15. SPICE CROPS

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Spices had profound influence on the course of history and civilization. The principal use of spices is in flavouring food and confectionery. Essential oils and oleoresins extracted from various plant parts are also used. Many of the spices are believed to have stimulative, carminative, astringent and aphrodisiac properties as they are used in native Ayurvedic and Unani systems of medicines.

India, is one of the major producers and exporters of spices. There are many spices cultivated in India. They are either annuals or perennials growing from tropical to temperate regions of the country. The most important of them are black pepper, cardamom, ginger, turmeric, tree spices and seed spices. A major expansion in the cultivation of spices is envisaged to increase production and export. Attempts to increase production and productivity of spices are bearing fruits with the release of high yielding varieties and development of improved production technologies. However, the annual production of these spices is almost static. Major bottlenecks in increasing production and productivity of spices are lack of adequate disease free planting material of high yielding varieties and non availability of effective control measures against major diseases and pests. In addition, the existing genetic variability in nutmeg, cinnamon, clove and vanilla is insufficient for meaningful crop improvement programmes.

In recent times, there is a growing awareness on the utility of plant biotechnology in these crops, for both agricultural and industrial purposes. In spices, many of which are perennials, biotechnology augments other crop improvement strategies. The commercial utilisation of plant biotechnology in spices can be principally in following areas.

- Rapid clonal multiplication of high yielding 'elite' genotypes to generate adequate good quality and disease free planting material.
- Exploiting somaclonal variation and utilisation of techniques like somatic cell hybridisation, anther culture, embryo rescue, etc., for crop improvement.

- *In vitro* selection for resistance to biotic and abiotic stresses.
- Production of flavour and volatile constituents in culture
- Biological control of diseases and pests, and
- Biofertilisers

However, production of quality, disease-free planting materials using micropropagation and biological control of major diseases have become commercially viable at present.

Micropropagation

Micropropagation is the true-to-type propagation of selected genotype using tissue culture techniques. Most spices - black pepper, cardamom, ginger, turmeric, cinnamon and clove are grown in plantation scale. The availability of good quality, disease free planting material is an important input in increasing their production.

Cardamom

Cardamom, *Elettaria cardamomum* Maton. (Zingiberaceae), a native to India, is the most important spice. The productivity of cardamom is hampered by 'Katte', 'Kokke kandu' and 'Nilgiri necrosis' diseases. Utilization of virus-free planting material is essential for disease management.

Being a cross pollinated crop, clones are ideal for generating true to type planting material from high yielding clumps. *In vitro* propagation helps in a large scale production of planting material. *In vitro* propagation methods for clonal propagation of cardamom from vegetative buds have been standardized (Nadgauda *et al.*, 1983); High multiplication of disease free planting material makes micropropagation more profitable than conventional methods.

Many commercial laboratories are engaged in a large-scale production of cardamom clonal planting material using micropropagation. Field evaluation of tissue-cultured plants of cardamom has been carried out at Indian Institute of Spices Research (IISR), Calicut. The results showed that the micropropagated plants performed on par with suckers of the original mother plant (Lukose, 1993).

Black pepper

India is one of the major producers and exporters of black pepper, *Piper nigrum* L.'Foot rot' caused by *Phytophthora capsici* is the major disease affecting pepper plantations. *In vitro* cloning of black pepper has been reported using seedlings and mature shoot tip explants Nazeem *et al.*, 1993; Philip *et al.*, 1992; Nirmal Babu *et al.*, 1993a;b) and tissue cultured plantlets were successfully established in field. The multiplication rate is around 6 shoots/culture in about 90 days. Phenolic exudates from the cut surface and bacterial contamination (Raj Mohan, 1985; Fitchet, 1988a;b) severely affect black pepper tissue culture.

Related species of Piper

The *Piper betle* is an economically important species cultivated extensively in India. The leaves of which are used as a masticatory. The *Piper longum* L. (Indian long pepper) and *Piper chaba* Hunt. (Java long pepper) are another groups of medicinally important spices. Protocols for rapid clonal multiplication of *Piper longum* from shoot tip explants are available (Sarasan *et al.*, 1993; Rema *et al.*, 1995). Micropropagation of *P.chaba* (Nirmal Babu *et al.*, 1994; Rema *et al.*, 1995), *P. betle* (Nirmal Babu *et al.*, 1992c) has been standardized. Micropropagated plantlets of these species are being evaluated for their field performance at the IISR,Calicut.

Ginger

Ginger, *Zingiber officinale* Rosc. (Zingiberaceae), is an important tropical spice. Crop improvement programmes in ginger are hampered by the absence of seed set leading to limited variability. Somaclonal variation could be an important source of variability that could be exploited to evolve high-yielding and 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 has been reported (Hosoki and Sagawa, 1977; Nadgauda *et al*, 1980; Pillai and Kumar, 1982; 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.

Field evaluation of tissue cultured plants at the IISR, Calicut indicated that they cannot be used directly for commercial planting, since it takes 2 crop seasons for the micropropagated plants to develop normal-sized rhizomes that can be used as seed rhizomes.

Turmeric

India is the major producer and exporter of turmeric (*Curcuma longa* L.) . Curcumin is the important colouring material obtained from turmeric rhizomes. Successful micropropagation in turmeric has been reported (Nadgauda *et al.*, 1978; Shetty *et al.*, 1982). This technique could be used to produce disease-free planting material of elite plants. Micropropagation of turmeric has been standardized at the IISR, Calicut using young vegetative buds as explants. They responded readily to culture conditions producing 8-10 adventitious shoots in 40 days of culture (Nirmal Babu *et al.*, 1993a).

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 also be micropropagated (Barthakur and Bordoloi, 1992; Vincent *et al.*, 1992; Sajina *et al.*, 1997a).

Vanilla

The *Vanilla planifolia* Andr. is the natural source of the flavouring substance, vanillin. Vanilla is commercially propagated by means of stem cuttings. *In vitro* culture will help in rapid multiplication of planting material. Micropropagation of vanilla using apical meristem was standardized for a large scale multiplication of disease free and genetically stable plants (Cervera and Madrigal, 1981; Kononowicz and Janick, 1984; Philip and Nainar, 1986; George *et al.*, 1995; Minoo *et al.*, 1997). Reports are available for plant regeneration from root (Philip and Nainar, 1986, Ravindran *et al.*, 1996).

Saffron

The dried styles and stigma of its flowers are used as a spice. Saffron is a triploid and sterile genotype propagated vegetatively by means of corms. Reports are available on the micropropagation of saffron. Successful micropropagation, somatic embryogenesis and regeneration were also reported in saffron (Sarma *et al.*, 1995).

Tree spices

Cinnamon (*Cinnamomum verum Presl.),* cassia *(Cinnamomum cassia* Blume.) and curry leaf (*Murraya koenigii* Spreng.) 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 of these spices. Micropropagation of *Cinnamomum verum* have been reported by Jagdishchandra and Rai (1986), Mini *et al.* (1997). Curry leaf could also be cloned using techniques developed by Hazarika *et al.* (1995) and Rajendra and D'Souza (1995). Micropropagation of *Tamarindus indica* was reported by Venkateswarlu and Mukhopadhyaya (1995)

Seed and herbal spices

Fennel, thyme, lavender, mint, basil, oregano, marjoram, sage, celery and anise are some of the important herbal spices. Micropropagation protocols for many seed and herbal spices - coriander, celery, thyme, lavender, anise, savory, ocimum, oregano, basil, sage, fennel, parsley, dill, lavender and fennel were standardized (Sajina *et al.*, 1997a; Cellarova, 1992; Venkataraman and Ravishankar, 1986;Rech and Pires, 1986; Toth and Lacy, 1992; Furmanowa and Ozszowska, 1992; Hunault and Du-Manoir, 1992; Panizza and Tognoni, 1992; Miura *et al.*, 1987). Though these protocols have no immediate commercial potential, they can be used for production of biomass if needed, since in most of these, plant parts can be used directly for extraction of flavouring substance.

Biological Control of Diseases Of Spices

Crop losses caused by disease have been identified as one of the

major production constraints in spices such as black pepper, cardamom, ginger and turmeric (Sarma *et al.*, 1994). The economically important diseases in these crops are soil borne and are caused by fungi belonging to *Phytophthora, Pythium* and *Rhizoctonia.* Soil borne diseases are difficult to manage with chemicals. But, these organisms are amenable for biological control using antagonistic fungi such as *Trichoderma.*

Phytophthora Foot Rot of Black Pepper

The *Phytophthora* foot rot is the major disease on black pepper causing severe economic damages (Anandaraj *et al.*, 1989). Caused by *Phytophthora capsici,*it affects all parts of the plant, from roots to shoots. The expression of symptoms depends upon the site of infection and extent of damage (Anandaraj and Sarma, 1995). The fungus is active during the wet monsoon period and remains dormant in soil during the inter-monsoonal dry periods. Contaminated soil is the main source of initial inoculum which builds up at the onset of wet period (Anandaraj, 1997). The disease management strategy includes production of disease free planting material, phytosanitation, cultural practices, chemical and biological control.

Production of disease free planting material

The pathogens attacking nursery stock of black pepper are *Phytophthora capsici, Radopholus similis* and *Meloidogyne incognita.* They also affect feeder roots of adult plants causing slow decline disease. Often these organisms are carried inadvertently through the planting material to the plantations. Hence, production of disease free planting material is the first step in disease management. The nursery mixture should be sterilized by soil solarization by covering with a transparent polythene sheet and exposing the soil to sunlight for 35 - 45 days. All pathogen propagules are killed due to the heat generated under the polythene sheet. After solarization the mixture is fortified with propagules of beneficial organisms such as vesicular arbuscular mycorrhiza and antagonistic fungi *Trichoderma* spp.and *Gliocladium virens.*

Biological control in the field

The population of *P. capsici* builds up with the increase in soil moisture and growth of feeder roots of black pepper (Anandaraj, 1997).

To check the population of *P. capsici Trichoderma* sp. or *Gliocladium sp.* is multiplied on organic substrates and applied to the base of the vines twice during the season (Sarma *et al.*, 1996). The efficacy of biological control in the management of *Phytophthora* foot rot of black pepper has been demonstrated in over 2,000 ha in farmers' plots.

Capsule rot and Rhizome rot of cardamom

Capsule rot of cardamom caused by *Phytophthora meadii* and rhizome rot by *Pythium vexans* and *Rhizoctonia solani* are soil borne diseases, appearing during the wet monsoon period. Application of biocontrol agents mainly *Trichoderma* spp. is helpful (Suseela Bhai *et al.*, 1993, Sarma *et al.*, 1996). The rhizome-rot caused by *Pythium vexans* and *R. solani* also occurs in the nursery resulting pre and post emergence damping off.

This is effectively managed by solarization of nursery beds followed by the application of *Trichoderma sp.* at the time of raising nursery. Application of biocontrol agents in the nursery not only protects the seedlings but protect the plants against rhizome rot pathogens.

Rhizome rot of Ginger

Rhizome rot or soft rot of ginger is caused by *Pythium aphanidermatum, P. myriotylum* and *P.vexans. P. aphanidermatum* is the most predominant one (Sarma, 1994). Ginger yellows caused by *Fusarium oxysporum* f.sp. *zingiberi* is a major disease in the northern part of India (Dohroo, 1995). These diseases are also managed successfully by applying bioagents *Trichoderma sp.* along with soil solarization (Sarma *et al.*, 1996).

***Mass multiplication of* Trichoderma**

Several isolates of *Trichoderma* were obtained from black pepper rhizosphere from healthy areas including the Western Ghat forests having black pepper plants growing naturally. These organisms were screened for their antagonistic potential against foot rot pathogen *P. capsici.* The efficient isolates are multiplied for a large scale application in the fields. These antagonists essentially prevent the population build up of pathogens by different modes of actions including competition, antibiosis and hyperparasitism. For the successful colonization of these

introduced organisms the soil must contain sufficient organic material. The large scale multiplication was tried on agricultural byproducts - oil cakes, coffee husk, tea waste and matured coconut water (Sarma *et al.*,1996, Anandaraj and Sarma, 1994). Since all these substrates supports good growth of biological control agents, materials available at each locality could be used to multiply biological control agents.

Future Prospects

Protocols for micropropagation are available for most of the spices with high commercial potential. However, more information need to be generated in scaling up of these technologies for their large scale multiplication at a moderate cost.

Though sporadic information are available on utilization of biotechnology in developing new plant types, commercial varieties are not yet available in spices. Research in this direction need to be strengthened. Production of flavours and flavour components from cell cultures and scaling up of these technologies for industrial production is another important area which needs special attention especially in spices. A few promising reports are available in vanilla.

Since spices are export-oriented crops, a lot of emphasis should be laid on pesticide-free produce for the export. Integrated disease management strategy involving biological control has been developed for the management of *Phytophthora* foot-rot on black pepper, rhizome-rot on ginger and cardamom and capsule rot of cardamom. These techniques were also demonstrated in farmers' fields with convincing results. A large collection of biological control agents is maintained at the IISR, Calicut. The *Trichoderma* spp. although are used primarily against pathogenic fungi, they also colonize the egg masses of nematodes infesting black pepper. The *Verticillium chlamydosporium* was found to affect both fungi and also nematodes. Efficient isolates effective against both would be ideal for integrated management strategies.

Evolving efficient isolates of biological control agents and developing inexpensive multiplication and delivery systems would go a long way in popularizing biological control agents. Such a technology also would pave the way for private entrepreneurs taking up this technology.

Molecular characterization of the efficient strains of biocontrol agents and patenting, developing suitable carrier media, standardizing techniques

to maintain the shelf-life and strict quality control, and also evolving new efficient strains of biological control agents through biotechnological methods needs to be developed to meet the ever growing challenges posed by the disease causing organisms.

The biological control methods standardized for managing soil borne diseases of spice crops needs to be adopted in larger areas, the main constraint being the non availability of the planting material. Since the technology has already been proved in farmer's fields, production of biocontrol agents needs to be commercialized so that the technology reach the growers.

Similar approaches hold good promise in utilization of biofertilizers. Development of efficient biotechnologies, especially for cloning, developing new varieties, minimisation of use of chemical fungicides, insecticides and fertilisers by an integrated approach using biocontrol agents and biofertilizers will help in sustainable production of clean spices.

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