present BYMY isolate with other isolate/strains of BY MY from different geographical locations taken for comparison ranged from 80-98% and 85-98% while it was 80-97% and 87-95% with isolate/strains of BYMV from India. The present BYMY isolate shared maximum nucleotide identity of 98% with BY MY isolate/strains 'S22N' infecting gladiolus from Japan and 'Msdvl' on *Masdevallia* spp. from Germany, while maximum deduced amino acid sequence identity of 98% was

observed with isolate/strains 'S22N' infecting gladiolus from Japan. The percent identity of nucleotide and deduced amino acid sequence of the present isolate with two BYMY isolates from Karnataka (Doddaballapura 'VMO' and Shimoga 'YM23') infecting vanilla in India (unpublished), was found to be 97% and 95% respectively indicated that the present BYM V isolate (YP) from Madikeri, Karnataka might be a strain different from the BYMV from Doddaballapura and Shimoga isolates. These results also indicate different strain s of BYMY infecting vanilla in India. A vanilla plant exhibiting severe stunting, mosaic and leaf deformation was found to be associated with CMV. Primer pair A1B1/A1B2 (specific for CMV coat protein region), amplified a - 660 bp region of the viral genome was cloned and sequenced. The sequence contained 657 bp of that potentially codes for 218 amino acids. Sequence analysis revealed a 100% nucleotide and deduced amino acid sequence identity to the CMV isolate already reported that infect vanilla in India (AY754359). Vanilla plants exhibiting mild chlorotic streaks runing parallel to the venation on young leaves followed by mild necrosis on old leaves were found to be associated with CymMV. In the present study a RNA isolation protocol was developed and used for the detection of BCMV, BYMV, CMV and CymMV infecting vanilla in field conditions by RT- PCR. RT-PCR was carried out on 30 vanilla isolates from different geographical regions of Karataka and Kerala with primer pair specific for each of the four viruses. Results that 6% each of the vanilla samples were infected with BCMV, BYMV and CMV while 46% of samples were found infected with CymMV. Out of the two samples infected with CMV, one was found co-infected with BCMV while the other was co-infected with CymMV indicating occurrence of mixed infections in nature. Also, in this investigation two mRT-PCR reactions (one for the detection of BCMV and CMV, and the other for BY MY and CyMV) were standardized.

OTHERS

1. Detection of plant-based adulterants in selected market samples of spices using DNA barcoding technique (TH 180)

Ms. A. Parvathy Viswanath/University of Calicut/2017/Guide: Dr. B. Sasikumar

In its powdered form, turmeric [*Curcuma longa* L. (Zingiberaceae)], a spice of medical importance, is often adulterated lowering its quality. Objective: The study sought to detect plant-based adulterants in traded turmeric powder using DNA

barcoding. Accessions of *Curcuma longa* L., *Curcuma zedoaria* Rosc. and cassava starch served as reference samples. Three barcoding loci, namely ITS, rbcL, and matK, were used for PCR amplification of the reference samples and commercial samples representing 10 different companies. PCR success rate, sequencing efficiency, occurrence of SNPs, and BLAST analysis were used to assess the potential of the barcoding loci in authenticating the traded samples of turmeric.The PCR and sequencing success of the loci rbcL and ITS were found to be 100%, whereas matK showed no amplification. ITS proved to be the ideal locus because it showed greater variability than rbcL in discriminating the *Curcuma* species. The presence of *C. zedoaria* could be detected in one of the samples whereas cassava starch, wheat, barley, and rye in other two samples although the label claimed nothing other than turmeric powder in the samples. Unlabelled materials in turmeric powder are considered as adulterants or fillers, added to increase the bulk weight and starch content of the commodity for economic gains. These adulterants pose potential health hazards to consumers who are allergic to these plants, lowering the product's medicinal value and belying the claim that the product is gluten free. The study proved DNA barcoding as an efficient tool for testing the integrity and the authenticity of commercial products of turmeric.

2. Identification and sequencing of novel target genes in burrowing nematode (*Radopholus similis*) and docking studies with naturally occurring compounds (TH 164)

Ms. O.B. Rosana/Mangalore University/2016/Guide: Dr. Santhosh J. Eapen

Damage and toxicological tribulations led banning of highly efficient synthetic nematicides that are commonly used for management of nematodes. Promising approaches, involving specific control measures that are safe to the environment and human beings, are urgently needed to prevent proliferation of these destructive pests. Historically, multiple biological processes in nematodes have been targeted by synthetic nematicides for suppressing them. Bioprospecting of nematicidal natural compounds with precise mode of action targeting different biological functions and processes of nematodes could strengthen our arsenal against the devastating nematodes. Safety is an essential component of delivering a new anti-parasitic molecule; ideally, starting with nematicidal plant-derived compounds can contribute a lot in developing safer molecules. Therefore, identifying target proteins vital for nematode parasitism and survival is the first step in discovering nematode specific

compounds. Thus new mode of action chemistries for nematode management will be pursued by employing traditional phenotypic screening and target-based approaches. Eight potential target genes were identified from *Radopholus similis* ESTs; they were amplified and used for molecular modeling and molecular docking interaction studies with phytochemicals. Hundreds of compounds from 15 plants were subjected to *in silico* bioactivity screening; further, 158 phytochemicals with nematicidal potential were short-listed through molecular docking interaction studies. Subsequently, *in vitro* bioassays, LD50 calculation, gene expression studies, *in vitro* protein inhibition assay, bioassay for assessing nematode mobility, attraction and infectivity and *in planta* pot experiments were conducted to validate the efficacy of results obtained through *in silico* screening. The bioinformatics mediated approach backed up with experimental validations could confirm the potential of these natural compounds. They could be developed as nematicides or can be used as a model for synthesizing novel products that are environmentally safer and ideal for eco-friendly management of nematodes.

3. Isolation, characterization and evaluation of plant growth promoting bacteria for seed spices (TH 150)

Ms. Y. K. Bini/Mangalore University/2013/Guide: Dr. M. Anandaraj

Seed spices are those crops of which the fruit or seeds are used as spice. They are very important crops as they are used as spices, condiments and medicines, the world over. There is a great export potential for seed spices, as India contributes to 0. 83 million tonnes (55.7 %) of world trade. Although there are about 20 seed spice crops, coriander (*Coriandrum sativum* L.), cumin (*Cuminum cyminum* L.), fennel (*Foeniculum vulgare* M.) and fenugreek (*Trigonella foenum-graecum* L.) are most important. The population of the introduced organisms in these locations was monitored during the crop season. Application of PGPR at the time of planting lasted throughout the crop season and the population was found in rhizosphere. The effect of PGPR on the yield of coriander showed that irrespective of the climatic and soil conditions the use of PGPR significantly affect the yield of coriander. In all the centres the PGPR treated coriander yielded more compared to control. So also for other crops evaluated in farmers' field namely cumin, fennel and fenugreek. The farmers were convinced about the beneficial effects not only in terms of growth and yield but also reduced incidence of pests and diseases. After successful evaluation of PGPR technology in farmers' plots across the country the technology has been popularized by developing entrepreneur to take up the technology to the farmers. Thus the study on isolation, characterization,

identification and evaluation of PGPR for seed spice resulted in obtaining two efficient PGPR strains, FK14 and FL18. The evaluation of these PGPR for seed spices in various locations of India resulted in obtaining two promising PGPR strains *Pseudomonas putida* FK14 (FN257488) and *Microbacterium paraoxydans* FL18 (FN257488) for seed spice crops *viz*., coriander, cumin, fennel and fenugreek. Though PGPR offer an environmentally sustainable approach to increase crop production and health, their introduction into the phyllosphere, spermosphere or rhizosphere may be moderately effective or sometimes totally ineffective under field conditions. This may be due to the variation in climatic conditions that suppress growth and survival of biocontrol agents. However, in this study, the two strains of PGPR showed positive effects on seed spices suggesting that they are effective under varying climatic and soil conditions. The application of molecular tools for enhancing our ability to understand and manage the rhizosphere will lead to new products with improved effectiveness. Besides, the basic knowledge on molecular signalling mechanisms between related strains and species has to be understood for the development of a better formulation that could suppress a broad spectrum of pathogens and pests besides plant growth promotion. The results obtained from this study do indicate the possibility of developing a microbial consortium of the two PGPR strains for growth promotion and biocontrol in seed spices.

4. Development of PCR based methods to detect *Phytophthora capsici* from soil and plants (TH 144)

Ms. N. Sheji Chandran/Mangalore University/2010/Guide: Dr. M. Anandaraj

In the present investigation, PCR based detection methods were developed that are sensitive to detect the pathogen *P. capsici* from both infected tissue and soil.The DNA extraction methods standardized by addition of a step for removal of RNA has resulted in reducing the time required for DNA extraction and purification. Protocols were found to be very effective and rapid since it required only 2hrs for the whole procedure. It also proved to be simple as it did not require the use of expensive methods. This could be applied to process large number of samples at a time. Simple, rapid efficient and labour-effective protocols were standardised for the extraction of DNA from infected host tissues. The procedure of DNA isolation from host samples described in the study worked well for host materials such as root, stem, fruit and leaf of all infected plants like black pepper, cocoa, capsicum, Java long pepper and arecanut. Simple, rapid and efficient protocols were standardized for the extraction of DNA from soil samples by addition of skimmed milk at the grinding stage along with liquid

nitrogen. The quantity and quality of the DNA obtained by these methods were suitable for peR amplification and other molecular assays like restriction digestion, Southern hybridization reactions. ITS-I' DNA analysis the ITS-rRNA region of *Phytophthora* isolates were amplified using the universal primers ITS-6 and ITS-4 and obtained amplicon size ranging from 800 bp to 1000 bp. Variation in the amplicon size was observed within *P. capsici* isolates of black pepper and also for *P. capsici* of different host plants. RFLP finger prints clearly showed the existence of three genetic groups on black pepper and supported the existence of three subgroups within *P. capsici*. The results showed that ITS RFLP variation occurred within and between different geographical locations. The its A sequences obtained for 21 *Phytophthora* isolates consisted of the entire sequence of ITS I, ITS 2 and S.8S; partial sequences of 28 Sand 18S regions. All the r-DNA gene sequences were submitted to GenBank with the following accession numbers: AM422703, AM422704, AM42270S EUSISI67, EUSISI68, EUSISI69, EUSISI70, EUSISI71, EUSISI72, EUSISI73, EUSISI74, EUSISI7S, EUSISI76, FN2S7934, FN2S7938, FN2S7936, FN2S7937, FN257935, FN257939, FN257940, FN257942, FN257943. The *P. capsici* ITS-I' DNA sequences included nine from black pepper (*Piper nigrum* L); two form capsicum (*Capsicum annum* L); one each from java long pepper (*Piper chaba* Hunter); cocoa (*Theobroma cacao* L); nutmeg (*Myristica fragrans* Hoult). The *P. palmivora* sequences included one each from bougainvilla (*Bougainvillea buttiana* L) and coconut (*Cocos nucifera* L). *P. meadii* included two from cardamom (*Elletaria cardamom* Maton) and one from papaya (*Carica papaya* L). There was 169 a lone sequence from *P. nicotianae* from betel vine (*Piper betel* L). There was also a ITS-rDNA sequence from *Pythium cucurbitacearum* isolated from of black pepper rhizosphere. Development of species specific primers ITS-rDNA Based on this study molecular techniques for rapid detection of three *Phytophthora* species namely. *P. capsici, P. palmivora* and *P. meadii* were developed by designing species specific primers from ITS r-DNA sequences. Among *P. capsic* primers, primer II with 22mer forward and 24mer reverse could amplify 370 bp from all *P. capsic* isolates. The sensitivity of the specific primers was found to be 0.1 ng DNA for specific PC R and I pg in nested PCR. Genetic variation among *P. capsici* isolates from different locations was assessed using thirty RAPD markers. Cluster analysis of RAPD data of *Phytophthora* isolates differentiated *P. capsici* into three RAPD groups at 52 % similarity level. This study has enabled to develop a SCAR marker from RAPD analysis to detect *P. capsici* from host plants and soil. The methods presented here allowed

detection of species of *P. capsici* in soil and host tissues. This is the first report of RAPD SCAR maker to detect *P. capsici* from environmental samples. The nucleotide sequence data of the PCR clones obtained from SCAR marker in this study showed a short sequence similarity with virulence associated genes of *Phytophthora*. Based on this study oligonucleotide probes were developed from both ITS and RAPD SCAR, that can be used as a detection tool for *P. capsici.* The RAPD based probe and primer developed in this study can be used for development of detection kit for *P. capsici* from soil and plants. This is the first report of RAPD SCAR marker and probe to detect *P. capsici* from environmental samples in India.

5. Detection of probable plant based adulterants in selected powdered market samples of spices using molecular techniques (TH 138)

Ms. K. Dhanya/Mangalore University/2009/Guide: Dr. B. Sasikumar

The present study could develop PCR based methods to detect plant based adulterants in powdered spice samples. The extraction of DNA from food matrices for subsequent use in PCR is often considered to be a problem. The present work has shown that the DNA extracted from spice powders and their adulterants, by the methods developed/standardized, could be successfully used as template DNA in PCR with regard to its low operating cost and ability to distinguish between different plant species RAPD-PCR served as a useful starting point for adulterant detection in spice powders. Adulterant specific RAPD markers identified in the study were authenticated using simulated samples of spice powders and were also found helpful in detecting adulterants in marketed spice powders. Conversion of adulterant specific RAPD marker in to Sequence Characterized Amplified Region (SCAR) markers increased the reliability of the discrimination method. The SCAR markers specific to papaya seed (P1 and P2), *Piper attenuatum* (P3) and *P. galeatum* (P4) developed in the study could detect the presence of these adulterants in black pepper powder. The SCAR markers Z1 and B1 could detect *Ziziphus nummularia* and red beet pulp adulteration, respectively in chilli powder, Like wise the *Curcuma zedoaria* and *C. malabarica* specific SCAR markers, C1 and C3, could detect the presence of these wild species in turmeric powder. Out of the eight market samples of black pepper powder tested using SCAR markers, two samples were found to be adulterated with papaya seed. One out of six market sample of chilli powder tested was found to be adulterated with powdered *Z. nummularia* fruits and four out of the six market samples of turmeric powder was found to be adulterated with the rhizomes of *C. zedoaria*, *C. malabarica* thus unfortunately confirming the occurrence of

plant based adulteration in commercial samples of spice powders. All the SCAR markers developed in the study could detect adulteration even at a lower concentration of 10 g adulterant per kg of spice powder. The present study is the first report on the development of SCAR markers for the detection of adulterants in spice powders. This method is simple, rapid, and highly sensitive. It has the potential to be developed into a quantitative analytical method and commercial PCR kits for large-scale screening of spice powders to detect and prevent adulteration. Since spice powders are value added product traded globally, its adulteration is a major concern of the sanitary and phytosanitary issues of the WTO agreement. This work has much relevance in this context as India is one of the major suppliers of the commodity in the word market. The technology developed in this study can be utilized by approved authorities/agencies for adulterant detection/authentication in/of spice powders. The markers can be used for primary routine screening of batches, allowing the immediate rejection of suspected samples or even in case of disputes arising from the quality of a lot. The work has thus public health significance besides protecting the traded commodities from adulteration and unscrupulous trade practices.

6. Genitic analysis in coconut (TH 183)

Mr. C.G. Narayanan Namboothri/Mangalore University/2005/

Guide: Dr. V.A. Parthasarathy

The three F2, populations showed wide variations within the populations showing segregation for different vegetative, reproductive and fruit component traits towards both the parents, namely, the dwarf COD (Chowghat Orange Dwarf) parent and tall WCT (West coast tall parent). The appearance of transgressive sergeants towards both extremes was also observed for many of the characters studied. The tendency for maintaining the hybridity in the F, generation was also recorded for different traits. The wide range of fruit weight observed in the F2, populations indicates the polygenic effects on this character and heterozygosity for the character in the parents. The transgressive segregation observed in the F2, populations for fruit length and breadth exceeded the parental values and it indicates the choice of selection for further breeding programme. Wide range of segregation was observed for nut weight with transgressive segregants in the F2 populations. The transgressive segregation and wide variation for shell weight in the F2 populations showed the heterozygosity of the parents and the polygenic control for this character in coconut. Transgressive segregation observed for weight of kernel and weight of copra in the F2, populations

indicates the possibility of selection for obtaining higher kernel weights from the F2 populations. The phenotypic variance estimated for all the characters were greater in magnitude than the genotypic variations. Many of the characters showed genotypic and phenotypic variation very low or negligible. Some of the characters showed negligible genotypic variation but high phenotypic variation indicating effect of environmental factors. The traits *viz*., number of leaves on the crown, total leaves produced, number of spikelets, number of female flowers per inflorescence, total number of inflorescences produced, number of fertile inflorescences produced and per cent set after 90 days exhibited positive significant correlation with nut yield and could be considered as major contributing characters to the nut yield. The pattern of segregation of the genotypes in the F2, populations of D x T coconut was confirmed by isozyme banding pattern data of the enzymes namely esterase and peroxidase. Heterozygosity of the parents was again proved by the isozyme banding pattern in F2 populations, F1 hybrids and WCT parent. The homozygosity of the dwarf COD parent was confirmed by the lesser enzyme polymorphism observed. Cluster analysis showed the pattern of grouping of genotypes according to the intensity of segregation in the F2, populations. A clear 1:1 ratio of sergeants in the F2 populations (towards both WCT parent and COD parent) showed how the F2 progenies are getting segregated towards the parental lines.

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