TISSUE AND CELL CULTURE RESEARCH IN SPICES

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India is the land of spices and over 100 spices which are grown which are grown in about two million hectares. The annual production of spices in India is around 2.2 million tonnes and accounts for about 47 per cent of the global trade. Black pepper, cardamom, ginger, turmeric, vanilla, capsicum, cinnamon, clove, nutmeg, tamarind, coriander, cumin, fennel, fenugreek, saffron, thyme, basil, oregano, celery, anise are a few spices of relevance. The productivity of many of these crops is low due to lack of high yielding, pest and disease resistant varieties and absence of genetic variability in other important agronimic characters. The recent increase in our ability to study and manipulate plant tissues has resulted in development of efficient technologies for commercial clonal plant propagation, development of new breeding lines *via* somaclonal variation, anther culture, protoplast fusion and transgenics. Significant progress was made in spices biotechnology also.

Black pepper

In vitro culture methods for cloning as well as plant regeneration from callus of black pepper have been reported using shoot tips, nodal segments and apical meristems from both mature and juvenile tissue. The

Fig. 1. Micropropagation of black pepper

Fig. 2. Cryopreserved embryos of black pepper

tissuecultured plantlets were successfully hardened by transferring the well rooted platnlets into polybags. In field tissue cultured plants are on par with vegetatively propagated plants. Micropropagation techniques are available for many related species of Piper, such as *P.betle* L. (betel vine), *P.longum* L. (Indian long pepper), *P.chaba* Hunt. (Java long pepper), *P. colubrinum* Link. and *P.barberi* Gamble. (Babu *et al* 1997). Attempts on somaclones for tolerance to *Phytophthora* foot rot resistance resulted in identification of tolerant somaclone among the regenerated plantlets (nazeem et al 199.). Successful isolation and culture of protoplasts were reported in *P.nigrum*. Preliminary reports are available on *Agrobacterium* mediated gene transfer system in *P.,nigrum* (Sasikumar and Veluthambi 1996a,b). Synthetic seeds, consisting of somatic embryos or shoot buds enclosed in calcium alginate was reported in pepper (Sajina *et al.* 1997) for disease free plant movement, propagation, conservation and exchange of germplasm.

Cardamom

In vitro methods for clonal propagation of cardamom are available (Nadgauda *et al*, 1983, Rao *et al*1982). Plant also were regenerated from immature floral buds. Many

Fig. 3. Syn seeds in cardamom

commercial laboratories are using micropropagation for cloning of cardamom planting material. Field evaluation of tissue cultured plants of cardamom showed that the micropropagated plants performed on par with suckers (Lukose, 1993). Protoplasts could be isolated successfully from leaf mesophyll tissues, collected from *in vitro* grown plantlets and cell suspension cultures of cardamom. Sajina *et al* (1997) reported development of synthetic seeds in cardamom.

Ginger

Crop improvement programs in ginger are hampered by lack of seed set leading to limited variability and hence biotechnological tools will be ideal for adoption. Clonal multiplication of ginger as well as regeneration of plantlets through callus phase was reported by many workets (Hosoki and Sagawa, 1977, Nirmal Babu *et al*, 1992, Nirmal Babu *et al* 1998). Micropropagation will reduce the risk of disease spread through infected seed rhizomes. Field evaluation of somaclones indicated high variability with regard to various agronmic characters and other yield attributes and a few promising high yielding lines were identified (Samsudeen K. 1996, Nirmal Babu, 1997). In nature, ginger fails to set fruit. By supplying required nutrients to immature inflorescences, it was possible to effect *in vitro* pollination and to develop 'fruit' and subsequently plants (Valsala *et al*, 1997, Nirmal Babu *et al*, 1998). *In vitro* formed micro rhizomes are an important source of disease-free planting material and these were induced in ginger also (Bhat *et al*, 1994). Technology for synthetic seeds is available for ginger (Sharma *et al*, 1994, Sajina *et al* 1997). A preliminary study on transformation of cardamom was attempted using biolistic process and GUS gene was successfully expressed in the bombarded callus tissue (Nirmal Babu, 1998).

Turmeric

Micropropagation of turmeric was standardized (Nadgauda *et al*, 1978). Variants with high curcumin content were isolated from tissue culture plantlets (Nadgauda *et al*, 1982). Raghu Rajan, 1997 reported induction of micro rhizomes in turmeric.

Fig. 4. Turmeric microrhizomes

Other zingiberaceous taxa

Many economically and medicinally important zingiberaceous species like *Amomum subulatum* (large cardamom), *Curcuma aromatica* (kasturi turmeric), *C. amada* (mango ginger), *Kaempferia galanga, K.rotunda, Alpinia spp. etc.*, could be micropropagated (Geetha *et al*, 1997, Nirmal Babu *etal* 1997)

Vanilla

Micropropagation of vanilla using apical meristem was standardized for large scale multiplication of disease free and genetically stable plants (Philip and Nainar, 1986). Ovule culture was standardised to generate highly variable segregating progenies (Minoo *et al*, 1997). Embryo rescue was successfully employed to produce

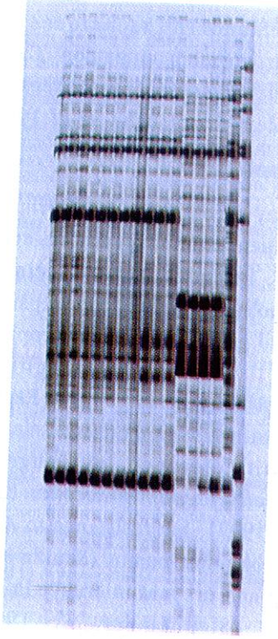

Fig. 5. AFLP frofiles in vanilla progenies

interspecific hybrid between *V. planifolia* and *V. aphylla*.

Tree spices

In perennial tree spice identification and clonal multiplication of high-yielding genotypes becomes an immediate priority due to pre bearing pe-

Fig. 6. Multiple shoots in cinnamon

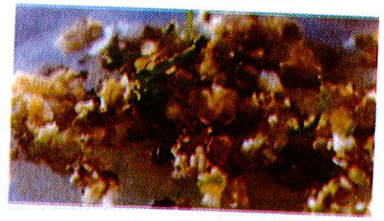

Fig. 7. Somatic embroyogenesis in cinnamon

riod. Standard techniques for micropropagation cinnamon, Cassia, Curry leaf and camphor trees are available (Nirmal Babu *er al* 1997).

Seed and herbal spices

Many herbal spice like coriander, anise, peppermint, spearmint, celery, thyme, lavender, savory, ocimum, oregano, basil, sage, fennel, parsley, dill and garlic are easy to propagate using tissue and callus cultures. (Nirmal Babu *et al* 1997). Reports are also available *on vitro* selection for salt tolerance in fenugreek, *Trigonella foenum-graeceum* (Settu *et al,* 1997), for resistance to *Alternaria* blight in cumin (Shukla *et al,* 1997) and drought tolerance in coriander (Stephen *et al,* 1997). Somaclonal variation and virus elimination for improvement of garlic has been reported by Koch and Solomon (1994). Lacy *et al* (1996) identified MSU - SHK 5, a somaclonally derived *Fusarium* yellows resistant line in celery. Micropropagation and *in vitro* proliferation of saffron stigma was also reported (Ding *et al,* 1981).

Capsicum (*Paprika*)

Chilly (*Capsicum annum* L.) is one of the major spice crops of India. Micropropagation and plant regeneration in chilly was reported using various explants (Agarwal, 1988). George and Narayana Swamy (1973) reported development of haploid capsicum through androgenesis.

In Vitro conservation of germplasm

IISR holds the world's largest collection of spices 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 reclacitrant and heterozygous. Hence storage of germplasm *in vitro* conservation of pepper, cardamom, herbal spices, vanilla and ginger germplasm in *in vitro* gene bank by slow growth was developed as a safe additive to field gene banks. (Geetha *et al,* 1995, Nirmal Babu *et al,* 1998). About 500 accessions of spices germplasm are currently kept in *in vitro* repository of IISR. Cryopreservation of black pepper and cardamom seeds in liquid nitrogen (LN_2) was also reported by Choudhary and Chandel (1994; 1995).

Production of secondary 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. Production of flavour components and secondary metabolites *in vitro* using immobilised cells is an ideal system and production of saffron and capsaicin (Ravishankar *et al,* 1995; Venkataraman and Ravishankar, 1997), *in vitro* synthesis of crocin, picrocrocin and safranel from saffron stigma (Himeno and Sano, 1995) and colour components from cells derived from pistils (Hori *et al,* 1988) are available. Production of essential oils from cell cultures (Ernst, 1989) and accumulation of essential oils by *Agrobacterium tumefaciens* transformed shoot cultures of *Pimpinella anisum* (Salem and Charlwood, 1995) and production of anethole from cell cultures of *Foeniculum vulgare* (Hunault *et al,* 1989) were reported. Production of rosmarinic acid in suspension cultures of *Salvia officinalis* (Hippolyte *et al* (1992), production of phenolic flavour compounds using cultured cells and tissues of vanilla (Dorenburg and Knorr 1996) in vitro production petroselinic acid from cell suspension cultures of coriander (Kim *et al* 1996) are also available.

Though the feasibility of *in vitro* production of spice principles has been demonstrated, methodology for scaling up and reproducibility need to be developed.

Protoplast Culture

Protoplast is an important tool for parasexual modification of genetic content of cells. Successful isolation and cutlute of protoplasts were reported in black pepper, *Piper colubrinum,* ginger, cardamom, vanilla and capsicum (Shaji *et al,* 1996, Nirmal Babu *etal* 1998). Organogenesis and plant regeneration from isolated protoplasts are available in chillies, fennel (Miura and Tabata, 1986), fenugreek (Multani, 1981), peppermint (Sato *et al,* 1993), garlic (Ayabe *et al,* 1995) and saffron (Isa *et al,* 1990) rtc.

Isolation of DNA and 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. Protocols were standardised for isolation of genomic DNA in black pepper, cardamom, ginger, turmeric and vanilla and DNA of over 200 lines of various spices were kept in DNA Bank. RAPD and AFLP polymorphism is being used to estimate genetic variability in selfed progenies and interspecific hybrids of vanilla, black pepper and cardamom cultivars.

Conclusion

The achievements in spices biotechnology so far are mainly in developing protocols for micropropagation of majority of the spices, which can be adopted wherever necessary. Conservation of genetic resourses in *in vitro* gene banks is another positive development. The future focus will be one use of molecular markers for genetic characterization of important plant types and application of recombinent DNA technology for production of resistant types to biotic and abiotic stress.

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