INTRODUCTION

Plant genetic resources, a subset of biodiversity, contain the genetic material, which is the source of the vast variety of plant life on the planet. Plant genetic resources hold the key to food security and sustainable agricultural development (Iwananga, 1994) and which include land races, primitive cultivars, advanced/improved varieties and wild relatives of crop plants. Activities that relate to conservation and use of plant genetic resources include: exploration and collecting, characterization and evaluation, conservation, assessment of variation and identification of useful genes, and exchange and genetic enhancement (Rao and Riley, 1994). Plant genetic resources in agric-horticultural crops and their wild relatives are of immense value to mankind as they provide food, fodder, shelter and industrial products. Plant breeders require reservoir of genetic variation for crop improvement. Due to the spread of high yielding varieties and selection pressure the genetic variability is gradually getting eroded resulting in large-scale depletion of variability. This situation thus demands priority action to conserve such germplasm (Frankel, 1975).

Plant genetic resources constitute genotypes or populations of cultivars (landraces, advance/improved cultivars), genetic stocks, wild and weedy species, which are maintained in the form of plants, seeds, tissues, etc. The wealth of genetic diversity currently available holds vast potential. However, the genetic resources are non-renewable and are among the most essential of the world’s natural resources. It is essential that these be well conserved, be it at species, genepool or ecosystem level, for present and future generations (V. Ramanatha Rao et al., 1994). Thus the need for conservation is stressed.

India is one of the twelve identified centres of origin of cultivated plants and has two of the eighteen identified mega diversity ‘hotspots’. thus possessing a rich wealth of plants comprising fruits, vegetables, spices and ornamentals.

India is considered as the land of spices. In India, 53 major spices are grown most of which are tropical thus playing a major role in the country’s economy. Spices like blackpepper, cardamom, ginger, turmeric, cinnamon, chilli, vanilla, fennel, fenugreek, coriander and cumin form the economic backbone of large number of people in India.
Pepper, ginger and turmeric are the spices, which are native to this country having originated and diversified the country has numerous cultivated varieties and their related wild species.

Black pepper, known as the’ King of spices’, is the most important and most widely used spice in the world. Black pepper is a woody climber, grown in the South Western region of India, comprising of the states of Kerala, parts of Karnataka, Tamil Nadu and Goa. Cardamom, referred as the ‘Queen of spices’ is a large perennial, herbaceous rhizomatous monocot, belonging to the family Zingiberaceae. It belongs to the genus *Elettaria* and species *cardamomum* (Maton). It is a native of the moist evergreen forests of the Western Ghats of southern India. The cardamom of commerce is the dried ripe fruit (capsules) of cardamom plant. Because of its very pleasant aroma and taste it is highly valued from ancient times. Turmeric is the rhizomes of *Curcuma longa* L. (syn. *C. domestica* Val.) and is an important spice in India, South East Asia and Indonesia and is indispensable in the preparation of curry powders. In addition to its use as a spice, it has medicinal properties and also used as a dye (Filho *et al*. 2000). Turmeric powder is a main ingredient in many of the cosmetics. Ginger (*Zingiber officinale*) is an important commercial spice crop, grown from very ancient times in India. Though ginger is also cultivated in the West Indies, Sierra Leone, Brazil, China, Japan and Indonesia, India still is the largest producer of dry ginger in the world. Vanilla the ‘princess of Spice’

Conservation of these plant genetic resources has become an urgent necessity in view of the several threats to their survival. Conversion of orchard lands into residential and commercial plots has led to the loss of several unique and rare collections of these genetic resources. Deforestation, habitat degradation and over-exploitation have caused considerable loss of diversity in spices. Realising this threat, several conservation strategies have been developed.

**The concept: core collection**

The potential use of plant genetic resources contained in large germplasm collections could be greatly enhanced by constituting sub-samples called core collections or core subsets. A core collection should include the maximum genetic variation contained in the whole collection with minimum duplication (Frankel 1984). Obviously, the quality of
core collection is dependent upon good passport and evaluation data on the accessions that constitute the whole collection. The sampling strategies for obtaining a core collection are mainly focused on grouping the accessions into homogenous groups or clusters initially and then selecting sub-samples from each group to obtain a pooled core collection.

**CONSERVATION METHODS**

Conservation can be done on-site (*in situ*) and off-site (*ex situ*).

*In situ conservation:*

The conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and in the case of domesticates or cultivated species, in the surroundings where they have developed their distinctive properties.

**Protected areas:**

Protected areas are widely regarded as instrumental for *in situ* conservation of wild relatives. Wild relatives of crops may occur beyond the influence of farming, in natural and semi-natural ecosystems and their conservation may well fit into the existing system of natural reserves. A disadvantage of protected area conservation is that the conserved material is not readily available for agricultural use. Also, with limited opportunity for management, little characterization and evaluation can be done on the germplasm, restricting its use as a genetic resource (Maxted et al., 1997). Conservation on farm and home gardens, which are reservoir of diversity for fruits, vegetables, are other methods of *in situ* conservation.

*Ex situ conservation*

a) Seed storage:

Storing genetic diversity, as seed is the best researched, most widely used and most convenient method of *ex situ* conservation. Requirements include adequate drying, i.e. seed moisture contents as low as 3% for oily seeds and 5% or more for starchy seeds. Appropriate storage temperature (-18°C is recommended for long-term storage) and careful production of quality seed to ensure the greatest longevity (Rao and Jackson, 1996). Recent research shows that very low moisture contents could be sub-optimal and care is needed. However, the seeds of many crop species, especially tropical shrubs and
trees, will lose viability if dried (recalcitrant seeds). Seeds of some species can be dried to some extent but cannot survive low-temperature storage and are intermediate in storage characteristics. In addition, seeds of wild relatives do not always behave similarly to the seed of domesticates, and optimal storage conditions have to be individually determined.

Most national gene banks now rely on cold storage facilities for seed maintenance. However these depend on a reliable electricity supply that can represent a problem in some countries. To overcome this problem, alternative approaches to low temperature storage have been developed, including the so-called ‘ultra-dry seed’ technology. Drying seeds to moisture content as low as 1% (in the case of oily seeds) or approximately 3% (starchy seeds) and hermetic packaging allows storage for long periods at room temperature. Care must be taken to prevent over-drying of the seeds (Walters and Engels, 1998). Another alternative is storing seeds in liquid nitrogen. Besides the danger of over-drying the (orthodox) seeds, seed size is important for cryopreservation. But this approach might have advantages under circumstances where electricity supply is unreliable.

b) Pollen storage:

The technique of pollen storage is comparable with that of seed storage, since pollen can be dried (less than 5% moisture content on a dry weight basis) and stored below 0°C. There are limited reports on the survival and fertilizing capacity of cryopreserved pollen more than five years old (Towill, 1985). Pollen might represent an interesting alternative for the long-term conservation of problematic species (IPGRI, 1996). However, pollen has a relatively short life compared with seeds (although this varies significantly among species) and viability testing can be time-consuming and uneconomical. Pollen has therefore, been used to a limited extent in germplasm conservation (Hoekstra, 1995). Other disadvantages of pollen storage are the small amount produced by many species, the lack of transmission of organelle genomes via pollen, the loss of sex-linked genes in dioecious species and the general inability to regenerate into plants (Hoekstra, 1995). An advantage is that pests and diseases are rarely transferred by pollen (excepting some virus diseases). This allows safe movement and exchange of germplasm as pollen.
c) Field genebanks:

Field genebanks are used for the conservation of clonal crops, where seed is recalcitrant and for crops that rarely produce seed. Management may be the same as used during routine farming and cultivation methods can be adapted to local circumstances. Conserved material can be readily characterized and evaluated and then accessed for research and commercial use. Some natural selection may take place within and between accessions, but management is designed to prevent it. Major constraints faced by field genebanks include costs and all the natural hazards of farming, including pests and diseases, drought, flood, cyclones etc. (Engelmann and Engels, 2002).

d) In vitro conservation:

When conservation method is susceptible to unavoidable hazards, as with field genebanks an alternative, complementary method should also be used. In vitro conservation involves maintenance of explants in a sterile, pathogen-free environment and is widely used for the conservation and multiplication of species that produce recalcitrant seeds, or do not produce seeds (Engelmann, 1997).

Various in vitro conservation methods are used. For short-and medium-term storage the aim is to increase the intervals between subcultures by reducing growth. This is achieved by modifying the environmental conditions, including the culture medium to realize so-called slow-growth conservation. The most widely applied technique is temperature reduction (varying from 0-5°C for cold tolerant species to 9-18°C for tropical species) that can be combined with a decrease in light intensity or storage in the dark (Engelmann, 1997) and adjustment of the growth medium Alternatives to standard slow-growth conservation include modification of the gaseous environment of cultures, desiccation and encapsulation of explants.

For small volumes, long-term storage is practicable through storage of cultures in cryopreservation at ultra-low temperature, usually by using liquid nitrogen (-196°C). At this temperature all cellular divisions and metabolic processes are virtually halted and consequently, plant material can be indefinitely stored without alteration or modification.
**e) DNA storage:**

This is more recently developed technique is increasing in importance. DNA from the nucleus, mitochondria and chloroplasts is now routinely extracted and stored. The advantage of storing DNA is that it is efficient and simple and overcomes many physical limitations and constraints that characterize other forms of storage. The disadvantage lies in problems with subsequent gene isolation, cloning and transfer but, most importantly, it does not allow the regeneration of live organisms (Maxted et al., 1997).

**Genetic stability:**

An important prerequisite for any conservation technique is that the regenerants produced from the conserved material should be true-to-type. There are ample evidences to indicate that under certain culture conditions the materials undergo genetic changes (somaclonal variations) and as a consequence lose their integrity and uniformity. This would be highly undesirable in spices varieties where the purpose is not only to conserve a genotype but also retain its specific quality traits. Thus testing for the genetic stability of *in vitro* conserved materials is of utmost importance. Besides morphology, one of the most widely used methods is to observe for cytological changes like polyploidy, aneuploidy and chromosomal breakages. Sophisticated biochemical and DNA-based techniques have enabled more critical analysis of the genetic stability of *in vitro* materials.

Spices such as black pepper, cardamom, ginger, turmeric, vanilla etc., are propagated vegetatively and their germplasm is conserved in clonal field repositories. Serious soil-borne diseases and high risk of mixing up of germplasm due to replanting in the same area especially in Zingiberaceous taxa, threaten most of these crops. Thus, formulation of *in vitro* conservation strategies is a complement to field gene banks.

The *in vitro* gene bank at IISR was set up to develop *in vitro* conservation strategies for short as well as long-term conservation through slow growth and cryopreservation respectively and utilize morphological, biochemical and molecular tools for characterization of *in vitro* conserved plants to estimate their genetic fidelity. This method offers a safe alternative or additive to field gene bank.
Black Pepper, known as the ‘king of spices’, is the most important and most widely used spice in the world. Black pepper is a woody climber, grown in the South Western region of India, comprising of the states of Kerala, parts of Karnataka, Tamil Nadu and Goa. The humid tropical evergreen forest bordering the Malabar Coast (Western Ghats is one of the hot spot areas of plant bio-diversity on earth) is the center of origin and diversity for both the king of spices (pepper) and queen of spices (cardamom).

Piper Linn.Gen.Pl.ed.1: 333,1737:Type species *P. nigrum* L. Species of this genus are perennial, scandent or woody climbers or creepers, shrubs or small trees. The genus in general is characterized by very small, highly reduced flowers, closely packed to form spikes. Most of the South Indian taxa are dioecious but cultivated black pepper is bisexual. There are many Piper species occurring in Western Ghats, which include *P. longum, P. chaba, P. pseudonigrum, P. suganthi*, Rare and endangered species like *P. hapnium, P. silentvalleyensis, P. barberi* etc, high elevation species like *P. mullesua, P. schmidtii, P. wightii*, Ornamental pepper like, *P. ornatum, P. magnificum* etc

The details of related Piper species are given in the Table:

The cultivars of black pepper might have originated from the wild ones through domestication and selection. Over hundred cultivars are known, but many of them are getting extinct due to various reasons like devastation of pepper cultivation by diseases such as, foot rot and slow decline, replacement of the traditional cultivars by a few high yielding varieties etc. Cultivars density is richest in the state of Kerala followed by the state of Karnataka. Most of the cultivars are bisexual forms. The cultivar density of Black pepper is represented in Table:

The Western Ghats is very high in endemic species, unfortunately it is also one of the most ecologically threatened areas due to large-scale encroachments and human settlements that have taken place during the past hundred years. To save the pepper genetic resources, the Indian Institute of Spices Research has established a National Repository for the *ex situ* conservation of Pepper germplasm and is conserved at four stages (Ravindran and Nirmal Babu 1994a).
i) In the nursery gene bank, where each accession is trailed in bamboo splits in serial order and are under continuous multiplication and maintenance.

ii) In the clonal repository where 10 rooted cuttings of each accession is maintained.

iii) In the field gene bank where the accessions are planted for preliminary yield evaluation and characterization

iv) In the in vitro and cryogene banks.

Indian Institute of Spices Research holds the world’s largest collection of pepper germplasm, which is at present conserved in clonal field repositories, where they are threatened by serious diseases. Storage of germplasm in seed banks is not practical as they are vegetatively propagated and seeds are recalcitrant and heterozygous. Hence storage of germplasm in in vitro is a safe alternative.

Protocols for in vitro conservation by slow growth of pepper and its related species viz., P.barberi, P.colubrinum, P.betle and P.longum were standardized by maintaining cultures at reduced temperatures, in the presence of osmotic inhibitors, at reduced nutrient levels and by minimizing evaporation loss by using closed containers. The conserved materials of all the species showed normal rate of multiplication when transferred to multiplication medium after storage. The normal sized plantlets when transferred to soil established with over 80% success. They developed into normal plants with out any deformities and were morphologically similar to mother plants.

Pepper seeds are recalcitrant and the seed viability decreases with reduction in moisture content. Seeds desiccated to 12% & 6%moisture contents were successfully cryopreserved in liquid nitrogen at –196°C, with a survival rate of 45% & 10.5% respectively (Chaudhury and Chandel 1994).
CARDAMOM

Introduction:

Cardamom is a large perennial, herbaceous rhizomatous monocot, belonging to the family Zingiberaceae. It belongs to the genus Elettaria and species cardamomum (Maton). It is a native of the moist evergreen forests of the Western Ghats of southern India. The cardamom of commerce is the dried ripe fruit (capsules) of cardamom plant. This is often referred as the ‘Queen of spices’ because of its very pleasant aroma and taste and is highly valued from ancient times. It is grown extensively in the hilly regions of South India at elevations of 800-1300m as an under crop in forestlands. Cardamom is also grown in Sri Lanka, Papua New Guinea, Tanzania and Guatemala. It is grown on a commercial scale in Guatemala, which incidentally is also now the largest producer of cardamom.

The present scenario:

Today, cardamom production is concentrated mainly in India and Guatemala. The productivity has increased in recent years due to the use of high-yielding varieties, better agro-production technology and better awareness of phytosanitation and control of diseases and insect pests.

The present research and development efforts are directed towards: 1) Developing high yielding, location specific cultivars for the major cardamom growing areas. 2) Evolving high production technology for the major growing tracts. 3) Evolving lines tolerant or resistant to clump rot and virus diseases. 4) Selection/ breeding for high quality and 5) Evolving drought-and heat-resistant lines.

The Western Ghats, the center of diversity for cardamom, has undergone much climatological changes during the past century due to the devastation of forest habitat. As a result, prolonged drought became an annual feature in the cardamom growing areas, leading too sharp decline in cardamom production and productivity. In view of this situation, the need of the hour is the development of lines tolerant or resistant to heat and moisture stress.
Varieties:
Based on the nature of panicles, three varieties of cardamom are recognized (Sastri, 1952). The var. Malabar is characterized by prostrate panicle and var. Mysore possesses erect panicle the third type var. Vazhukka is considered a natural hybrid between mysore and malabar and its panicle is semi-erect or flexuous.

Crop Improvement:
Use of genetically superior planting materials and improved cultural practices are the two important means to enhance crop productivity. The lack of superior genotypes and frequent vagaries of drought and unprecedented upsurge of diseases and pests are some of the major factors that contribute to low productivity in cardamom. Varieties with high yield potential, superior capsule quality and wider adaptability are essential to increase productivity. Selection of clones having resistance/tolerance to major diseases and pests as well as drought also should get due importance in planned breeding programme of this crop.

Germplasm:
Cardamom, being a cross-pollinated crop and propagated mostly through seeds, natural variability is fairly high. An assembly of diverse genetic stocks of any crop is the raw material from which a new variety can be molded to suit the requirements of farmers and end users. Hence, collection, conservation, evaluation and exploitation of germplasm deserve utmost importance in breeding strategies. Conservation of cardamom genetic resources under in situ situation does not exist, though natural population occurs in protected forest areas, especially in the Silent Valley Biosphere Reserve, where a sizable population of cardamom plants in its natural setting exists. Ex situ conservation of cardamom germplasm is being undertaken mainly by four organizations like, Indian Institute of Spices Research Regional Station, Appangala, Coorg, Karnataka, Indian Cardamom Research Institute, Myladumpara, Idukki, Kerala, Cardamom Research Centre, Pampadumpara, Kerala, Regional Research Station, Mudigere, Karnataka. Ex situ conservation in cardamom is being maintained as field gene banks and they are used for preliminary evaluation, maintenance as well as for characterization. Characterization involves morphological, agronomical as well as chemical characters.
Ex situ conservation is always at risk due to a variety of reasons, mainly biotic and abiotic stress factors. The prevalence of virus diseases is a serious threat to ex situ conservation of germplasm. An alternative is in vitro conservation and establishment of an in vitro gene bank, which is a safe alternative in protecting the genetic resources from epidemic diseases.

**Cryo-conservation:**

Choudhary and Chandel (1995) attempted cryo-conservation of cardamom seed. They tried to conserve seeds at ultra-low temperature by either (i) suspending seeds in cryovials in vapor phase of liquid nitrogen (-150°C) by slow freezing or (ii) by direct immersion in liquid nitrogen (-196°C) by fast freezing. The result showed that seeds possessing 7.7-14.3% moisture content could be successfully cryo-preserved with 80% germination when tested after one-year storage in vapor phase of liquid nitrogen (at -150°C).

**Ginger (Zingiber officinale Rosc.)**

Ginger (Zingiber officinale Rosc.) was first described by Rheede (1692) in *Hortus Indicus Malabaricus* (Ravindran *et al*, 1994; Nayar and Ravindran, 1995). The name is believed to have originated from the Sanskrit word *Singabera* meaning ‘shaped like a horn’, and this has further evolved to the Greek word *Zingiberi* and subsequently the Latin word *Zingiber*. Ginger has a long reported history as a spice. Its origin lies in either India or China, where it was mentioned in 500 BC in the writings of the philosopher Confucius. By the 14th century ginger was the most common spice after black pepper (Morris and Mackley, 1997).

Fischer (1928) reported seven species from South India including *Z. officinale*. Recently Sabu (1991) described eight species from Western Ghats and adjacent areas.

In India ginger is cultivated in about 77,610 ha and produced 263170 tonnes in the year 1999–2000. India earned a foreign exchange of Rs. 2295.40 lakhs by exporting about 6580 tonnes of ginger during 2000–2001 (DGCI & S, 2001). India is the largest producer and exporter of dry ginger contributing to 50% of world’s production. China,
Taiwan, Nigeria, Jamaica, Australia and Japan are the other important ginger producing countries.

Ginger is propagated vegetatively by rhizome pieces, weighing 20–40 gm each, with one or two good undamaged buds. Due to the incidence of major soil and seed borne diseases and pests, seed rhizomes are collected from disease and pest free plantations. The optimum time for harvesting ginger in India is at 245–260 days after planting.

Several cultivars are recognized in India, which differ in rhizome size and yield and fibre and moisture contents. The cultivars are generally are named after the area in which they are mostly cultivated. Some of the popular cultivars are Maran, Nadia, Jorhat, Burdwan, Wynad Local, Karakkal, Himachal, Kurrupumpadi, Narsapattam, China, Rio-de-Janeiro and Jamaica. The last three are exotic cultivars from China, Brazil and Jamaica, respectively. Maran, an Assam cultivar, is popular because of its high yield and high percentage recovery of dry ginger.

Conservation of ginger germplasm is being done in two ways namely, as nucleus gene bank (in concrete tubs in nursery, for safeguarding the material from soil borne pathogens, maintained under 50% shade (to avoid the Phyllosticta leaf spot incidence) and avoid mixing up of varieties) and in in vitro gene bank by slow growth. The National Conservatory for Ginger Germplasm at IISR, Calicut has 659 accessions, all being maintained in cement tubs. The other important centres of ginger conservation are High Altitude Research Station, Pottangi (Orissa) and Department of Vegetable Crops, Dr. Y.S. Parmar University of Horticulture and Forestry, Solan (Himachal Pradesh). Small collections are maintained in a few other centres also.

Turmeric (*Curcuma longa* L.)

Turmeric is the rhizomes of *Curcuma longa* L. (syn. *C. domestica* Val.) and is an important spice in India, South East Asia and Indonesia and is indispensable in the preparation of curry powders. The name turmeric is believed to have originated from the Latin word *terra merita*, meaning merit of the earth. The turmeric he found in Southern China fascinated Marco Polo, “There is also a vegetable which has all the properties of
true saffron, as well the smell and the colour, and yet it is not really saffron”. Turmeric is much revered by Hindus and associated with fertility. In Malaysia a paste of turmeric is spread on the mother’s abdomen and on the umbilical cord after childbirth, not only to warn off evil spirits, but also for its medicinal value, as turmeric is known to be antiseptic (Morris and Mackley, 1997; Khanna, 1999). In addition to its use as a spice, it has medicinal properties and also used as a dye (Filho et al. 2000). Turmeric powder is a main ingredient in many of the cosmetics.

The genus *Curcuma* is mainly distributed in the Indo-Malayan region and about a 100 species are known. Baker (1886) described 27 species in *Flora of British India*. He subdivided the genus into three sections – *Exantha*, *Mesantha* and *Hitcheniopsis*. The section *Exantha* consists of 14 species including turmeric and other economically important species such as *C. angustifolia* Roxb. (Indian arrow root), *C. aromatica* Salisb. and *C. zedoaria* Rosc. From South India, 16 species of *Curcuma* were reported (Sabu, 1991), out of which 9 are endemic to India.

India is the largest producer and exporter of the crop. Turmeric is cultivated in about 1,61,300 ha with a production of over 653600 tonnes. India earned a foreign exchange of Rs. 9106 lakhs by exporting about 34,500 tonnes of ginger during 2000–2001 (DGCI&S, 2001). Turmeric was domesticated in southern or South East Asia. Turmeric requires a hot and moist climate (Purseglove et al. 1981) and can be cultivated in most areas of the tropics and subtropics.

Genetic resources of turmeric include popular cultivars/types, high yielding selections, local cultivars/types and semi wild and related species. Turmeric types are known by trade names, based on the appearance, rhizome thickness, colour intensity, aroma and hardness of the core and duration (Rao and Rao, 1994). Turmeric cultivars/types of Andhra Pradesh are classified into three groups namely, short duration ‘Kasturi’ types, medium duration ‘Kesari’ types and long duration types belonging to *C. longa*. Some of the popular cultivars are, Duggirala, Mydukur, Armoor, Sugandham, Erode, Salem, Alleppey and Mannuthy local (Muthuswamy and Ahamad Shah, 1982; Srirama Rao, 1982; Rao and Rao, 1994).

Efforts have been made to identify and select turmeric types with high yield potential, curcumin content and curing percentage. Some of the important released
varieties are Krishna, Suvarna, Roma, Suguna, Sudarsana, Prabha, Prathibha and BSR-2 (Pujari et al., 1986; Edison et al., 1991; Sasikumar et al., 1996; Chezhiyan and Shanmugasundaram, 2000).

Turmeric is always propagated vegetatively by finger or rhizomes with one or two buds. Leaf spot caused by *Taphrina maculans* Butl. is commonly present in India, wherever the crop is grown. Rhizome rot caused by *Pythium graminicolum* Subram. is another serious disease reported in India. The shoot borer, *Conogethes punctiferalis* Guen. is the most important pest of turmeric (Sarma, et al., 1994).

Turmeric is grown in most of the states in India, and there is rich cultivar diversity. Cultivation of turmeric is concentrated in Andhra Pradesh, Tamil Nadu, Maharashtra, Madhya Pradesh, Uttar Pradesh and Bihar. The total turmeric production in India is around 550,000 tonnes from an area of about 1,40,000 ha. Some cultivars such as Alleppey grown in Central Kerala are regarded as high yielding and of high quality. The cultivars are mainly known after the place where the particular type is grown traditionally. Velayudhan et al. (1990; 1991; 1994; 1999) described 6 new species, identified 21 different morphotypes, 6 taxonomic groups and several promising lines in *Curcuma*.

Collection and conservation of genetic resources of turmeric is mainly being carried out by Indian Institute of Spices Research (IISR), Calicut and National Bureau of Plant Genetic resources (NBPGR) Regional Station, Thrissur. The National Conservatory for turmeric at IISR has currently more than 800 accessions, all maintained in large cement tubs to maintain purity.

VANILLA

*Vanilla planifolia* Andrews(syn V.fragrans)(Salisb.)(Ames) is native to the humid tropical rain forests of South-eastern Mexico, Central America, the West Indies and northern part of South America. Vanilla is the most important spice from the west. It is the only member of Orchidaceae which has a real economic value in the food and related industries, owing to its unique flavour and pleasant aroma, other counterparts being well known for its most attractive flowers. The major vanilla growing countries are Madagascar, Indonesia,
India, Mexico, the Comoros and Reunion. Other countries where vanilla is cultivated to a limited extent are French Polynesia, Tonga, Guadeloupe and Zimbabwe. The cultivation of vanilla spread after the discovery of America by Columbus and now it accounts for about 0.75% of the total world trade in spices.

**Economic Importance**

Vanilla is one of the expensive spices traded in the global market. The substance chiefly responsible for the fragrance, flavour and pleasant aroma of the vanilla beans is vanillin (C8H8O3). Vanilla essence is largely used in the preparation of ice creams, chocolates, bakery products, puddings, pharmaceuticals, liquors and perfumes. Vanilla is the only member now in the ‘Orchid family’, which is a high valued crop and hence the name “Orchid of Commerce”. The vanilla flavour industry is based on the processed beans of the vanilla plants. With the advent of chemical technology to produce Vanillin/ethyl vanillin, these synthetic substitutes have taken over the use of vanilla beans. Natural vanilla beans, however, is still the most preferred flavour and is also used to enhance sweetness perception of foods, especially bakery products.

The total area under vanilla cultivation in the world during 2002 was 38066 hectares with the production of around 4956 metric tones. During 2000 the global trade of vanilla accounted for 103.17 million US$. The aggregate global demand for vanilla is estimated at above 2000 to 3000 metric tones a year.

**Systematics**

Vanilla belongs to the family Orchidaceae, an advanced group of monocotyledons. One hundred and ten species of vanilla are reported (Purseglove et al., 1988) consisting of terrestrial, climbing, epiphytic and saprophytic species. The basic chromosome number for the genus vanilla is x=16 and V. planifolia is a diploid with 2n=32. As far is known there are few, if any, recognized cultivars of V. planifolia. In countries where vanilla has been introduced, variability is likely to be highly limited. The material is propagated vegetatively hence of clonal origin.

**Habitat**

In its original habitat vanilla was seen wild as a climber. Vanilla requires warm and moist conditions of humid tropics and thrives well between 10oN and 20oS latitudes having a
well distributed rainfall of 150-300cm with a temperature range of 25-32°C and comes up well up to 1500m above msl.

**Morphology**

Vanilla planifolia is a herbaceous perennial vine, climbing up trees or other supports to a height of 10-15m by means of adventitious roots. The stem is long, cylindrical, succulent and branched. It is 1-2cm in diameter and is dark green and photosynthetic with stomata. The internodes are 5-15cm in length. Leaves are large, flat, fleshy, subsessile, alternate, oblong-elliptic to lanceolate. They are 8-25cm long and 2-8cm broad. The tip is acute to acuminate and the base somewhat rounded. Venation is parallel and the veins are distinct. The petiole is thick, short and canalized above. The stout racemose inflorescences are axillary, usually simple but rarely branched. The rachis is stout and 4-10mm in diameter. The bracts are rigid, concave and persistent. Flowering is seen usually from December to May and it takes 45-60 days from the initiation of inflorescence to flowering. Flowers are large, waxy, pale greenish yellow, bisexual and zygomorphic. The fruit is a capsule, which is dehiscent in planifolia and in trade it is known as a bean. The bean is pendulous, narrowly cylindrical, obscurely three angled, 10-25cm long and 5-15mm in diameter. It becomes aromatic on processing. The vanilla bean contains thousands of seeds. Seeds are very minute black and globose in shape, about 0.3mm in diameter.

In vanilla under normal conditions seed germination is scanty. But now methods are perfected for in vitro germination of seeds on culture media under sterile conditions. This can be successfully utilized in hybridization programmes for rescuing embryos of artificial hybrid thereby creating improved variant lines.

**Other species**

Vanilla is a pantropical genus of about 110 species, of which only three viz. *Vanilla planifolia*, *V. pompona* Schiede and *V. tahitensis* J.W Moore are commercially exploited and cultivated throughout the tropics. Only *V. planifolia* is commercially cultivated in India. *V. andamanica* Rolfe, *V. pilifera* Holtt, *V. walkeriae* Wight and *V. wightiana* Lindl. ex J.D. Hook. are native to India. *V. andamanica* is known only from the Andaman group of islands. *V. pilifera* was originally described from Malaya and later recorded from
Thailand. It is found growing in the Mikir Hills of Assam. *V. walkeriae* is found in Sri Lanka and Southern India. *V. wightiana* is endemic to southern India, occurring in the states of Kerala, Karnataka, Andhra Pradesh, *Vanilla aphylla*, previously known from Thailand, Laos, Vietnam, Malaya and Java is found occurring in India also.

**Plant protection**

Vanilla is susceptible to many fungal diseases and viral infection. Rots of various plant parts such as root, stem, leaf, bean and shoot tip are the common fungal diseases. They are generally caused by Fusarium sps, Sclerotium sps, Phytophthora sps and Colletotrichum sps. Judicious usage (spraying and soil drenching) of appropriate fungicides such as Bordeaux mixture (1%), Bavistin (0.2%), Copper Oxychloride (0.2%), and Akomin (0.4%) has been recommended for their management. Vanilla viral diseases are caused by virus such as mosaic virus and necrosis poty virus. The disease is sap transmitted. Affected plants are to be destroyed and its vine should not be used for new planting. In this aspect in vitro conservation can be a safe additive to the field gene bank.

The germplasm of *Vanilla planifolia* is threatened by deforestation and over collecting and bad management. With this conservation of genetic resources of vanilla is so important for the future crop improvement programmes in this crop.
EXPERIMENT NO: 1

1. To conserve the core collections of black pepper, cardamom, ginger, turmeric and vanilla germplasm in *in vitro* gene banks.

Introduction:

The potential use of plant genetic resources contained in large germplasm collections could be greatly enhanced by constituting sub-samples called core collections or core subsets. A core collection should include the maximum genetic variation contained in the whole collection with minimum duplication (Frankel 1984). Obviously, the quality of core collection is dependent upon good passport and evaluation data on the accessions that constitute the whole collection. The sampling strategies for obtaining a core collection are mainly focused on grouping the accessions into homogenous groups or clusters initially and then selecting sub-samples from each group to obtain a pooled core collection.

**BLACK PEPPER**

Indian Institute of Spices Research holds the world’s largest collection of pepper germplasm, which is at present conserved in clonal field repositories, where they are threatened by serious diseases. Storage of germplasm in seed banks is not practical as they are vegetatively propagated and seeds are recalcitrant and heterozygous. Hence storage of germplasm *in vitro* is a safe alternative.

**MATERIALS & METHODS**

The data used for the present study pertains to a collection of 3220 accessions of black pepper which include 727 cultivars, 213 karimunda lines, 75 kottanadan lines, 1136 hybrids, olines, cytotypes, 651 wild relatives of black pepper which include piper spp like *P. argyrophyllum*, *P. betle*, *P. hapnium*, *P. crocatum* (exotic), *P. colubrinum* (exotic), *P. magnificum* (exotic), *P. arboreum*, *P. chaba*, *P. longum*, *P. schmidtii* (high elevation spp) and related genera like *Pothomorphe subpeltata*, *Peperomia* spp etc maintained at the national conservatory of black pepper germplasm at IISR, Calicut.

As in most crop plants the primary objective of pepper breeding is increase in yield. This primary objective has been broaddened towards evolving varieties combining high yield, quality and resistance to biotic and abiotic stresses. Pepper, blessed with the
twin advantages of vegetative propagation and viable sexual reproduction, offers much
scope for exploitation of hybrid vigour as well as selection breeding. Clonal selection,
hybridization, open pollinated progeny selection, mutation and polyploidy have been
used in improving pepper. Recently biotechnological approaches are also being
developed mainly for developing pathogen resistance.

Selection for drought tolerance:
Since rainfall is not distributed uniformly black pepper suffers due to moisture
stress. So screening of pepper germplasm for drought tolerance was carried out using
criteria like: Stomatal resistance, transpiration rate, leaf water potentials, chlorophyll to
carotenoid ratio etc (Vasantha et al., 1989, 1990). Drought tolerance were observed in
cultivars like Kottanadan, Neelamundi, etc

Clonal selection:
Many of the popular pepper cultivars exhibit considerable intracultivar variability.
In Kerala, the state having the maximum genetic diversity of black pepper-the most
popular cultivar is Karimunda. The Indian Institute Of Spices Research (IISR) carried
out a clonal selection in this cultivar. Based on yield & quality two lines were selected
for release to farmers. These lines were named Sreekara and Subhakara.
A generalized procedure for clonal selection in black pepper is given as a flow chart
Clonal selection in cultivar Kuthiravally carried out by the Pepper Research Station,
Panniyur, affiliated to Kerala Agricultural University, has resulted in Panniyur-4, PLD-2
is a selection from Cultivar Kottanadan by the Central Plantation Crops Research
Institute, Research Centre, Palode.

Selection from germplasm:
Two promising lines (Panchami and Pournami) identified in the germplasm
collections of the IISR were compared against two promising cultivars, Panniyur-1 and
Karimunda and with introduced Kuching, the ruling cultivar in Malaysia. Of the two
lines, one was a high yielding line from an elite mother vine of cultivar Aimpiriyan, and
the other was cultivar Ottaplackal. The latter was found to be tolerant to root knot
nematode Meloidogyne incognita. The performance of these two cultivars were much
superior to the prevailing varieties (Ravindran et al.1992).
Selection in OP progenies:

Pepper being heterozygous and propagated mainly through cuttings, segregation of characters can be expected in the open pollinated and selfed progenies. Because of the geitonogamous mode of pollination the open pollinated progenies are comparable to selfed off springs. There is thus a fair chance to locate useful genotypes in open pollinated progenies. Comparative genetic variability within open pollinated progenies of a few varieties of black pepper was reported by Ibrahim et al. (1986). Selection in OP progenies was carried out at the Pepper Research Station, Panniyur. Two cultivars Panniyur 2 and Panniyur 5 were developed through selection in op progenies of cultivars Balankotta and Prumkodi respectively.

Intercultivar Hybridization:

Considerable intercultivar variability exists in pepper for yield, quality and morphological features. Genetic improvement through hybridization generally involves three main steps. 1) Selection of parents. 2) Production of progeny. 3) Selection of superior genotypes to be developed into clones (varieties). Hybridization work in pepper was started by 1959 in India at the Pepper Research Station, Panniyur. Evaluation of F1 progenies of many crosses led to the hybrid Panniyur 1, a selection from F1 of a cross between cv. Uthirancotta X Cheriyakanikkadan. This hybrid was released in 1966. A second hybrid Panniyur 3, was also developed from the F1 population involving the same parents in early 1990s. No information is available on the breeding value of various varieties used as parents. General or specific combining ability of pepper cultivars are not worked out due to the perennial nature of the vine. In the absence of such information the available gene pool is used at random for intercultivar hybridization. At IISR, cultivars having promising characters are being used as parents. A large number of crosses involving many cultivars were made and the progenies tested for yield in preliminary trials. The promising F1 plants were multiplied and planted for comparative yield evaluation. A few hybrids having desirable yield and adaptability for higher elevation also selected.
Selection of hybrids with *Phytophthora* resistance:

The F1 plants were subjected to a preliminary selection in the nursery, eliminating the unhealthy and stunted ones. The others were subjected to screening for *Phytophthora* and the susceptible ones were further eliminated. The selected progenies were finally field planted for evaluation in progeny plots. The worth of individual plants was assessed visually in terms of vigour in growth, plant architecture, yielding potential and susceptibility to pests and diseases. The promising ones were multiplied and evaluated in replicated and multilocal trials.

**Polyploidy and its Application:**

A natural triploid (2n(3x)=78) was located among the IISR germplasm (Vadakkan) and this has very bold fruits and low fruit setting. This triploid plant was used for generating a series of cytotypes. Principal components account for the variations among Black pepper cultivars:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Principal components</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf size index, Leaf length, Leaf breadth</td>
</tr>
<tr>
<td>2</td>
<td>Leaf thickness, Lower epidermal thickness, Upper epidermal thickness</td>
</tr>
<tr>
<td>3</td>
<td>Leaf length-spike length ratio, Spike length, Peduncle length</td>
</tr>
<tr>
<td>4</td>
<td>Guard cell length and Guard cell breadth</td>
</tr>
<tr>
<td>5</td>
<td>Fruit size and fruit shape</td>
</tr>
<tr>
<td>6</td>
<td>Leaf shape and leaf base</td>
</tr>
<tr>
<td>7</td>
<td>Stomatal frequency and mesophyll thickness</td>
</tr>
<tr>
<td>8</td>
<td>Leaf shape (Orthotropic shoot) &amp; Colour of the emerging runner shoot tip</td>
</tr>
</tbody>
</table>

**Morphological traits and their descriptor states used for the core collection study of Piper species**

<table>
<thead>
<tr>
<th>Leaf shape</th>
<th>Ovate to ovate elliptic, Ovate to lanceolate, Ovate to elliptic, Cordate, Elliptic to elli-lanceolate, Ovate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf base</td>
<td>Round to attenuate, Round, Cordate, Acute</td>
</tr>
<tr>
<td>Leaf texture</td>
<td>Glabrous, Sparsely, Hairy on ribs, Hirsute</td>
</tr>
<tr>
<td>Leaf nature</td>
<td>Membraneous, Coriaceous</td>
</tr>
</tbody>
</table>
### Spike shape
- Filiform, Cylindrical, Globose

### Spike orientation
- Pendulous, Erect

### Spike texture
- Glabrous, Hirtellous

### Bract type
- Adnate, Peltate, Orbicular, Pedicelled, Connate, Fleshy cup, Stalked, Shallow cup below the ovary, Obconical, Angular free margins, Deeply copular, Stipitate, Adnate with free margins

### Fruit nature
- Free, Fused

### Fruit shape
- Obovate-Oblong, Spherical, Elliptical

### Fruit taste
- Bitter, Bitter/spicy*, Spicy, Pungent

* - Bitter initially, gently spicy later

**Agronomic and quality traits used for the core collection study of Black pepper hybrids and cultivars**

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yield (Kg/vine, fresh)</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>Maximum length of spike (cm)</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Mean spike length (cm)</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td>Maximum no. of berries in a spike</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Weight of fresh spike (Kg/vine)</td>
<td>11</td>
</tr>
<tr>
<td>6</td>
<td>Weight of threshed berries (Kg/vine)</td>
<td>12</td>
</tr>
</tbody>
</table>

### DEVELOPING SLOW GROWTH PROTOCOL

The prime requisite of slow growth storage strategy includes culture initiation and multiplication of the targeted crop species.

**Micropropagation**

The micropropagation protocols standardized at this Institute (Nirmal Babu et al. 1997) was used to multiply black pepper cultures to generate adequate material for *in vitro* conservation experiments.

**In vitro storage by minimal growth**
In black pepper, the cultures could be stored up to 360 days with 80% survival in half WPM medium supplemented with 15g/l each of sucrose and mannitol in sealed culture vessels & the growth rate was also reduced. Piper species like *Piper barbieri* & *Piper colubrinum* were also conserved using the same method.

The core collections of black pepper germplasm established at *in vitro* repository are the following:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Black pepper cultivar names</th>
<th>Plant characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sreekara</td>
<td>IISR Released variety, High quality, 11% oleoresin</td>
</tr>
<tr>
<td>2</td>
<td>Subhakara</td>
<td>IISR Released variety, High quality</td>
</tr>
<tr>
<td>3</td>
<td>Panchami</td>
<td>IISR Released variety, Late maturing type, suited to all pepper growing regions</td>
</tr>
<tr>
<td>4</td>
<td>Pournami</td>
<td>IISR Released variety, Tolerant to root knot nematode</td>
</tr>
<tr>
<td>5</td>
<td>IISR Thevam</td>
<td>'Good yielding, hardy variety, medium quality, tolerant to foot rot disease, 4.25Kg fresh yield/vine</td>
</tr>
<tr>
<td>6</td>
<td>Panniyur-1</td>
<td>Suited to most pepper regions, ideal for white pepper preparation</td>
</tr>
<tr>
<td>7</td>
<td>Panniyur-2</td>
<td>Shade tolerant</td>
</tr>
<tr>
<td>8</td>
<td>Panniyur-3</td>
<td>Suited to most pepper regions</td>
</tr>
<tr>
<td>9</td>
<td>Panniyur-4</td>
<td>Stable yielder</td>
</tr>
<tr>
<td>10</td>
<td>Panniyur-5</td>
<td>Tolerant to nursery diseases &amp; shade</td>
</tr>
<tr>
<td>No</td>
<td>Name</td>
<td>Characteristics</td>
</tr>
<tr>
<td>----</td>
<td>--------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>11</td>
<td>PLD-2</td>
<td>High quality, suited to all pepper growing regions</td>
</tr>
<tr>
<td>12</td>
<td>Perumkodi</td>
<td>Fruits bold, quality medium, alternate bearer</td>
</tr>
<tr>
<td>13</td>
<td>Poonjaranmunda</td>
<td>Large, round leaves, tolerant to drought</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Very old vine, loose setting, no disease</td>
</tr>
<tr>
<td>15</td>
<td>Kaniakkadan</td>
<td>Fruit bold, oblong, medium quality</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate yielder, medium quality</td>
</tr>
<tr>
<td>16</td>
<td>Karivilanchi</td>
<td>Medium large leaves, small spike, medium berries</td>
</tr>
<tr>
<td>17</td>
<td>Bilimalligesara</td>
<td>Escape from screening against Radophilus similis (nematode)</td>
</tr>
<tr>
<td>18</td>
<td>Kuthirugunda</td>
<td>Good yielder, high spiking intensity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Good setting</td>
</tr>
<tr>
<td>19</td>
<td>Op. Karimunda</td>
<td>High pungency, polymorphic branching habit</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medium quality, predominantly female</td>
</tr>
<tr>
<td>20</td>
<td>Jeerakamundi</td>
<td>Late maturing, regular yielding</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less important cultivar</td>
</tr>
<tr>
<td>21</td>
<td>Karimkotta</td>
<td>Good yield, moderate quality</td>
</tr>
<tr>
<td>22</td>
<td>Kottanadan 1487</td>
<td>Introduced from Panniyoor Research Station</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Escape from pepper field</td>
</tr>
<tr>
<td>23</td>
<td>Cholamundi</td>
<td>High yielder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short spike, bold berries, used for white pepper preparation</td>
</tr>
<tr>
<td>24</td>
<td>Kumbanadan</td>
<td>High yielding, good fruit setting</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less important cultivar</td>
</tr>
<tr>
<td>25</td>
<td>Murithottan</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>Uddagharae</td>
<td></td>
</tr>
</tbody>
</table>
Cardamom is a large perennial, herbaceous rhizomatous monocot, belonging to the family Zingiberaceae. It belongs to the genus Elettaria and species cardamomum (Maton). It is a native of the moist evergreen forests of the Western Ghats of southern India. The cardamom of commerce is the dried ripe fruit (capsules) of cardamom plant. This is often referred as the ‘Queen of spices’ because of its very pleasant aroma and taste and is highly valued from ancient times. It is grown extensively in the hilly regions of South India at elevations of 800-1300m as an under crop in forestlands. Cardamom is also grown in Sri Lanka, Papua New Guinea, Tanzania and Guatemala. It is grown on a commercial scale in Guatemala, which incidentally is also now the largest producer of cardamom.

**MATERIALS & METHODS**

The data used for the present study pertains to a collection of 314 cultivated germplasm and 13 wild and related taxa of cardamom from the collection of cardamom germplasm maintained at the IISR Regional Station, Appangala, Coorg, Karnataka.

Cardamom is amenable to both sexual and vegetative propagations, hence techniques such as selection, hybridization, mutation and polyploid breeding are used as means for genetic up gradation of the crop.

**Selection:**
The breeding strategies employed in clonal selection pathway is given in Fig. It is concluded that panicles per plant, fresh weight of capsules per plant, nodes per panicle and internodal length of panicle are useful characters for improvement in yield of cardamom. Patel et al (1997,1998) also suggested use of traits like panicles per bearing tiller, panicles per clump, recovery ratio and capsules per panicle as criteria for selection for yield in cardamom.

Selection for biotic stress tolerance:

At Indian Institute of Spices Research, Cardamom Research Centre at Appangala, efforts have been made to survey and collect disease escapes from hot spot areas of Katte disease. Collections of Natural Katte Escapes (NKE) lines from such surveys were then subjected to artificial inoculation through the use of insect vectors. The plants that have not taken up infection even after repeated screening were field evaluated again in a hot spot area. Some of these resistant lines are also high yielding, comparable to the released varieties, both in yield and quality (IISR, 1997). One particular collection has been shown to be resistant to rhizome rot caused by Phytophthora sp. This rhizome rot resistant (RR1) line was tested in comparative yield trials along with the NKE lines. This line has given consistently good yield in all the years. Its negative point is the lower percent of bold capsules.

Selection for drought tolerance:

Selection of drought tolerant cardamom genotypes were carried out using parameters such as relative water content, membrane leakage, stomatal resistance and specific leaf weight and significant variations have been noted among cultivars (IISR, 1997).

Hybridization:

The breeding strategies employed for the crossbreeding pathway for cardamom improvement is given in Fig. The popular cardamom variety namely Vazhukka possibly originated as a natural cross between var. Malabar and var. Mysore. Since cardamom is amenable to both sexual and vegetative propagation, hybridization is a very useful tool for crop improvement. As only one species occurs in India, crossing in cardamom is confined to intra-specific levels. Because of its perennial, cross-pollinated and
heterozygous nature, the conventional methods for evolving homozygous lines in cardamom are time consuming.

**Callus culturing and somaclonal variations:**

Callus regeneration protocols are important for generating somaclonal variations for future crop improvement use. An efficient system for callus regeneration is essential to produce large number of somaclones and such a system has been reported earlier by Rao et al. (1982) and was also standardized at Indian Institute of Spices Research. High amount of variability was noticed among the somaclones for morphological characters in the culture vessel itself (Ravindran et al., 1997). The most striking morphological variant is a needle leaf variant having small needle-shaped leaves, that multiply and root profusely in the same medium, but its rate of establishment in the nursery and field is reported to be low. At Indian Institute of Spices Research, standardization of cell culture system for large-scale production of callus through somatic embryogenesis for enhancing genetic variability was carried out. The somaclones thus produced are being subjected to evaluation for virus resistance and other characters (Nirmal Babu, unpublished). Geetha et al. (1995) and Nirmal Babu et al. (1994,1999) reported conservation of cardamom germplasm in *in vitro* gene bank by slow growth at IISR. The above workers carried out various trials to achieve an ideal culture condition under which the growth is slowed down to the minimum without affecting the physiology or genetical make up of the plant. The slow growth is achieved by the incorporation of agents for increasing the osmotic potential of the medium such as mannitol. They found that half strength MS without growth regulators and with 10 g/l each of sucrose and mannitol was the best for *in vitro* storage of cardamom under slow growth. By using the above medium in screw capped vials the subculture interval could be extended to one year or more, when incubated in 22±2°C at 2500 lux of light and at 10h photoperiod. Low temperature storage at 5°C and 10°C was found to be lethal for cardamom, as the cultures did not last more than three weeks (Geetha et al.1995).

**Parameters used for the study of three different varieties of cardamom**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Malabar</th>
<th>Mysore</th>
<th>Vazhukka</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Adaptability</td>
<td>Lower elevation (600-1000m msl)</td>
<td>Higher elevation (900-1200m msl)</td>
<td>Higher elevation (900-1200m msl)</td>
</tr>
</tbody>
</table>
2 Tolerance to drought
Withstand long dry spell (4-6 months)
Needs well distributed rain

3 Plant stature
Dwarf (2-3m) Tall (3-5m) Tall (3-5m)

4 Leaf
Short petiole Long petiole Long petiole

5 Bearing nature
Early short span of flowering Late, long flowering span Late, long flowering span

6 Panicle
Prostrate Erect Semi erect

7 Capsule colour
Pale/golden yellow Green Green

### Morphological and Quality characters used for the study of core collections in cardamom:

<table>
<thead>
<tr>
<th>S.No</th>
<th>Characters</th>
<th>S.No</th>
<th>Characters</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Plant height(cm)</td>
<td>9</td>
<td>Panicle length(cm)</td>
</tr>
<tr>
<td>2</td>
<td>Total tillers/plant</td>
<td>10</td>
<td>No:of nodes/panicle</td>
</tr>
<tr>
<td>3</td>
<td>Productive tillers/plant</td>
<td>11</td>
<td>Internode length(cm)</td>
</tr>
<tr>
<td>4</td>
<td>Leafy stem diameter(cm)</td>
<td>12</td>
<td>Capsule length(cm)</td>
</tr>
<tr>
<td>5</td>
<td>No:of leaves/plant</td>
<td>13</td>
<td>Capsule breadth(cm)</td>
</tr>
<tr>
<td>6</td>
<td>Leaf length(cm)</td>
<td>14</td>
<td>Essential oil % (v/w)</td>
</tr>
<tr>
<td>7</td>
<td>Leaf breadth(cm)</td>
<td>15</td>
<td>1,8-Cinelole %</td>
</tr>
<tr>
<td>8</td>
<td>No:of panicles/plant</td>
<td>16</td>
<td>Terpinyl acetate %</td>
</tr>
</tbody>
</table>

### DEVELOPING SLOW GROWTH PROTOCOL

The prime requisite of slow growth storage strategy includes culture initiation and multiplication of the targeted crop species.

**Micropropagation**

The micropropagation protocols standardized at this Institute (Nirmal Babu *et al.* 1997) was used to multiply cardamom cultures to generate adequate material for *in vitro* conservation experiments.

**In vitro storage by minimal growth**

In cardamom vegetative bud explant was used and cultures were established in MS (Murashige and Skoog, 1962) basal medium with 0.5 mg/l kinetin. The established
cultures are being multiplied for the production of sufficient amount of material for *in vitro* storage.

Cardamom cultures were multiplied in MS medium supplemented with 0.5 mg/l NAA and 1.0 mg/l BAP, with an average of 8 shoots per culture, in about 90 days of culture. In cardamom the cultures could be stored up to 12 months with 85% survival in half MS medium supplemented with 10g/l each of sucrose and mannitol in sealed culture tubes.

**ANNEXURE-II**

**Detailed expenditure statement for the year 1998 – 2002**

*Establishing in vitro conservatory of spices germplasm*


---

**Statement of expenditure during 1998-99 (in Rs.)**

<table>
<thead>
<tr>
<th>Heads</th>
<th>Opening balance</th>
<th>Amount sanctioned</th>
<th>Amount released</th>
<th>Amount spent</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fellowship*</td>
<td>-</td>
<td>1,90,440*</td>
<td>96000</td>
<td>1,03,150</td>
<td>-7,150</td>
</tr>
<tr>
<td>Recurring contingency &amp; TA</td>
<td>-</td>
<td>2,20,000</td>
<td>2,20,000</td>
<td>86,542</td>
<td>1,33,458</td>
</tr>
<tr>
<td>10% Institutional charges</td>
<td>-</td>
<td>31,600</td>
<td>31,600</td>
<td>Nil</td>
<td>31,600</td>
</tr>
<tr>
<td>Non Recurring contingency</td>
<td>-</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>-</td>
<td><strong>4,42,040</strong></td>
<td><strong>3,47,600</strong></td>
<td><strong>1,89,692</strong></td>
<td><strong>1,57,908</strong></td>
</tr>
</tbody>
</table>

**Statement of expenditure during 1999-2000**

<table>
<thead>
<tr>
<th>Heads</th>
<th>Opening balance</th>
<th>Amount sanctioned</th>
<th>Amount released</th>
<th>Amount spent</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fellowship*</td>
<td>-7,150</td>
<td>1,90,440*</td>
<td>2,35,200</td>
<td>1,89,585</td>
<td>38,465</td>
</tr>
<tr>
<td>Recurring contingency &amp; TA</td>
<td>1,33,458</td>
<td>2,20,000</td>
<td>2,20,000</td>
<td>1,24,833</td>
<td>2,28,625</td>
</tr>
<tr>
<td>10% institutional charges</td>
<td>31,600</td>
<td>31,840</td>
<td>31,840</td>
<td>Nil</td>
<td>63,440</td>
</tr>
<tr>
<td>Non Recurring contingency</td>
<td>-</td>
<td>Nil</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1,57,908</strong></td>
<td><strong>4,42,280</strong></td>
<td><strong>4,87,040</strong></td>
<td><strong>3,14,418</strong></td>
<td><strong>3,30,530</strong></td>
</tr>
</tbody>
</table>
## Statement of expenditure during 2000 - 2001

<table>
<thead>
<tr>
<th>Heads</th>
<th>Opening balance</th>
<th>Amount sanctioned</th>
<th>Amount released</th>
<th>Amount spent</th>
<th>Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fellowship*</td>
<td>38,465</td>
<td>1,98,720*</td>
<td>86400</td>
<td>1,39,461</td>
<td>-14596</td>
</tr>
<tr>
<td>Recurring contingency &amp; TA</td>
<td>2,28,625</td>
<td>2,20,000</td>
<td>110000</td>
<td>2,22,314</td>
<td>1,16,311</td>
</tr>
<tr>
<td>10% Institutional charges</td>
<td>63,440</td>
<td>32,080</td>
<td>16040</td>
<td>95,520</td>
<td>-16,040</td>
</tr>
<tr>
<td>Non Recurring contingency</td>
<td>-</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>3,30,530</strong></td>
<td><strong>4,50,800</strong></td>
<td><strong>2,12,440</strong></td>
<td><strong>4,57,295</strong></td>
<td><strong>85,675</strong></td>
</tr>
</tbody>
</table>
### ANNEXURE-11 (Continued..)

#### Statement of expenditure during 2001-2002

<table>
<thead>
<tr>
<th>Heads</th>
<th>Opening balance</th>
<th>Amount sanctioned</th>
<th>Amount released</th>
<th>Amount spent</th>
<th>Balance (Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fellowship</td>
<td>-14,596</td>
<td>-</td>
<td>1,62,000</td>
<td>84059</td>
<td>63345</td>
</tr>
<tr>
<td>Recurring cont. &amp; TA</td>
<td>1,16,311</td>
<td>-</td>
<td>-</td>
<td>36403</td>
<td>79,908</td>
</tr>
<tr>
<td>10% Instit. charges</td>
<td>-16,040</td>
<td>-</td>
<td>16040</td>
<td>33167</td>
<td>-33,167</td>
</tr>
<tr>
<td>Non Recurring contingency</td>
<td>-</td>
<td>Nil</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>85,675</td>
<td>1,78,040</td>
<td>1,53,629</td>
<td>1,10,086</td>
<td></td>
</tr>
</tbody>
</table>

#### Expenditure committed during March, 2002 (Actual expenditure met in July, 2002)

<table>
<thead>
<tr>
<th>Heads</th>
<th>Opening balance</th>
<th>Amount sanctioned</th>
<th>Amount released</th>
<th>Amount spent</th>
<th>Balance (Rs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fellowship</td>
<td>63345</td>
<td>-</td>
<td>-</td>
<td>337</td>
<td>63,008</td>
</tr>
<tr>
<td>Recurring cont. &amp; TA</td>
<td>79,908</td>
<td>-</td>
<td>-</td>
<td>47396</td>
<td>32,512</td>
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<tr>
<td>10% Instit. charges</td>
<td>-33,167</td>
<td>-</td>
<td>-</td>
<td>62353</td>
<td>-95,520</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1,10,086</td>
<td>-</td>
<td>-</td>
<td>1,10,086</td>
<td>0</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Year</th>
<th>Amount Sanctioned</th>
<th>Opening balance</th>
<th>Amount released</th>
<th>Amount spent</th>
<th>Balance provision@</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998–1999</td>
<td>4,42,040</td>
<td>-</td>
<td>3,47,600</td>
<td>1,89,692</td>
<td>1,57,809</td>
</tr>
<tr>
<td>1999–2000</td>
<td>4,42,280</td>
<td>1,57,809</td>
<td>4,87,040</td>
<td>3,14,418</td>
<td>3,30,530</td>
</tr>
<tr>
<td>2000–2001</td>
<td>4,50,800</td>
<td>3,30,530</td>
<td>2,12,440</td>
<td>4,57,295</td>
<td>85,675</td>
</tr>
<tr>
<td>2001–2002</td>
<td>-</td>
<td>85,675</td>
<td>1,78,040</td>
<td>1,53,629</td>
<td>1,10,086</td>
</tr>
<tr>
<td>*2002 May</td>
<td>-</td>
<td>*1,10,086</td>
<td>-</td>
<td>*1,10,086</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>13,35,120</td>
<td>-</td>
<td>12,25,120</td>
<td>12,25,120</td>
<td>0</td>
</tr>
</tbody>
</table>

* The expenditure committed in March, 2002, and the payments made in July 2002
@ Amount under balance provision need not be released.

The amount sanctioned under fellowship is based on the following ICAR sanctions, granting additional funds under pay and allowances on account of revision of emoluments

1. F. No. 15(16)/ 95 Hort. 1 dated Sept. 5, 1997
2. F. No. 15 (16)/ 95-Hort.1, dated July 1998
3. **F. No. 15 (16)/95-Hort.1, dated 31 August, 2001** (The account is based on this final sanction)

Principal Investigator
Director
K Nirmal Babu
Date

Finance and Accounts Officer
Consolidated statement of expenditure for 1998–2002 of the ICAR Ad-hoc scheme

Establishing *In vitro* conservatory of spices germplasm (IISR, Calicut)


<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Am. Sanctioned</td>
<td>Am. released</td>
<td>Am. spent</td>
</tr>
<tr>
<td>Fellowship *</td>
<td>1,65,600</td>
<td>96000</td>
<td>1,03,150</td>
</tr>
<tr>
<td>Rec. Cont. &amp;TA</td>
<td>2,20,000</td>
<td>2,20,000</td>
<td>86,542</td>
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<tr>
<td>10% Instit. Charges</td>
<td>31,600</td>
<td>31,600</td>
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</tr>
<tr>
<td>Total</td>
<td>4,17,200</td>
<td>3,47,600</td>
<td>1,89,692</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HEADS</th>
<th>2001 - 2002</th>
<th>2002 **</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Am. Sanctioned</td>
<td>Am. released</td>
</tr>
<tr>
<td>Fellowship *</td>
<td>-</td>
<td>1,62,000</td>
</tr>
<tr>
<td>Rec. Cont. &amp;TA</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Non. Rec. Cont.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10% Instit. Charges</td>
<td>-</td>
<td>16040</td>
</tr>
<tr>
<td>Total</td>
<td>-</td>
<td>1,78,040</td>
</tr>
</tbody>
</table>

* The expenditure committed in March, 2002, and the payments made in July, 2002

* The amount sanctioned under fellowship is based on the following ICAR sanctions, granting additional funds under pay and allowances on account of revision of emoluments

4. F. No. 15(16)/ 95 Hort. 1 dated Sept. 5, 1997
5. F. No. 15 (16)/ 95-Hort.1, dated July 1998
6. F. No. 15 (16)/ 95-Hort.1, dated 31 August, 2001 (The account is based on this final sanction)

Principal Investigator      Finance and Accounts Officer      Director
K. Nirmal Babu
Date


Barthakur M P and Bordoloi D N. 1992. Micropropagation of


Barghchi, M. 1986.


Guha S and Maheswari S C. 1964. In vitro 


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