Introduction
India is blessed not only with rich biological diversity but also with the associated indigenous knowledge. The country is one of the twelve mega diversity centres and harbors 3 of the 28 global hotspots (Chauhan, 1996). It is estimated that up to 100,000 plants, representing more than one third of all the world's plant species, are currently threatened or face extinction (BGCI, 2005). Preservation of the plant biodiversity is essential for plant improvement and provides various compounds to the pharmaceutical, food and crop protection industries. Plant genetic resources which include land races, primitive cultivars, advanced/improved varieties and wild relatives of crop plants hold the key to food security and sustainable agricultural development (Iwananga, 1994).

Conservation of germplasm in seed genebanks by storage of desiccated seeds at low temperature is the most efficient, economical and preferred. But this method is not applicable to crops that do not produce seed (e.g., ginger) or with recalcitrant seed (cardamom & black pepper) as well as to plant species that are propagated vegetatively to preserve the unique genomic constitution of cultivars. Conservation in clonal field repositories is the cheapest alternative. But the field collections are exposed to risks of pests, diseases and adverse weather conditions in addition to being labour-intensive. These risks can be mitigated using in vitro conservation strategy which is also labour-intensive. Somaclonal variation is another factor to be considered and at best in vitro conservation can be used only for medium term conservation. Hence, cryopreservation or freeze-preservation at ultra-low temperature (-196°C, i.e., the temperature of liquid nitrogen) is a sound additive to conventional ex situ approaches for the long-term conservation of base collections of plant genetic resources, since under these
conditions, biochemical and most physical processes are completely arrested and the
plant material can be stored for unlimited periods (Withers & Engelmann, 1998).

**Spices Genetic Resources**

India is considered as the land of spices, over 53 major spices are grown. Spices like black pepper, chilli, ginger, turmeric, cardamom, fennel, fenugreek, coriander and cumin form the economic backbone of large number of people in India.

Cardamom, *Elettaria cardamomum* Maton, considered the “Queen of Spices”, is a large, herbaceous, rhizomatous perennial, belonging to the family Zingiberaceae. Cardamom is native to the moist evergreen forests of the Western Ghats of Southern India and is propagated both through seeds and clonally through suckers. Being a cross-pollinated, seeds are heterozygous. Serious diseases of viral and bacterial origin, such as katte, Nilgiri necrosis and Azhukal disease threaten crop cultivation as well as field germplasm repositories (Venugopal, 2001). Cardamom is also infested by various insects and nematode pests, among which thrips (*Sciothrips cardamomi* Ramk.) shoot and capsule borer (*Conogethes punctiferalis* Guen.) root grub (*Basilepta fulvicorne* Jacoby) and root knot nematode (*Meloidogyne incognita* Kofoed et White) is important (Premkumar and Madhusoodanan, 1995, Ramana and Eapen, 1992). Hence development of alternate strategies for conservation is important.

Ginger, *Zingiber officinale* Rosc. also belongs to the family Zingiberaceae is not known to occur in the truly wild state but is under cultivation since ancient times. Ginger is believed to have originated in Southeast Asia and is propagated only through vegetative means. India is the largest producer of ginger with rich cultivar diversity (Lawrence, 1984). It is cultivated from time immemorial in India and China (Ravindran
et al., 2005). Over 800 accessions of ginger germplasm are available at National Conservatory for Ginger (Ravindran et al., 2005) at Indian Institute of Spices Research (IISR). The major constraints involved in the conservation of the germplasm of ginger are the two soil-borne diseases—rhizome rot caused by Pythium spp. (P. aphanidermatum, P. myriotylum and P. vexans) and the bacterial wilt caused by Ralstonia solanacearum (Pseudomonas solanacearum). Added to this, infection by leaf fleck virus also poses serious problems for conservation. These diseases are extremely difficult to control under field conditions. In vitro conservation of ginger germplasm is safe and complementary. Conservation of ginger germplasm under in vitro conditions by slow growth was standardized at IISR (Geetha, 2002). At IISR, over 100 unique accessions of ginger are conserved in vitro as medium-term storage of germplasm (Geetha, 2002; Nirmal Babu et al., 2005; Ravindran et al., 2005). The possibility of storage at relatively high ambient temperatures (24-29°C) by subjecting the ginger and related taxa to stress factors was explored (Dekker et al., 1991). In vitro conservation of ginger requires subculturing at 8-12-months intervals (Geetha, 2002) and can ensure only medium-term storage and cryopreservation is more appropriate for long-term conservation of ginger germplasm.

India, is also the center of origin and diversity for black pepper (Piper nigrum L, family Piperaceae) considered the ‘king of spices,’ one of the most widely used spice in the world. Pepper is predominantly propagated using stem cuttings as seeds are heterozygous and seed progenies are not true to type. Piper barberry, a closely related species, is very rare and is reported to be almost extinct (Subramanyam and Henry, 1970). 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. 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.

Efficient technologies for cryopreservation of germplasm in cardamom, ginger and black pepper are not yet optimized. Only one report each are available on cryo conservation of black pepper and cardamom, using seeds (Chaudhury & Chandel, 1994; 1995). Heterozygous nature of seeds in these crops makes these conservation techniques not suitable for germplasm conservation.

For long-term conservation of the germplasm cryopreservation is the only current method without subculture conferring genetic stability with minimum space and maintenance requirements (Engelmann, 1997). Storage of clonal materials in liquid nitrogen (LN) as a base collection is the goal of many genebanks (Reed et al, 2000), but for efficiency and reliability each species requires the development of an appropriate cryopreservation protocol. It is important that cryopreservation is not seen as a full replacement for conventional ex situ approaches (Withers and Engelmann, 1997). It serves the field gene bank as an additional tool to improve the conservation of germplasm and can also be used for efficient pest and pathogen free germplasm exchange.

In the case of black pepper and cardamom, the few published studies in this area have used seeds (Chaudhury and Chandel, 1994; 1995). As per literature available, the most relevant strategy for long-term conservation of vegetatively propagated
species, cryopreservation of shoot tips (a genetically stable plant material), has not been investigated for the above crops.

The present study aims at developing cryopreservation strategies for safe long term conservation of base germplasm in important spice crops such as black pepper, cardamom and ginger. In addition this study also attempts to the genetic fidelity of cryopreserved material by molecular markers.