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Spices biotechnology

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ABSTRACT

In recent times, biotechnological tools have supplemented various conventional approaches in conservation, characterization, improvement and utilization for increasing production and productivity of spices. In many spices, viable micropropagation technologies are available for commercial production and generation of disease - free planting material. Somaclonal variation is important in crops where natural variability is low and a few useful somaclonal variants have been identified in ginger, turmeric and vanilla. Protoplast technology is also available for capsicum, black pepper, fennel, fenugreek, garlic, saffron and peppermint. *In vitro* cryopreservation, Synseed and Micro-rhizome technologies are available for safe propagation, conservation, movement, and exchange of spices germplasm. Studies are in progress for *in vitro* production of flavour and colouring compounds like capsaicin, vanillin, anethole, crocin, picrocrocin, saffranal, etc. using immobilized and transformed cell cultures. Use of molecular markers for crop profiling, fingerprinting, molecular taxonomy, identification of duplicate hybrids, estimation of genetic fidelity and tagging of genes for marker aided selection (MAS) is gaining importance. Isolation of important and useful genes and development of transgenics is in the preliminary stage.

Key words: Spice crops, micropropagation, somaclonal variation, DNA fingerprinting, secondary metabolites

INTRODUCTION

Spices and herbs are aromatic plants, parts of which are used to flavour culinary preparations, in confectionery, and in medicines and perfumery. Spices and herbs are grown throughout the world; different plant species are grown in different regions. India is a rich repository of spices with over 100 species of herbs and spices being grown. Black pepper, cardamom, ginger, turmeric, vanilla, capsicum, cinnamon, clove, nutmeg, tamarind, pimenta, etc., constitute the major spices. Seed spices like coriander, cumin, fennel, fenugreek, dill, caraway, anise and herbal spices like saffron, lavender, thyme, oregano, celery, anise, sage and basil are also important. Crop improvement aims to increase productivity and quality of a target crop to meet increasing human demands. Lack of high yielding, pest and disease resistant varieties, and a limited genetic variability in some crops, is a major production constraint in spices. Use of biotechnological tool stands to play a major role in achieving the above through commercial propagation, development of novel varieties and new breeding lines *via* somaclonal variation, anther culture, protoplast fusion, bioreactor and recombinant DNA technologies for improving, conserving and utilizing the diversity and increasing the utility of spices.

Micropropagation and Plant regeneration

High and rapid rate of multiplication coupled with additional advantage of obtaining disease-free planting material makes micropropagation an important and viable alternative to conventional propagation.

Black pepper and related species

Methods for micropropagation of black pepper have been reported using various explants from both mature and juvenile tissues (Broome and Zimmerman, 1978; Lissamma Joseph *et al,* 1996). Phenolics and endogenous bacterial contaminants severely hamper establishment in black pepper cultures. Treating explants with fungicides prior to routine sterilization followed by frequent transfer to fresh medium, use of activated charcoal and antibiotics in culture media have been suggested for reducing phenolic interference and systemic contamination. Efficient plant regeneration protocols are essential for genetic manipulation of any crop species. Plants have been successfully regenerated from callus cultures of many *Piper* species. Plant regeneration

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was reported from shoot tip and leaf, with or without an intervening callus phase, (Bhat *et al*, 1995).

Techniques for somatic embryogenesis in black pepper are reported by Nair and Gupta (2003). Cyclic somatic embryogenesis from maternal tissues like integuments has tremendous potential for automated micropropagation. These systems are useful for transgenic experiments for transfer of *Phytophthora* resistance. Methods for micropropagation of medicinally important species of piper viz., *Piper longum P. chaba* and *P. betle* have also been developed (Sarasan *et al*, 1993). Plants were regenerated from leaf and stem explants of related species of black pepper like *Piper longum*, *P. betle*, *P. chaba*, *P. attenuatum* and *P. colubrinum* through both direct and indirect organogenesis (Bhat *et al*, 1992;1995). Somatic embryogenesis is also reported in betelvine (Johri *et al*, 1996).

Cardamom

Efficient and commercially viable technology for rapid clonal propagation of cardamom is available (Vatsya *et al*, 1987). Many commercial laboratories use micropropagation techniques for large-scale production of clonal material.

Successful high-frequency regeneration of plantlets from cardamom has been reported. Attempts on anther and microspore culture were reported to be inconsistent in plant regeneration from anther derived callus on MS medium.

Ginger

Clonal multiplication of ginger has been reported by many workers (Rout *et al*, 2001). Micropropagation helps in production of pathogen-free planting material in ginger where diseases often spread through infected seed rhizomes. Regeneration of plantlets through callus has been reported from leaf, vegetative bud, ovary and anther explants. Ginger fails to set fruit in nature. However, in *vitro* pollination could be effected to overcome prefertilization barriers to develop the 'fruit' and subsequently, plants could be recovered from these fruits (Valsala *et al*, 1997).

Turmeric

Technologies for micropropagation turmeric of for production of disease-free planting material were developed (Nadgauda *et al*, 1978; Yasuda *et al*, 1988, Rahman *et al*, 2004; Prathanturarug *et al*, 2003, 2005). Organogenesis and plant regeneration has been reported in turmeric by various workers (Shetty *et al*, 1982; Praveen *et al*, 2005).

Renjith *et al* (2001) reported *in vitro* pollination and hybridization using two short duration types VK-70 and VK-76 and reported seed set and seed development. This reduces breeding time and helps in recombination breeding which had not been attempted in turmeric earlier.

Other Zingiberaceous taxa

Protocols for micropropagation of many economically and medicinally important zingiberaceous species like *Amomum subulatum* (large cardamom), *Curcuma aromatica* (kasturi turmeric), *C. domestica* var. 'Koova', *C. aeruginosa, C. caesia* , *C. amada* (mango ginger), *Curcuma domestica [C. longa], C. zedoaria Kaempferia galanga, K. rotunda, Alpinia* spp., *Alpinia conchigera, Alpinia galanga, etc have been* developed. (Barthakur and Bordoli, 1992; Chang and Criley, 1993). Yasuda (1988) reported successful callus induction from rhizomes. Prakash *et al* (2004), Lakshmi and Mythili (2003) and Rahman *et al* (2004) reported efficient plant regeneration through somatic embryogenesis from leaf base-derived callus of *Kaempferia galanga* L.

Vanilla

Micropropagation of vanilla has been standardized for large-scale multiplication of disease-free plants (Cervera and Madrigal, 1981; Kononowicz and Janick, 1984; Geetha and Shetty, 2000).

In vitro germination of vanilla seeds and selection of useful genotypes from segregating progenies is also reported. This technique was also used to rescue interspecific hybrids between cultivated *V. planifolia* and wild *V. aphylla* through embryo rescue.

In vitro propagation of *Vanilla tahitiensis* (Mary Mathew *et al*, 2000) and endangered species of *Vanilla-V. wightiana* , *V. andamanica, V. aphylla* and *V. pilifera* was also reported to save these species from extinction.

Successful plant regeneration from shoot and seed derived callus was reported in vanilla (Davidonis and Knorr, 1991; Nirmal Babu *et al*, 1997). This efficient system can be used for creation and exploitation of somaclonal variation in this crop where the existing variation is limited.

Tree spices

Micropropagation protocols have been reported in many tree spices like cinnamon, nutmeg, cassia, clove, camphor, curry leaf, pomegranate, camboge and tamarind (Zhang and Stoltz, 1981; Mascarenhas, *et al* 1987; Mathew and Hariharan, 1990; Hazarika *et al*, 1995; Mini *et at*, 1997; Mallika, et al, 1997; Bhuyan *et al*, 1997; Huang *et al*, 1998; Nirmal Babu *et al*, 2000; Mehta *et al*, 2000).

Plant regeneration through somatic embryogenesis has been reported in *Cinnamomum verum* and *C. camphora.* Induction of somatic embryogenesis from zygotic embryos of *Syzygium cumini* and nutmeg was reported by Iyer *et al,*. (2000).

Seed and herbal spices

Micropropagation protocols for many seed and herbal spices are available. These include coriander, fennel, anise, peppermint, spearmint, celery, thyme, lavender, savory, ocimum, oregano, basil, sage, fennel , parsley, sweet marjoram, dill and garlic (Bhojwani, 1980; Ahuja, 1982; Miura, *et al,* 1987; Cellarova, 1992; Furmanowa and Ozszowska, 1992; Hunault and Du-Manoir, 1992; Panizza and Tognoni, 1992; Toth and Lacy, 1992; Patnaik and Chand, 1996; Vandemoortele *et al,* 1996; Sajina *et al,* 1997; Iyer and Pai, 1998).

Plant regeneration has been successfully induced from callus cultures of peppermint, coriander, celery, cumin, fennel, lavender, anise, parsley, poppy, oregano, dill, caraway and sage (Ratnamba and Chopra, 1974; Sehgal, 1978; Chand and Roy, 1981; Jha *et al,* 1982; Ammirato,1983; Van Eck and Kitt, 1990;1992; Neena Kumari and Sarathy, 1992; Kataeva and Popowich, 1993; Onisei *et al,* 1994; Okamoto *et al,* 1994; Donovan *et al,* 1994; Hunault and Maatar, 1995; Kim *et al,* 1996; Sajina *et al,* 1997; Sastry *et al,* 1997).

Propagation through somatic embryogenesis and *in vitro* flowering and seed set in coriander was reported by Stephan and Jayabalan (2001). *In vitro* flowering and seed formation in cumin has been reported. Bertaccini *et al* (2004) used micropropagation for elimination of mite-brone virus and for establishment of virus-free garlic (*Allium sativum*).

Plant regeneration from anther and microspore cultures has been reported in fennel and celery.

Capsicum

Micropropagation and plant regeneration in chilli was reported using various explants (Agarwal 1988; Anu *et al,* 2004).

Development of haploid capsicum through androgenesis is reported. New approaches for induction of pollen embryogenesis in *Capsicum annuum* were reported by Gonzalez *et al* (1996) and Regner (1996). Occurrence of unreduced gametes and ploidy restoration in haploid peppers (*Capsicum annuum*) was reported.

Saffron

Reports are available on micropropagation and plant regeneration in saffron. *In vitro* proliferation of saffron stigma was also reported (Homes *et al,* 1987; Ilahi *et al,* 1987; Yang *et al,* 1996).

Field evaluation of tissue cultured plants

Black pepper and related species

Large-scale field evaluation of tissue cultured black pepper plants, in over 30 ha in all the pepper growing districts of Kerala, indicated that tissue cultured plants were superior to conventional propagules in field establishment, plant height, internodal length, number of laterals per unit area, number of spikes per unit area, fruit set, mean yield, dry weight, oil content, oleoresin content, etc. Preliminary field performance of micropropagated plantlets of *Piper longum, P. chaba* and *P. betle* indicated that these were on par with conventionally propagated plants (Nirmal Babu *et al,* 2003).

Cardamom

Large-scale field evaluation of tissue cultured plants of cardamom was carried out by the Spices Board of India and the IISR. Results showed that micropropagated plants performed on par with suckers.

Ginger and turmeric

Field evaluation of tissue cultured plants of ginger and turmeric indicate that micropropagated plants require at least two crop seasons to develop rhizomes of normal size that can be used as seed rhizomes for commercial cultivation. Tissue cultured plants of kasturi turmeric, mango ginger, *Kaempferia galanga,* etc. also show a similar pattern.

Salvi *et al* (2002) reported in turmeric that micropropagated plants showed significant increase in shoot length, number of tillers, number and length of leaves, number of gingers and total fresh rhizome weight per plant compared to conventionally propogated plants. Variations among regenerated plants have been reported in *Kaempferia galanga.* Anu *et al,*(2004) reported variation among somaclones and their seedling progeny in *Capsicum annuum.*

Estimation of genetic fidelity in micropropagated pepper using RAPDs

Genetic fidelity of micropropagated plants of black pepper was confirmed by Nirmal Babu *et al* (2003). RAPD (Random Amplification of Polymorphic DNA) profiling and morphological characterization indicated that the micropropagation protocol can be used for commercial cloning of black pepper. Genetic uniformity of micropropagated *Piper longum* using RAPD profiling was reported by Ajith *et al* (1997) and Parani *et al* (1997).

In ginger, RAPD profiles did not show any polymorphism among micropropagated plants. However, Nirmal Babu *et al* (2003) reported RAPD profile differences in micropropagated ginger.

Salvi *et al* (2001) reported that RAPD analysis of regenerated plants in turmeric showed variation. Genetic stability and uniformity of *Foeniculum vulgare* Mill. plants regenerated through organogenesis and somatic embryogenesis was reported by Bennici *et al* (2004).

Somaclonal variation

Induction and utilization of somaclonal variation was attempted in many spices to develop genotypes resistant to biotic and a biotic stresses.

In black pepper, a few *Phytophthora* foot rot tolerant somaclones were identified through *in vitro* selection of calli using crude culture filtrate and toxic metabolites isolated from *Phytophthora capsici.*

Attempts to induce somaclonal variation in cardamom resulted in identification of a few Katte virus tolerant somaclones (Nirmal babu *et al,* 1997).

In ginger, field evaluation of somaclones indicated variability and resulted in identification of a few promising, high yielding lines with tolerance to rhizome rot (Nirmal Babu *et al*, 1996; Nirmal Babu, 1997). RAPD characterisation of these somaclones also showed profile variations indicating genetic differences Isolation of *Pythium*-tolerant ginger by using culture filtrate as the selecting agent has also been reported.

Variants with high curcumin content were isolated from tissue cultured plantlets of turmeric. Root rot disease tolerant clones of turmeric cv. Suguna were isolated using continuous *in vitro* selection technique against pure culture filtrate of *Pythium graminicolum* (Gayatri *et al*, 2005).

Variation in essential oil composition of plants regenerated from protoplasts of peppermint was reported. Reports are also available on *in vitro* selection for salt tolerance in fenugreek, *Trigonella foenum-graecum; in vitro* selection for resistance to *Alternaria* blight in cumin and drought tolerance in coriander through tissue culture has also been showed. Somaclonal variation and virus elimination for improvement of garlic has been reported. Ghosh *et al* (1997) reported generation of virus free plants by thermotherapy and meristem culture in garlic. MSU - SHK 5, a somaclonally derived *Fusarium* yellows resistant line in celery has been identified.

Microrhizomes

Microrhizomes form an important source of disease-free planting material in rhizomatous crops like ginger and turmeric and are ideally suited for germplasm exchange, transportation and conservation.

In vitro induction of microrhizomes in ginger, turmeric and *Kaempferia* is reported by many workers (Bhat *et al,* 1994; Nirmal Babu, 1997; Raghu Rajan, 1997; Sunitibala *et al,* 2001; Nirmal Babu *et al,* 2003).

Microrhizome derived plants had more tillers but the plant height was smaller. They gave fresh rhizome yield ranging from 100- 800 g per plant with an estimated yield of 10 kg per 3m^2 bed. *In vitro* formed rhizomes were found to be genetically more stable compared to micropropagated plants (Nirmal Babu *et al,* 2003).

Synthetic seeds

Artificial or synthetic seeds can be an ideal system for low-cost plant movement, propagation, conservation and exchange of germplasm.

Synthetic seeds were developed by encapsulationg *in vitro* developed small shoot buds in 3% calcium alginate in black pepper, cardamom, ginger, turmeric, camphor, cinnamon, celery, lavender and fennel. These synthetic seeds could be stored from 7 to 10 months in sterile water with over 80 % viability (Redenbaugh *et al,* 1986; Pratap 1992; Sharma *et al*, 1994).

Protoplast culture

The protoplast is a naked cell and absence of the cell wall makes a protoplast suitable for a variety of manipulations that are not normally possible with intact cells. Hence, protoplast is an important tool for parasexual modification of genetic content of cells.

Successful isolation and culture of protoplasts was reported in *P. nigrum* and *P. colubrinum* (Shaji *et al*, 1998). Plant regeneration, however, was observed only in *P. colubrinum*. Protoplasts could be successfully isolated from *in vitro* grown leaf mesophyll tissues of cardamom, ginger and turmeric. These were cultured upto the microcalli stage (Nirmal Babu,1997; Geetha *et al*, 2000).

Isolation and fusion of protoplasts in vanilla is reported. Isolation of protoplasts from leaves of nutmeg has been reported by Iyer *et al* (2000).

Successful isolation and culture of protoplasts was reported in fennel (Miura and Tabata, 1986), fenugreek (Sen and Gupta, 1979), peppermint and garlic (Ayabe *et al*, 1995) and saffron (Isa *et al*, 1990). Suh Sang Ki and Park (1995) reported protoplast fusion and culture in garlic. Successful production of interspecific hybrids between peppermint and gingermint was reported by Sato *et al* (1996).

Organogenesis and plant regeneration from isolated protoplasts have been demonstrated in chillies (Fari and Czako 1981; Agarwal, 1988; Prakash *et al*, 1997).

Genetic transformation

Preliminary reports are available on *Agrobacterium* - mediated gene transfer in *P. nigrum* (Sasikumar and Veluthambi, 1996). They obtained primary transformants for kanamycin resistance in cotyledons using *Agrobacterium tumefaciens* binary vector strains LBA 4404 and EHA 105. Sim *et al* (1998) reported *Agrobacterium*-mediated transfer of GUS gene to black pepper. Nirmal Babu *et al* (2005) reported *Agrobacterium* - mediated transformation of black pepper with the gene for osmotin, a PR (Pathogenesis related) protein known to induce resistance to *Phytophthora.*

Preliminary experiments to standardize optimum conditions for gene delivery and efficiency of the plasmid vector pAHC25 and promoter Ubi-1 and transformation of cardamom using biolistic process resulted in transient expression of GUS gene in bombarded callus tissue.

A few reports are available on *Agrobacterium*-mediated genetic transformation of capsicum (Liu *et al*, 1990; Shivegowda *et al*, 2002). Regeneration of transgenic pepper plants resistant to TMV and CMV has been reported.

Molecular characterization and development of mapping populations

In recent times, there is increased emphasis on using molecular markers for characterization of genotypes for genetic fingerprinting, to identify and clone important genes, for marker assisted selection and in understanding inter-relationships at the molecular level.

Black pepper

In black pepper, molecular markers like RAPD, AFLP and ISSR were used for assessment of genetic variability to characterize important cultivars, varieties, related species to develop fingerprints and to study inter relationships (Pradeep Kumar *et al*, 2001). A mapping population was developed for preparation of the genetic map in black pepper (Nirmal Babu *et al*, 2003). Male parent-specific RAPD markers were used by Johnson *et al* (2005) to identify hybrids.

Jaramillo and Manos (2001) used phylogenetic analysis of sequences of the Internal Transcribed Spacers (ITS) of nuclear ribosomal DNA based on a world wide sample of the genus *Piper.*

In long pepper (*Piper longum*), Banerjee *et al* (1999) reported male sex associated RAPD markers. Genetic diversity among landraces of a dioecious *Piper betle* using molecular markers was reported by Anjali *et al* (2004).

Cardamom

Molecular techniques like RAPD, RFLP and ISSR polymorphism were used to characterize cardamom germplasm collections comprising important cultivars, varieties and related genera to develop fingerprints and to study inter-relationships. The study indicated no duplicates in the 100 lines characterized and that the Kerala and Karnataka populations were divergent as they formed two separate clusters in the phylogram. RAPD and ISSR profiling of 11 species representing 5 major, related tribes of cardamom indicated that *Ammomum* is closest to the cultivated cardamom (Nirmal babu *et al*, 2005). A protocol for isolation and molecular characterization of DNA from market samples of cardamom was standardized and can be used to identify different grades of commercial cardamom and to identify adulttrents if any (IISR Annual Report, 2004).

Ginger

RAPD profiling of various ginger cultivars and related species is in progress at the Indian Institute of Spices Research to study the inter- relationships and to identify core collections in the germplasm. Ninetysix accessions of ginger were analysed and interrelationships studied. Polymorphism detected is moderate to low in ginger. RAPD profiling of ginger somaclones and selected 'variants' among micropropagated, callus regenerated and microrhizome derived plants indicated differences in RAPD profiles.

Phylogenetic analysis of the tribe *Zingibereae* (Zingiberaceae) was performed by Ngamriabsakul *et al* (2003) using nuclear ribosomal DNA (ITS1, 5.8S and ITS2) and chloroplast DNA. The study suggested that the tribe *Zingibereae* and the genus *Curcuma* are monophyletic. Kress *et al* (2002) studied phylogeny of the gingers (Zingiberaceae) using DNA sequences of the nuclear internal transcribed spacer (ITS) and plastid *matK* regions and proposed a new classification of the Zingiberaceae.

Turmeric

Sasaki *et al* (2004) used single nucleotide polymorphism (SNP) analysis of the trnK gene to identify *Curcuma* plants.

Sasikumar *et al* (unpublished) studied over 96 Indian cultivars and related species of turmeric using RAPD profiling for establishing interrelationship. RAPD analyses showed good polymorphism among the 96 accessions studied. Five species of *Curcuma* were characterized using 12 primers. Intra species polymorphism in (*curcuma* was high compared to the interspecies polymorphism (IISR 2003, 2004).

An efficient protocol for isolation of high molecular weight DNA from dried. Powder samples of turmeric, including market samples, is described by Remya *et al* (2004). This will help in PCR-based detection of adulteration in marketed turmeric powder. Cao *et al* (2001) and Sasaki *et al* (2002) used sequence analysis of Chinese and Japanese *Curcuma* drugs on the 18S rRNA gene and trnK gene and the application of amplification-refractory mutation system analysis for authentication.

Vanilla

In the absence of classical phenotypic markers in perennial crops like vanilla, molecular markers such as RAPD and AFLP were used to establish genetic similarities and interrelationships in cultivars, seed progenies, somaclones and interspecific hybrids. Isoenzyme, RAPD and AFLP polymorphisms, supplemented by morphological characters, have been used to study the existing variability in cultivated vanilla, species interrelationships, identification of interspecific hybrids, and, fingerprinting of important genotypes. The study indicated limited variability among the cultivated collections of *V. planifolia* grown in India. *Vanilla tahitensis* was found to be closest to *V. planifolia*. Significant variations exist among selfed seed progenies of *V. planifolia*. This variation was further magnified when plant regeneration was through callus or when explants were grown in colchicine containing medium. Progeny obtained from crosses between *V. planifolia* and *V. aphylla* is truly hybrid, and thus, *in vitro* technology can be used for generation of variability in crop improvement (Minoo *et al*, 2006).

In tree spices, Shibu *et al* (2000) identified sex - *specific DNA markers for identifying female trees in* nutmeg. Yapwattanaphun *et al* (2004) used ITS sequence data to elucidate phylogenetic relationship in mangosteen (*Garcinia mangostana*) and its wild relatives (*Garcinia* spp.). Molecular characterization and preparation of molecular maps has been done in *Capsicum*. Arnedo-Andres *et al* (2002) developed RAPD and SCAR markers linked to the Pvr4 locus for resistance to PVY in capsicum. Blum *et al* (2002) reported mapping of the locus for pungency in *Capsicum*. Kang *et al* (2001) developed interspecific (*Capsicum annuum* x *C. chinese*) F2 linkage map in pepper using RFLP and RAPD markers. Caranta *et al* (1999) developed CAPS marker for the Pvr 4 locus for pyramiding potyvirus resistance genes in pepper.

Isolation of candidate genes

Work on isolation of genes responsible for agronomically important characters, especially for biotic and a biotic stresses, has also been attempted in spices.

In black pepper, programmes on isolation, cloning of genes and validation is in progress and a few putative, genomic and cDNA fragments associated with resistance genes have been isolated (IISR 2004, 2005; Johnson *et al*, 2005). Molecular cloning of a cDNA fragment encoding the defense related protein â-1,3-glucanase in black pepper (*P. nigrum* L.) and methyl glutaryl CoA reductase in *Piper colubrinum* has been reported. Bhat *et al* (2005) reported isolation and sequencing of CMV coat protein gene with reference to black pepper.

Chen *et al* (2005) reported cDNA cloning and characterization of a mannose-binding lectin from *Zingiber officinale* Roscoe (ginger) rhizomes.

Molecular cloning of mannose-6-phosphate reductase and its developmental expression in celery was studied by Everard *et al* (1997). Wang and Kumar (2004) reported that heterologous expression of *Arabidopsis* ERS1 causes delayed senescence in coriander.

Huh *et al* (2001) utilized the candidate gene approach to identify phytoene synthase as the locus for mature fruit color in red pepper (*Capsicum* spp).

Tai and Staskawicz (2000) constructed yeast artificial chromosome (YAC) library of hot pepper (*Capsicum annuum* L.) and identified clones from the Bs2 resistance locus.

Bai *et al* (2004) reported successful cloning and expression of *Crocus sativus* phytoene desaturase gene and preparation of antiserum. Tsaftaris *et al* (2004) reported isolation of three homologous AP1-like MADS-box genes in *Crocus sativus* L. and characterized their expression.

Conservation of genetic resources

In vitro conservation

Genetic resources of most spices are conserved either in seed gene banks or in field repositories. Storage of germplasm in seed banks is not practical in some crops as these are vegetatively propagated and seeds are either recalcitrant or heterozygous. Conservation of germplasm in *in vitro* gene banks and cryobanks is a viable and safe alternative.

Conservation of pepper, cardamom, ginger, turmeric, vanilla, seed and herbal spice germplasm *in vitro* in gene banks by slow growth was reported (Dekkers *et al*, 1991; Nirmal Babu *et al*, 1996, 1997,1999).

Conserved material of all the species developed into normal plants without any deformities and was morphologically similar to the mother plants. RAPD profiling of the conserved plants too showed genetic integrity. Suspensions of embryogenic cell lines of fennel, conserved at 4 ^{0}C for upto 12 weeks, produced normal plants upon transfer to normal laboratory conditions (Umetsu *et al*, 1995). Conservation of genetic resources in *in vitro* gene banks is now an established convention and gene banks for conservation of spice germplasm functions at the IISR and at the National Bureau of Plant Genetic Resources, New Delhi. About 500 accessions of spice germplasm are currently conserved the *in vitro* repository of IISR.

Cryopreservation

Cryopreservation of black pepper and cardamom seeds in liquid nitrogen (LN_2) has been reported. Plants could be successfully regenerated from cryopreserved seeds of capsicum and anise, and, technologies for cryopreservation of black pepper, cardamom, ginger, turmeric and vanilla germplasm - using vitrification and encapsulation methods is available. Choudhary and Chandel, (1995); eported cryopreservation of vanilla pollen for conservation of the haploid genome and for assisted pollination between species that flower during different seasons and successful fertilization was effected using cryopreserved pollen.

Production of secondary metabolites

Use of biotechnology for biosynthesis of secondary metabolites particularly in plants of pharmaceutical significance holds an interesting alternative to conventional production of plant constituents.

In vitro proliferation of the stigma of saffron, *Crocus sativus* and chemical analysis of metabolites produced through tissue cultures has been reported by Himeno *et al* (1988); Koyama *et al* (1987). *In vitro* metabolite production from saffron tissue cultures has also been demonstrated by Venkataraman *et al* (1989) and Vishwanath *et al* (1990).

Production of flavour components and secondary metabolites *in vitro* using immobilised cells is an ideal system for spice crops. Production of saffron and capsaicin was reported using cell cultures (Johnson *et al,* 1996). Reports on *in vitro* synthesis of crocin, picrocrocin and saffranal from saffron stigma (Himeno and Sano, 1995) and colour components from cells derived from pistils (Hori *et al,* 1988) are available for further scale up. Johnson *et al* (1996) reported biotransformation of ferulic acid vanillamine to capsaicin and vanillin in immobilised cell cultures of *Capsicum frutescens.*

Callus and cell cultures have been established in nutmeg, clove, camphor, ginger, lavender, mint, thyme, celery, etc. Cell immobilization techniques have been standardized in ginger, sage, anise and lavender (Ilahi and Jabeen, 1992). Production of essential oils from cell cultures (Ernst, 1989) and accumulation of essential oils by *Agrobacterium tumefaciens* transformed shoot cultures of *Pimpinella anisum* has been reported (Salem and Charlwood, 1995). Regulation of the shikimate pathway in suspension culture cells of parsley (Conn and McCue, 1994) and production of anethole from cell cultures of *Foeniculum vulgare* (Hunault *et al*, 1989) is also reported. Growth of shoot cultures and production of monoterpene by transformed shoots of *Mentha citrata* and *Mentha piperita* in flasks and fermentors was reported. Chavez et al (1996) reported biosynthesis of the sesqiterpene phytoalexin capsidol in elicited root cultures of chilli pepper. Production of rosmarinic acid in suspension cultures of *Salvia officinalis* has been discussed by Hippolyte *et al* (1992). Phenyl propanoid metabolism in suspension cultures of *Vanilla planifolia* was studied by Funk and Brodelius (1990 a, b). Reports on production of phenolic flavour compounds using cultured cells and tissues of vanilla are also available (Dorenburg and Knorr, 1996). *In vitro* production of petroselinic acid was reported from cell suspension cultures of coriander (Kim *et al*, 1996). Kintzios *et al* (2004) reported scaling up of micropropagation in *Ocimum basilicum* L. in an airlift bioreactor and accumulation of rosmarinic acid thereof.

Though the feasibility of *in vitro* production of spice principles has been demonstrated, methodology for scaling up and reproducibility needs to be developed before it can reach commercial levels. Once standardized, this technology can have tremendous potential in industrial production of important compounds like capsaicin, vanillin, crocin, picrocrocin, saffranal, myristicin, anethole, menthol and curcumin.

Micropropagation technology is available for rapid cloning of many spices. Technology for conservation of genetic resources in *in vitro* gene banks is another useful development. Molecular characterization of germplasm has made reasonable progress. Identifying markers for important agronomic characters will help in marker assisted selection to shorten breeding time. Application of recombinant DNA technology for production of transgenics resistant to biotic and abiotic stress has a long way to go in spices improvement. Although programmes have been initiated in many laboratories on *in vitro* secondary metabolite production, these techniques need to be refined and scaled up for possible industrial application. Considering commercial possibilities, intensification of biotechnological activities in spices is needed in the coming decades.

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