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Plant biotechnology — its role in improvement of spices

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ABSTRACT

Spices have played a significant role in the history of civilization. Crop improvement in spices is difficult and time consuming due to their long pre-bearing age and other crop specific problems. Biotechnology offers novel avenues for crop improvement of spices. This paper reviews the present status of biotechnology of spices with emphasis on the work done in India on micropropagation and rapid clonal multiplication of high-yielding elite genotypes to generate good quality planting material, exploiting somaclonal variation and genetic engineering techniques for crop improvement, *in-vitro* selection for resistance to biotic and abiotic stresses, *in-vitro* conservation for safe exchange of germplasm and production of flavour and volatile constituents in culture.

Key words: spices, crop improvement, somaclones, biotechnology

The ultimate aim of any crop-improvement programme is increasing the productivity to cope up with the ever-increasing human population. Plant biotechnology can be utilized to manipulate cell, tissue and organ in different ways for the benefit of mankind. The newly emerging areas of plant biotechnology such as gene mapping and genetic engineering assumes greater significance in this context and are being increasingly utilized in improvement of many crops.

Spices are of considerable importance for India and are a major source of foreign exchange among agricultural commodities. India has been traditionally known as the leader of spices and has a pre-eminent position in the production of these crops and accounts for about 47% of the global trade. India is also known to be a rich repository of spices and more than 100 species of spices are grown in about 2 million ha in the country with an annual production of 2.2 million tonnes fetching Rs 70 000 million of revenue and Rs 11 800 million worth of foreign exchange and play a crucial role in the economy of various states where they are grown (Trade Information, Spices Board 1997). However, the productivity of many of these crops is considerably low due to various factors such as inadequate availability of high-yielding varieties, absence of genotypes resistant to pests and diseases and absence of variability in many of the introduced crops like nutmeg (*Myristica fragrans* Houtt.) and clove (*Syzygium aromaticum* Merr. & Perry) besides labour intensive cultural operations.

In spices, plant cell and tissue culture have been utilized for micropropagation, production of somaclones, production of haploid plants, production of secondary metabolites from cell cultures, *in-vitro* conservation, protoplast culture, plant genetic transformation etc. This paper reviews the present status of spices biotechnology with emphasis on the work done in India.

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MICROPROPAGATION

Micropropagation is the true-to-type propagation of selected genotype using *in-vitro* techniques. Many spices like black pepper (*Piper nigrum* L.), cardamom [*Elettaria cardamomum* Maton], ginger (*Zingiber officinale* Rosc.), turmeric (*Curcuma domestica* Valeton; syn *C. longa* L.), cinnamon (*Cinnamomum zeylanicum* Breyn.; syn *Z. verum* Presl.), clove, etc are grown in plantation scale and large-scale availability of good quality, disease-free planting material is an important input in increasing production.

Cardamom

Cardamom, native to India, is one of the most important spices. Its productivity is hampered by diseases of viral etiology like '*Katte*', '*Kokke kandu*' and 'Nilgiri necrosis'. Utilization of virus-free planting material is considered the most important input in disease-management programmes as well as to check disease spread. Being a cross-pollinated crop, clones are ideal for generating true-to-type planting material from high-yielding clumps and *in-vitro* propagation helps in large-scale production of planting material. *In-vitro* propagation methods for clonal propagation of cardamom from vegetative buds have been standardized (Vatsya *et al.* 1987, Reghunath and Gopalakrishnan 1991). High rate of multiplication (Fig 1, *top left*) coupled with additional

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advantage of obtaining uniform and disease-free planting material makes micropropagation a preferred method over conventional method.

Many commercial laboratories are at present using micropropagation techniques for large-scale production of cardamom clonal planting material. Field evaluation of tissue-cultured plants of cardamom showed that the micropropagated plants performed at par with suckers of the original mother plant (Lukose *et al.* 1993).

Immature inflorescences form an excellent source for clonal multiplication of cardamom through tissue culture especially when other sources are prone to high rate of contamination. Kumar *et al.* (1985) reported the successful conversion of immature floral buds to vegetative buds and subsequently to plantlets.

Black pepper

India is one of the major producers and exporters of black pepper. It is also native to India. Collection and conservation of the precious genetic resources of black pepper is one of the priority programmes. Foot rot (*Phytophthora capsici*) is the major disease affecting pepper plantations. None of the existing genotypes have resistance to this.

In-vitro culture methods of black pepper have been reported using mature shoot tip explants (Philip *et al.* 1992) and from seedlings (Mathew and Rao 1984) and tissue-cultured plantlets were successfully established in field. The multiplication rate is around 6 shoots/culture in about 90 days (Fig 1, *top right*). Though there were earlier reports on micropropagation of black pepper (Fitchet 1988 a,b), phenolic exudates from the cut surface and bacterial contamination (Raj Mohan 1985, Fitchet 1988 a,b) severely hampered the establishment phase. Methods for reducing phenolic interference and systematic contamination in the *in-vitro* cultures have been reported (Nazeem *et al.* 1993).

***Related species of* Piper**

Piper betle is an economically important species, cultivated extensively, in India; the leaves of which are used as a masticatory. *Piper longum* L. (Indian long pepper) and *Piper chaba* Hunt. (Java long pepper) are another group of medicinally important spices. *Piper colubrinum* is a South American species found to be resistant to *Phytophthora capsici* and *Rhadopholus similis* which are the causative organisms of the dreaded diseases foot rot and slow decline of black pepper. *Piper barberi* Gamble is an endangered species from south India (Nirmal Babu *et al.* 1992 b).

Protocols for rapid clonal multiplication of *Piper longum* from shoot tip explants are available (Nirmal Babu *et al.* 1994, Rema *et al.*, 1995). Micropropagation of *P. chaba* (Rema *et al.* 1995), *P. betle* (Nirmal Babu *et al.* 1992 c), *P. colubrinum* (Nirmal Babu *et al.* 1996 a) and *P. barberi* (Nirmal Babu *et al.* 1996 b, Rema *et al.* 1995) has been standardized. Conversion of root meristem into shoot meristem and its subsequent development to plantlets were reported in *P. longum* and *P. colubrinum* (Nirmal Babu *et al.* 1993 b). Micropropagated plantlets of these species are being evaluated for their field performance at the IISR.

Ginger

Ginger is an important tropical spice originated probably in South Asia. Rhizome rot caused by *Pythium aphanidermatum*, bacterial wilt caused by *Pseudomonas solanacearum*, leaf spot caused by *Phyllosticta zingiberi* are the major diseases and shoot-borer (*Conogethes punctiferalis*) and rhizome scale (*Aspidiella hartii*) are the major pests affecting ginger.

Crop-improvement programmes in ginger are hampered by lack of seed set leading to limited variability. Somaclonal variation could be an important source of variability that could be exploited to evolve high-yielding, high-quality lines and to develop lines resistant to rhizome rot and bacterial wilt. Tissue-culture techniques could also be used for *in-vitro* pollination, embryo rescue and possible production of 'seed' in ginger.

Clonal multiplication of ginger from vegetative buds (Fig 1, *middle left*) is possible (Balachandran *et al.* 1990, Choi 1991). Ginger cultivation is threatened by rhizome rot disease caused by *Pseudomonas solanacearum* and *Pythium* spp. Diseases of ginger are often spread through infected seed rhizomes. Tissue-culture technique would help in the production of pathogen-free planting material of high-yielding varieties.

At the IISR field evaluation of tissue-cultured plants indicated that they cannot be used directly for commercial planting, as 2 crop seasons are required for the micropropagated plants to develop rhizomes of normal size that can be used as seed rhizomes.

Inflorescence culture and in-vitro *development of fruit* : In nature, ginger fails to set fruit. However, by supplying required nutrients to immature inflorescence in culture, it was possible to make the ovary develop *in-vitro* into 'fruit' (Fig 4, *top left*) and subsequently plants could be recovered from the fruits (Valsala *et al.* 1996). It was also possible to convert the immature floral buds into vegetative buds and their subsequent development into complete plants (Nirmal Babu *et al.* 1992 d).

Turmeric

Turmeric, of commerce is dried rhizome of *Curcuma longa* L. India is the major producer and exporter of this spice. Rhizome rot (*Pythium graminicolum*) is a major production constraint. Curcumin is important colouring material obtained from turmeric and development of varieties with high recovery of curcumin is the need of hour.

Yasuda *et al.* (1988) reported successful micropropagation of turmeric. This technique could be used for production of disease-free planting material of elite plants. Micropropagation of turmeric was standardized at the IISR using young vegetative buds as explants. They responded

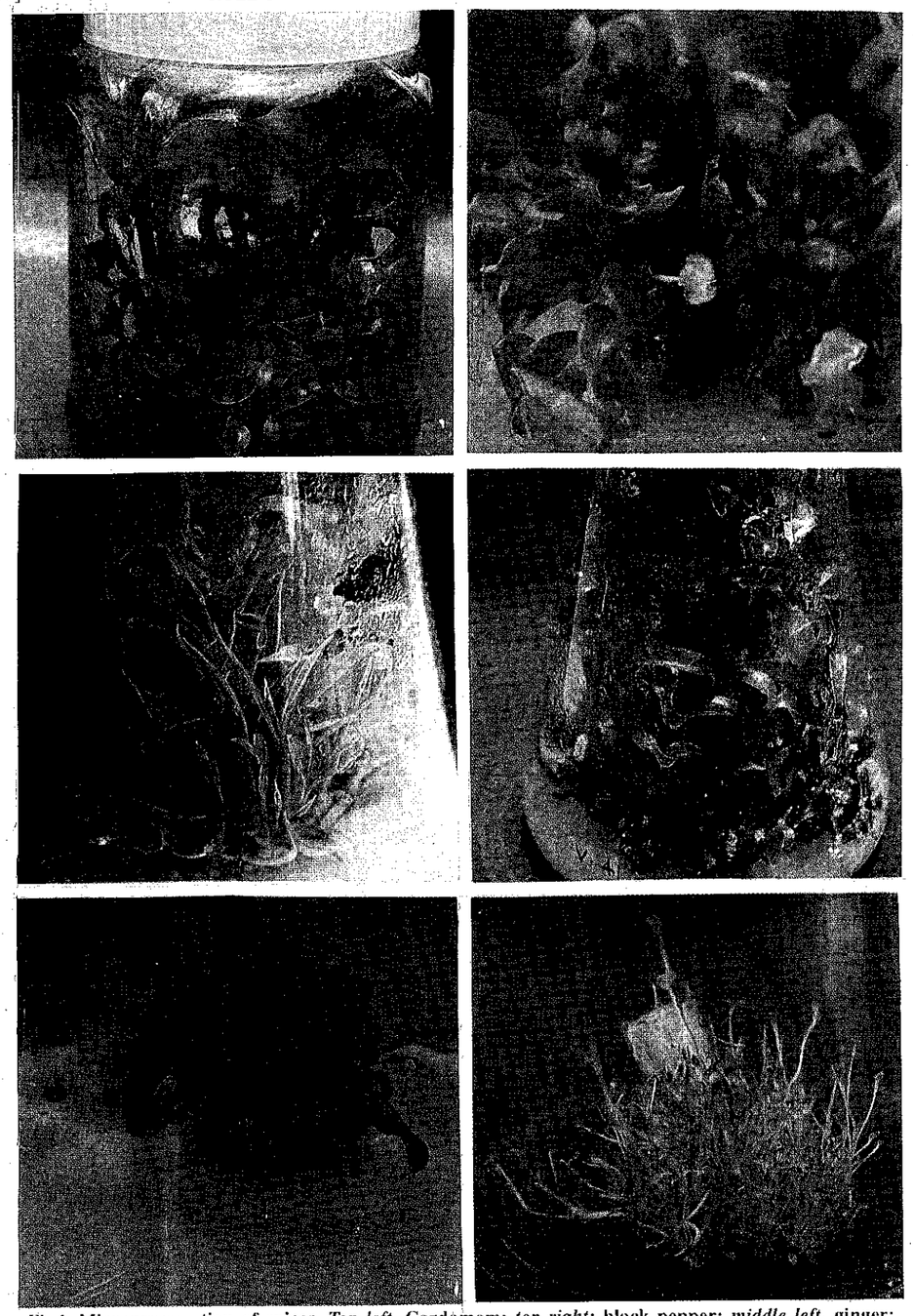

Fig 1 Micropropagation of spices. *Top left*, Cardamom; *top right;* black pepper; *middle left*, ginger; *middle right*, vanilla; *bottom left*; camphor; *bottom right;* thyme

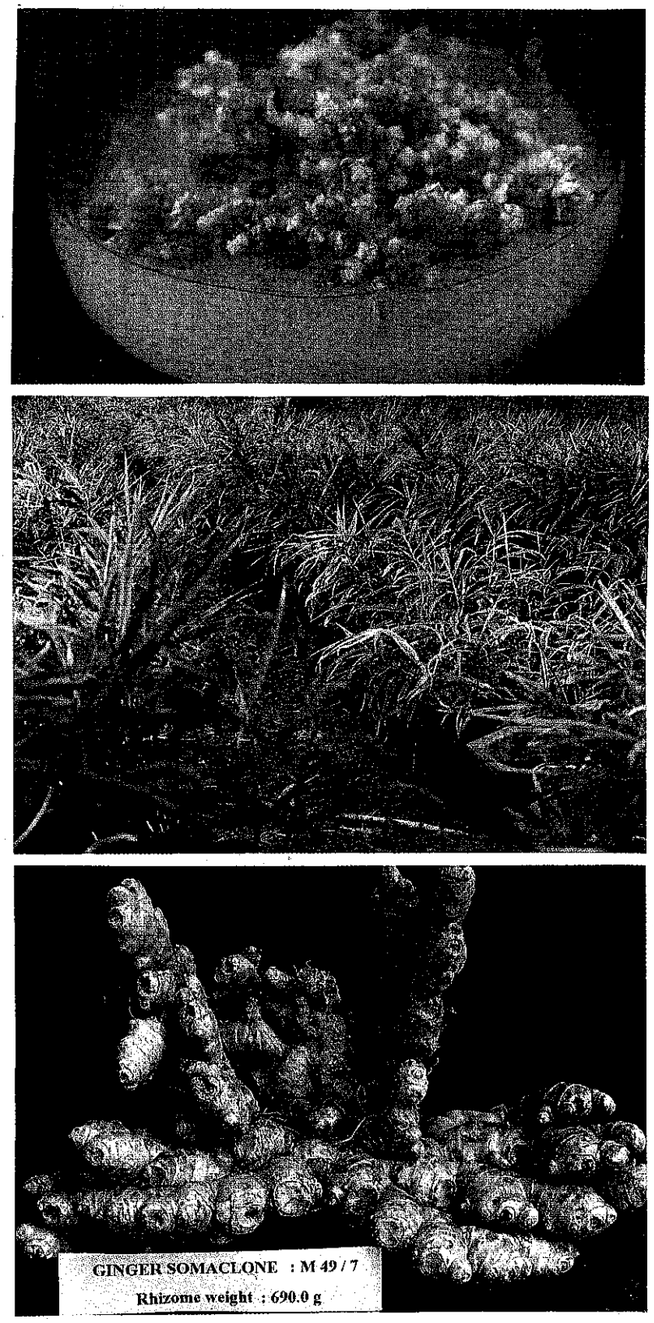

Fig 2 Plant regeneration from callus cultures. *Top*, Plant regeneration from ovary explants of ginger; *middle*, field evaluation of ginger somacloes; *bottom*, a promising ginger somaclones

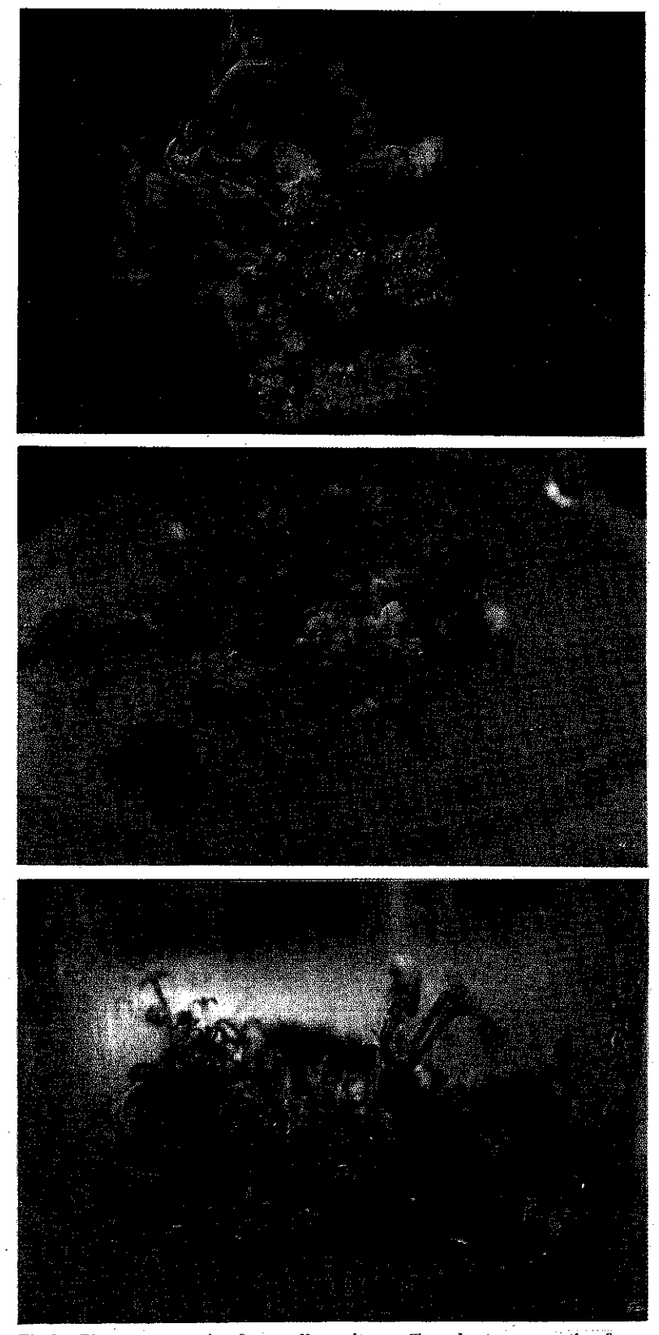

Fig 3 Plant regeneration from callus cultures. *Top*, plant regeneration from *Piper colubrinum*; *middle*, somatic embryogenesis in cinnamon; *bottom*, plant regeneration from lavender

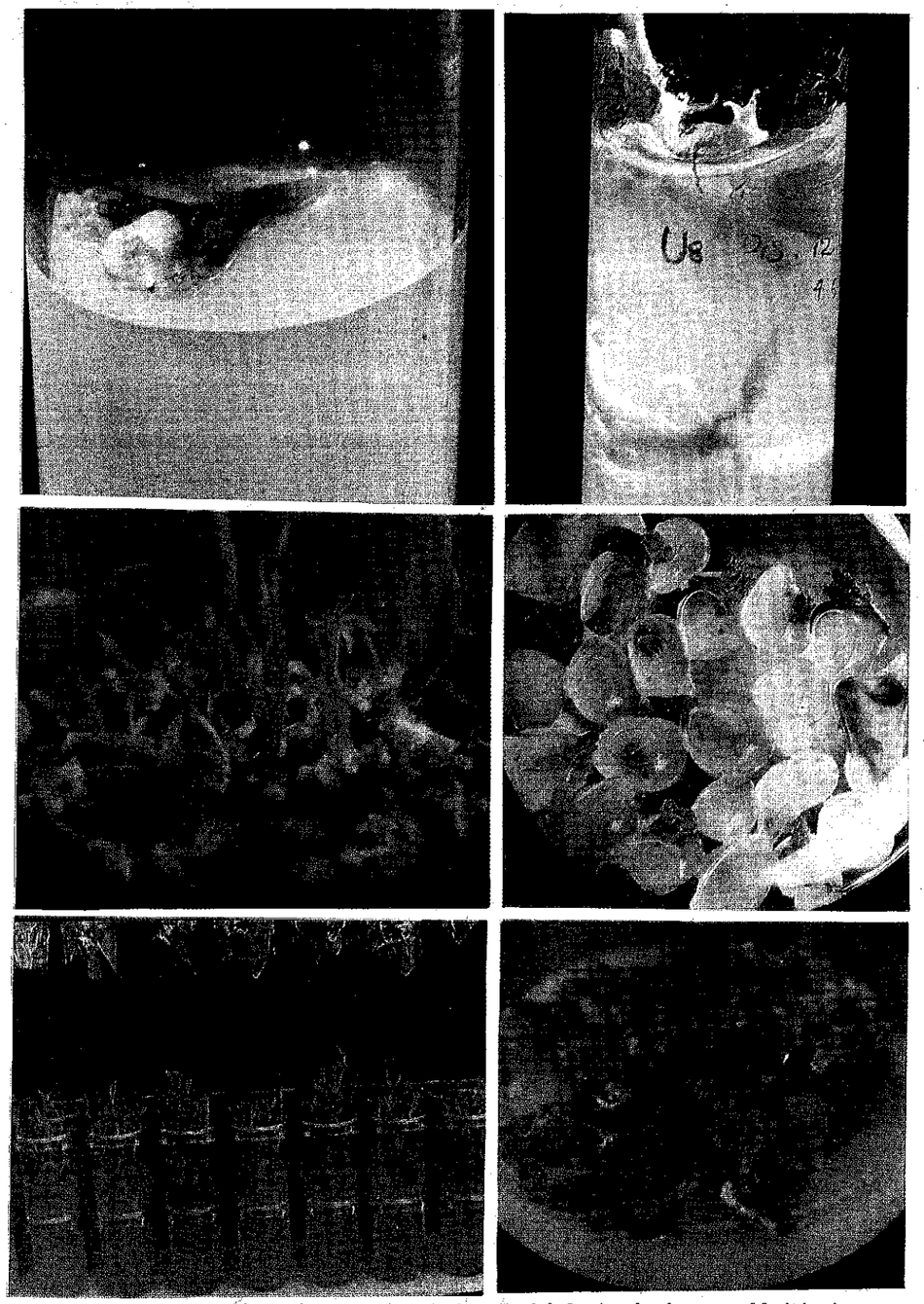

Fig 4 *In-vitro* propagation and conservation of spices. *Top left*, *In-vitro* development of fruit in ginger; *top right*, conversion of root meristem into shoot meristem in vanilla; *middle left*, seed culture of vanilla; *middle right*, synthetic seeds of camphor; *bottom left*, *in-vitro* conservation of cardamom by slow growth; *bottom right*, *in-vitro* proliferation of mace

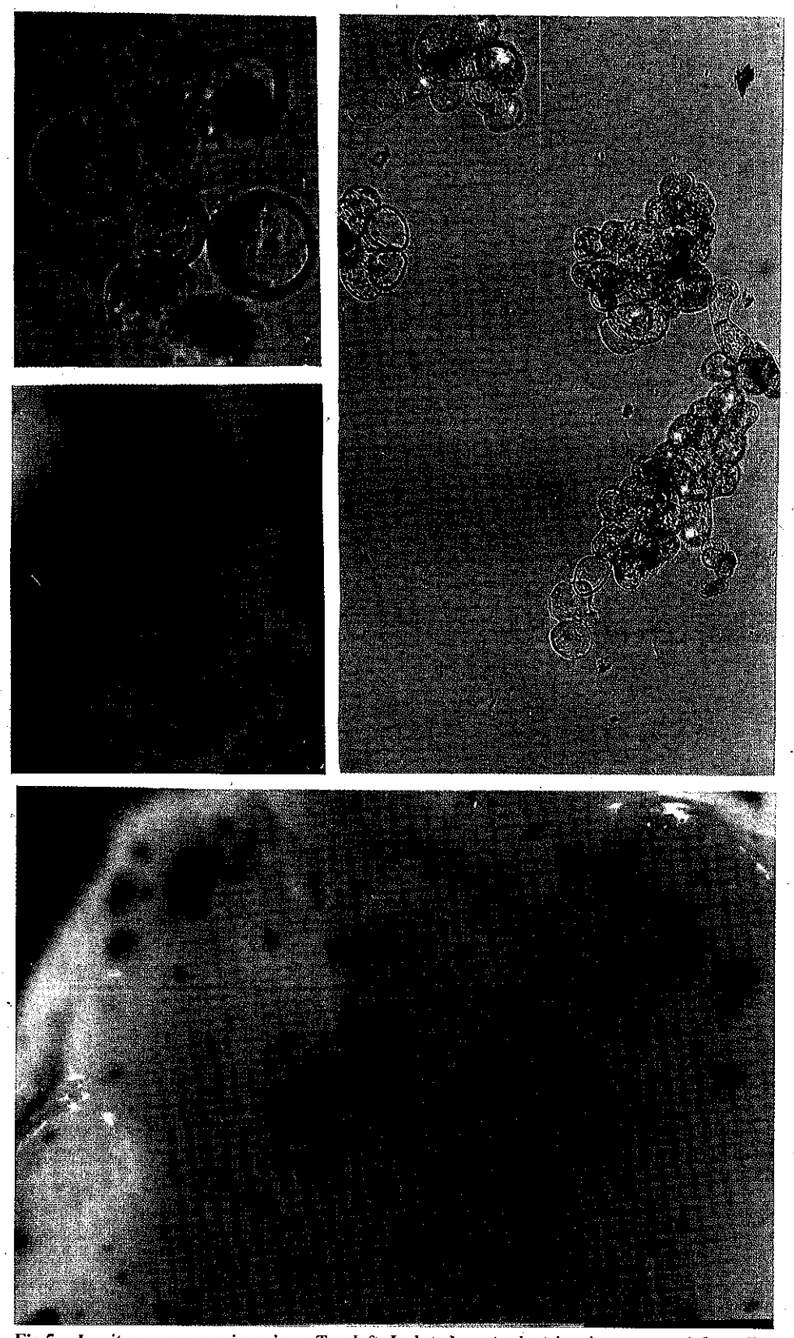

Fig 5 *In-vitro* responses in spices. *Top left*, Isolated protoplast in ginger; *top right*, cell-division from protoplasts of cardamom; *middle left*, development of oil cells in suspension cultures of ginger; *bottom*, transgenic expression of GUS in ginger embryogenic callus

readily to culture conditions producing 8–10 adventitious shoots in 40 days of culture (Nirmal Babu *et al.* 1993 a).

Other zingiberaceous taxa

Many other economically and medicinally important zingiberaceous species like *Amomum subulatum* (large cardamom), *Curcuma aromatica* (kasturi turmeric), *C. amada* (mango ginger), *Kaempferia galanga*, *K. rotunda* etc, could be micropropagated (Barthakur and Bordoli 1992, Vincent *et al.* 1993, Ravindran *et al.* 1996, Sajina *et al.* 1997). Reports are also available on micropropagation of *Alpinia* spp (Chang and Criley 1993, Thomas *et al.* 1996).

Vanilla

Vanilla planifolia Andr. is the natural source of the flavouring substance, vanillin. Practically there is no variability in the cultivated form of this spice, in India. Vanilla, though produces numerous minute seeds, do not germinate under natural conditions. Hence vanilla is commercially propagated by stem cuttings. Further, root rot (*Fusarium batatatis* var. *vanillae)* is a devastating disease which can wipe out plantations in a short period. *In-vitro* culture could be used for germination of seeds and selection of useful genotypes from segregating progenies, rapid multiplication and for getting disease-free planting material.

Micropropagation of vanilla using apical meristem (Fig 1, *middle right*) was standardized for large-scale multiplication of disease-free and genetically stable plants (Philip and Nainar 1986, George *et al.* 1995, Minoo *et al.* 1996a). Reports on conversion of root meristem to shoot meristem and subsequent development to plantlets (Fig 4, *top right*) were also available (Philip and Nainar 1986, Ravindran *et al.* 1996). Vanilla shows many meiotic and post-meiotic chromosomal abnormalities (Ravindran 1979). As a result of these aberrations, it is quite possible to get various cytotypes in the seed progenies. Culturing of seeds can thus give many genetically variant types. *In-vitro* germination of seeds has been reported (Fig 4, *middle left*) and the resultant progeny showed variations (Nirmal Babu *et al.* 1993a, Minoo *et al.* 1996).

Saffron

The dried styles and stigma of the flower is used as a spice. Saffron is a triploid and sterile genotype propagated vegetatively by means of corms.

Reports are available on the micropropagation and regeneration aspects in saffron. Successful micropropagation, somatic embryogenesis and regeneration were also reported in saffron (Ahuja *et al.* 1994, Sushma *et al.* 1995).

Tree spices

Clove, cinnamon, nutmeg, allspice [*Pimenta dioica* (L.) Merr.] etc. are some of the important tree spices grown in India. In all these perennial tree crops, identification and clonal multiplication of high-yielding genotypes becomes an immediate priority due to long pre-bearing period. Standardization of micropropagation methods will help in rapid multiplication of elite planting materials in this crop.

Micropropagation of clove (Jagadishchandra and Rai 1986, Mathew and Hariharan 1990) and *Cinnamomum verum* (Jagadishchandra and Rai 1986) has been reported. Successful micropropagation from shoot tip explants of mature trees has been recorded in cinnamon (Mini *et al.* 1996) and camphor (IISR 1996b) (Fig 1, *bottom left*). Micropropagation of *Murraya koenigii* (curry leaf) was reported by Hazarika *et al.* (1995) and Rajendra and D'Souza (1995).

Seed and herbal spices

Fennel, thyme, lavender, mint, basil, oregano, marjoram, sage, celery, anise etc. are some of the important herbal spices. Micropropagation protocols for 15 seed and herbal spices were standardized at the IISR including coriander, celery, thyme (Fig 1, *bottom right*), lavender, anise, savory, ocimum, oregano, basil, sage, fennel, parsley, dill, peppermint and spearmint (Sajina *et al.* 1996 a). Some of the earlier reports on micropropagation of herbal spices are on peppermint celery (Toth and Lacy 1992), thyme (Furmanowa and Olszowska 1992), fennel (Hunault and Du-Manoir 1992), lavender (Panizza and Tognoni 1992) etc. Clonal propagation of chemically uniform fennel plants through somatic embryoids was reported by Miura *et al.* (1987).

CALLUS REGENERATION AND PRODUCTION OF SOMACLONES

Efficient plant regeneration system is an important step in any genetic manipulation attempt for crop improvement.

Cardamom

Rao *et al.* (1982) reported the successful regeneration of plantlets from callus of seedling explants of cardamom. Plantlet formation via adventitious shoots from callus cultures was reported by Priyadarshan and Zachariah (1986). Protocols for organogenesis and plant regeneration from rhizome and vegetative bud-derived callus cultures were also standardized at the IISR (Nirmal Babu *et al.* 1993 a). This excellent regeneration system (with about 20–50 plantlets/culture) is being used at present for large-scale production of somaclones and selection of useful genotypes from them. High amount of variability could be noticed among the somaclones for the morphological characters in the culture vessels itself. Somaclones are being evaluated in the field at the IISR for the realistic estimation of the genetic variability.

Ginger

Regeneration of plantlets through callus phase has been reported (Kacker *et al.* 1993) from leaf explants. Techniques for plant regeneration from ovary (Fig 2, *top*) and vegetative buds were also standardized (Nirmal Babu *et al.* 1992 d, 1995). This system could be used for inducing somaclonal variability in ginger. This is very important in crops where

conventional breeding is hampered by lack of seed set. Somaclones could also be used for screening against disease resistance and biotic and abiotic stress. Field evaluation of somaclones (Fig 2, *middle*) indicated high variability with regard to various agronomic characters and other yield attributes. A few promising high-yielding lines (Fig 2, *bottom*) with tolerance to rhizome rot were identified from the somaclones (Samsudeen 1996, Nirmal Babu 1997).

In-vitro *selection:* *Pythium*-tolerant ginger could be isolated by using culture filtrate as the selecting agent. *In-vitro* selection for resistant types to *Pythium* and *Pseudomonas* is in progress at the IISR using culture filtrates of the pathogen, or pathotoxin as the selecting agent (IISR 1995).

Turmeric

Organogenesis and plantlet formation were achieved from the callus cultures of turmeric (Shetty *et al.* 1982). Variants with high curcumin content were isolated from tissue-cultured plantlets (Nadgauda *et al.* 1982).

Other zingiberaceous species

Successful plant regeneration and variations among regenerated plants were reported in *Kaempferia galanga* (Ajith Kumar and Seeni 1995).

Black pepper

Protocols for plant regeneration in black pepper were standardized and plants were regenerated from leaf-callus cultures (Nirmal Babu *et al.* 1993 a, b, Nazeem *et al.* 1993, Bhat *et al.* 1995). Plants could be regenerated from leaf tissues directly without intervening callus phase (NRCS 1991). These plant regeneration system could be useful for transfer of *Phytophthora* resistance from the wild to the cultivated *P. nigrum*. *In-vitro* screening of black pepper for tolerance to *Phytophthora* using concentrated culture filtrates of *P. capsici* was reported (Shylaja *et al.* 1996) and few tolerant lines were developed.

***Related species of* Piper**

Plants were regenerated from leaf and stem explants of *Piper longum*, *P. betle*, *P. chaba* and *P. colubrinum* (Fig 3, *top*) through direct and indirect organogenesis (Rema *et al.* 1995, Bhat *et al.* 1992, Bhat *et al.* 1995). In *P. betle* and *P.longum*, different explants from shoot, leaf and root developed multiple shoots and regenerated into plantlets either directly or through intervening callus phase (Nirmal Babu *et al.* 1992 b, Rema *et al.* 1995). Johri *et al.* (1996) reported regeneration of betle vine through somatic embryogenesis.

Vanilla

Plant regeneration from leaf and seed-derived callus was also successful in vanilla (Davidonis and Knorr 1991, IISR 1996a). This technique can be utilized for creation and exploitation of somaclonal variation.

Tree spices

A method for regeneration of plants through somatic embryogenesis from seedling explants in *Cinnamomum verum* (Fig 3, *middle*) was standardized at IISR (Mini *et al.* 1996). Somatic embryogenesis from hypocotyl callus in *C. camphora* was reported (Jagdishchandra and Sharief 1995).

Seed and herbal spices

Somatic embryogenesis in some species of Apiaceae has been discussed by Lourdes and Alfermann (1994). Regeneration of plants from callus cultures and potential sources of variation in tissue culture derived plants in celery (Donovan *et al.* 1994, Okamoto *et al.* 1994) and the frequency of somatic embryogenesis in fennel (Hunault and Maatar 1995), lavender (Onisei *et al.* 1994) have been studied. There was variation in essential oil composition of plants regenerated from protoplasts of peppermint (Okuyama *et al.* 1995). Plant regeneration was successfully induced from callus cultures of coriander, fennel, lavender (Fig 3, *bottom*), anise and sage (Sajina *et al.* 1996 a). Plant regeneration was possible from callus cultures from explants like embryos (Van Eck and Kitto 1990), seeds (Van Eck and Kitto 1988), leaf disc (Van Eck and Kitto, 1992) and stem (Lin and Staba 1961) of peppermint, dill (Ratnamba and Chopra 1974, Sehgal 1978) and coriander (Kataeva and Popowich 1993). Somaclonal variation and virus elimination for improvement in garlic were reported in Koch and Solomon (1994).

ANTHER CULTURE

Since the first report of androgenesis in *Datura* by Guha and Maheswari (1964) anther and microspore culture has become an important source for development of haploids and dihaploids. This is especially important in perennial crops like spices to understand their genetic architecture and in exploiting hybrid vigour.

Plant regeneration from anther callus was possible from diploid and tetraploid ginger (Samsudeen 1996, IISR 1996a). Callus formation, development of roots and rhizome like structures were reported earlier from excised ginger anthers cultured on MS medium containing 2,4-D and coconut milk (Ramachandran and Chandrashekharan Nair 1992).

Matsubara *et al.* (1995) reported callus formation followed by regeneration of adventitious embryos from microspores from anther and microspore cultures in fennel.

IN-VITRO RHIZOME FORMATION

Rhizome formation *in vitro* was successful in long-term cultures of ginger (Sharma and Singh 1995). *In-vitro* formed rhizome is an important source of disease-free planting material ideally suited for germplasm exchange, transportation and conservation similar to that of microtubers of potato.

SYNTHETIC SEEDS

Synthetic seed technology forms an ideal system for

propagation, conservation and exchange of plant material (Redenbaugh 1990).

Reports on synthetic seeds are available in ginger (Sharma *et al.* 1994), vanilla (George *et al.* 1995) and celery (Redenbaugh *et al.* 1986). Sajina *et al.* (1996 b) reported development of synthetic seeds in black pepper, cardamom, ginger, turmeric, vanilla, lavender, fennel, camphor (Fig.4, *middle right*), cinnamon by encapsulating the somatic embryos and shoot buds in sodium alginate.

IN-VITRO CONSERVATION

Though India is known to be the home of spices, ecological disturbances in recent years has eroded much of these valuable materials from its natural habitats. Conservation of it, which is our national heritage, is important. Preservation of the germplasm in *in-vitro* gene bank is a safe alternative to protect them from vagaries of nature.

Conservation of pepper, cardamom and ginger germplasm in *in-vitro* gene bank by slow growth was reported (Dekkers *et al.* 1991, Nirmal Babu *et al.* 1994, Nirmal Babu *et al.* 1996c).

Suspensions of embryogenic cell lines of fennel, conserved at 4 °C for up to 12 weeks produce normal plants on transfer to normal laboratory conditions (Umetsu *et al.* 1995).

Choudhary and Chandel (1994, 1995) reported cryopreservation of black pepper and cardamom.

Conservation of genetic resources in *in-vitro* gene banks (Fig 4, *bottom left*) is now an established convention and gene banks for conservation of spices germplasm function at the IISR, Marikunnu and National Bureau of Plant Genetic Resources, New Delhi. A total of 450 accessions of spices germplasm are currently kept in *in- vitro* repository of the IISR.

PRODUCTION OF SECONDARY METABOLITES

Biotechnology can be utilized to exploit the potential of spices for bio-production of useful plant metabolites. The use of tissue culture for the biosynthesis of secondary metabolites particularly in plants of pharmaceutical significance holds an interesting alternative to control production of plant constituents. This technique is all the more relevant in recent years due to the ruthless exploitation of plants in the field leading to reduced availability.

In-vitro *proliferation of mace and synthesis of flavour components in culture*

Nutmeg and mace are the 2 important spices obtained from nutmeg tree. Nutmeg is the kernel while mace is the dried aril that surrounds the seed. *In-vitro* proliferation of mace tissue (Fig 4, *bottom right*) has been reported (Nirmal Babu *et al.* 1992 a). A 10-fold increase in fresh weight of tissue within 2 weeks was observed. Proliferated tissue not only retained the colour but also the flavour of original mace. Gas chromatographic analysis of the mace oil extracted from the cultured tissue was similar to that of original in qualitative profile. This technique, if refined further, has tremendous potential for industrial production of mace tissue and *in-vitro* production of myristicin and myristic acid.

In-vitro *proliferation of saffron stigma*

Most of the reports in this crop concentrate on the *in vitro* proliferation of stigma and *in-vitro* synthesis of colour components and metabolites. Proliferation of stigma of saffron *in-vitro* and chemical analysis of metabolites produced through tissue cultures of *Crocus sativus* was reported (Sarma *et al.* 1990, 1991). Vishwanath *et al.* (1990) reported *in-vitro* metabolite production from saffron tissue cultures.

Callus and cell culture systems

Plant cells cultured *in-vitro* produce wide range of primary and secondary metabolites of economic value. Production of phytochemicals from plant cell cultures have been presently used for pharmaceutical products. Production of flavour components and secondary metabolites *in-vitro* using immobilized cells is an ideal system for spices crops. The Central Food Technological Research Institute, Mysore, has done pioneering work in this field especially in the production of saffron and capsaicin (Ravishankar *et al.* 1993, Venkataraman and Ravishankar 1996). Reports on the *in-vitro* synthesis of crocin, picrocrocin and safranel from saffron stigma, and colour components from cells derived from pistils (Hori *et al.* 1988) are available for further scaling up. Callus and cell cultures were established in nutmeg, clove, camphor, ginger (Fig 5, *middle left*), lavender, mint, thyme, celery etc. Cell immobilization techniques were standardized in ginger, sage, anise and lavender (Ravindran *et al.* 1996, Sajina *et al.* 1996 b). Production of essential oils from cell cultures (Ernst 1989) and accumulation of essential oils by *Agrobacterium tumefaciens* transformed shoot cultures of *Pimpinella anisum* was reported (Salem and Charlwood 1995). Accumulation of total essential oil by transformed shoot cultures was lower than the untransformed cultures, but the relative amount of principle component in the essential oils of the transformed shoots was similar to those present in the parent plant. Conn and McCue (1994) reported regulation of the shikimate pathway in suspension culture cells of parsley, and Hunault *et al.* (1989) reported production of anethole from cell cultures of *Foeniculum vulgare*. Growth and production of monoterpene by transformed shoot cultures of *Mentha citrata* and *M. piperata* in flasks and fermentors was possible (Hilton *et al.* 1995). Production of rosmarinic acid in suspension cultures of *Salvia officinalis* was discussed by Hippolyte *et al.* (1992). Phenyl propanoid metabolism in suspension cultures of *Vanilla planifolia* was studied by Funk and Brodelius (1990 a, b).

Though the feasibility of *in-vitro* production of spice principles could be demonstrated, methodology for scaling up and reproducibility need to be developed before it can reach commercial levels. Once standardized, this technology has tremendous potential in industrial production of impor-

tant compounds like capsaicin, vanillin, crocin, picrocrocin, safranol, myristicin, anethole, menthol and curcumin.

PROTOPLAST CULTURE

The protoplast is a naked cell, without cell-wall surrounded by plasma membrane, but is potentially capable of cell-wall regeneration (in case of plants), growth and division. The absence of cell-wall makes the protoplast suitable for a variety of manipulations that are not normally possible with intact cells such as uptake of cell organelles, micro-organisms, foreign genetic material to form genetically modified cell and also for production of somatic hybrid cells by fusion of 2 protoplasts. Thus protoplast is an important tool for parasexual modification of genetic content of cells (Vasil and Vasil 1980). Successful isolation of protoplast is a prerequisite for many genetic transformation experiments for developing transgenics.

Successful isolation and culture of protoplasts were reported in ginger (Fig.5, *top left*) cardamom (Fig 5, *top right*), black pepper, *Piper colubrinum*, and vanilla (Sim *et al.* 1995, IISR 1996a, Minoo *et al.* 1996b). Reports on organogenesis and plant regeneration from isolated protoplasts are available in chillies (Agarwal 1988), fennel (Miura and Tabata 1986), fenugreek (Sen and Gupta 1979), peppermint (Okuyama *et al.* 1995), garlic (Ayabe *et al.* 1995) and saffron (Isa *et al.* 1990). In garlic, protoplast isolation, fusion and culture was done by Suh Sang Ki and Park (1995). An interspecific hybrid could be produced between peppermint and gingermint (Sato *et al.* 1996).

GENETIC TRANSFORMATION

Recent advances made in developing techniques for transfer of foreign DNA into plant cells have aroused much interest in the possibility of utilizing recombinant DNA technology in crop improvement (Walder 1988). Among the more important and frequently used techniques of gene transfer are *Agrobacterium* or viral vector-based transformation and the transformation by direct uptake of naked DNA. Of these, the *Agrobacterium*-mediated gene transfer is most successful in plants especially in dicots. For many plant species methods were standardized for obtaining transgenic plants after *Agrobacterium*-mediated transformation. Leaves, roots, hypocotyls, petioles, cotyledons or seeds were used as targets for transformation (Kado 1991, Hooykaas and Schilperoort 1992, Zambryski 1992).

Agrobacterium-*mediated gene transfer*

Preliminary studies were carried out on *Agrobacterium* transfer system in *P. nigrum* (Sasikumar and Veluthambi 1993). Sim *et al.* (1995) reported *Agrobacterium*-mediated transfer of GUS to black pepper. Transformation system can be used for the transfer of disease resistance against *Phytophthora* foot-rot from *P. colubrinum* to *P. nigrum*.

Direct gene transfer by particle bombardment

A preliminary study on transformation of ginger was attempted using biolistic process to study the optimum conditions for gene delivery and the efficiency of the plasmid vector pAHC 25 and promoter Ubi-1 (maize ubiquitin) for transformation and gene expression in ginger (Fig 5, *bottom*) and cardamom embryogenic callus cultures. The GUS gene was successfully expressed in the bombarded callus tissue (IISR 1996, Nirmal Babu 1997).

Isolation of DNA and studies on biochemical or molecular markers

The recent advances in the mapping of the genome of important crop species through RFLP analysis and the use of PCR technology will be useful in genetic fingerprinting, in identification and cloning of important genes and in understanding of inter-relationships at molecular level.

Isozyme studies using PAGE of esterases and SOD indicated the presence of high variability among seed-generated progeny of vanilla (Minoo *et al.* 1996a).

The DNA was isolated from 3 species of *Piper* and 2 lines of ginger. RAPD profiles of black pepper and long pepper were recorded using 15 types of random primers. Most of them gave positive differences between these 2 species (IISR 1996a). Marker-aided studies were carried out for screening genetic stability in micropropagated plants of *Piper* (Ajith 1996).

Protocols for micropropagation are available for most of the spices. However, intensive research is needed for application of genetic manipulation and transgenic techniques for production of resistant types to biotic and abiotic stresses. Anther and pollen culture for production of homozygous lines will increase the speed and efficiency of conventional plant breeding. Use of molecular markers for genetic characterization of germplasm as well as plants need to be given importance especially in post-GATT scenario. Though programmes have been initiated in many laboratories in *in-vitro* secondary metabolite production, these techniques are to refined and scaled up for possible industrial production of these products. Because of their commercial possibilities, intensification of biotechnological activities in spices are important in the coming decade.

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