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Biotechnology in spices

Biotechnology has great potential in spice cultivation and industry. Many private organizations have already taken up commercial multiplication of cardamom through tissue culture. The possibility of production of flavour and volatile constituents in culture makes nutmeg and vanilla tissue culture a profitable proposition. At the National Research Centre for Spices the priorities are refining techniques for micropropagation of tree spices, *In-vitro* selection for disease resistance in black pepper and ginger, production of virus-free planting material in cardamom and conservation of the valuable spice germplasm *In-vitro* repositories.

OVER 80 species of spices are 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, small cardamom, ginger, turmeric, tree and seed spices. They earn foreign exchange equivalent of Rs 300 crores annually. The annual production of these spices is almost static. The major bottlenecks in increasing the production and productivity of spices are lack of adequate disease-free planting material of the high-yielding varieties and prevalence of major diseases and pests with no effective control measures. Besides, the existing genetic variability in nutmeg and clove is insufficient for any meaningful crop improvement programme.

In spices, most of which are perennials, biotechnology comes handy to augment the conventional crop improvement programmes. Particularly through:

- Micropropagation and rapid clonal multiplication of high-yielding genotypes to generate adequate quality and disease-free planting material
- Somaclonal variations and utilization of somatic cell hybridization and anther culture for crop improvement
- *In-vitro* selection for resistance to biotic and abiotic stresses
- Production of flavour and volatile constituents in culture
- *In-vitro* conservation of germplasm and its safe exchange

The principal use of spices is in flavouring food, confectionary, drinks and in perfumary. The essential oils and oleoresins extracted from various parts of the plants are also used. Many of the spices are believed to have aphrodisiac properties and are used in indigenous medicines. India, the legendary land of spices, is the major producer and exporter of spices.

The beginning

The National Research Centre for Spices (NRCS) of the Indian Council of Agricultural Research has been conducting research on crop improvement, management, protection and post-harvest technology of spices. Use of biotechnology in crop improvement programmes began in 1983, with the rapid clonal multiplication of cardamom at the Central Plantation Crops Research Institute. Tissue culture propagation in turmeric, ginger, black pepper and tree spices nutmeg, cinnamon, allspice and clove took off subsequently.

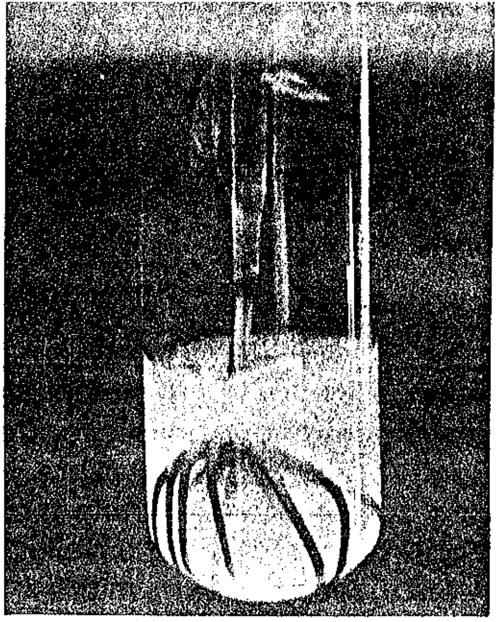
The Achievements

Small cardamom

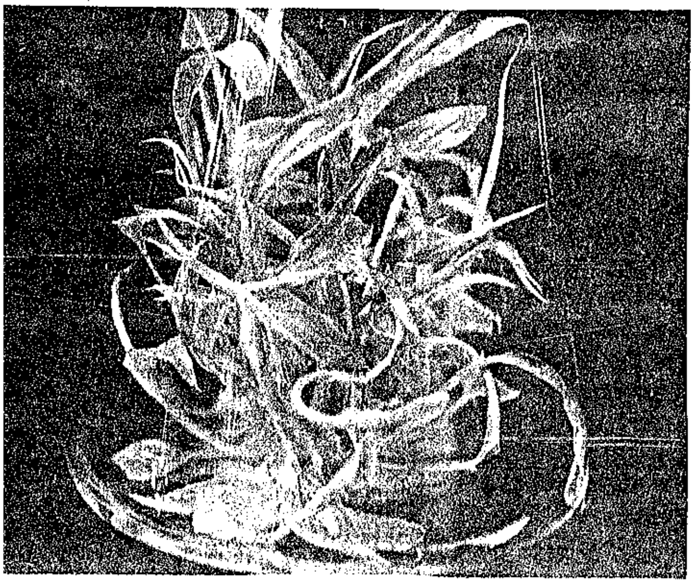
'Queen of spices', the small cardamom is the dried fruit of the perennial herb *Elettaria cardamomum* Maton.

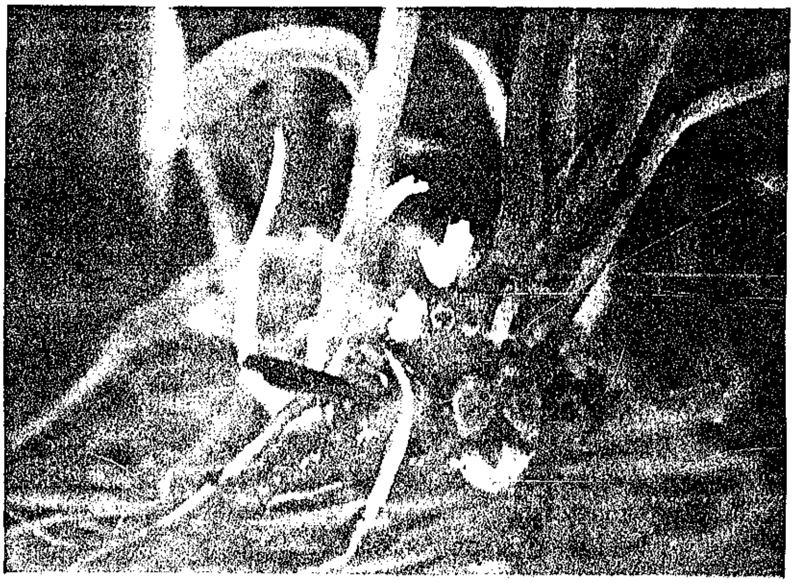
Clonal multiplication: Protocol for clonal multiplication from vegetative buds in small cardamom has been standardized. An average of 6 axillary shoots could be produced within 30 days of culture. This method is extensively used for raising clones of the high-yielding Coorg cardamom 'Selection 1' and for the production of 'katte'-

Multiple shoots in shoot tip cultures of black pepper. Shoot tips in black pepper may be utilized for the production of planting material.



Shoots produced from the shoot tips strike healthy roots *in vitro* in black pepper.



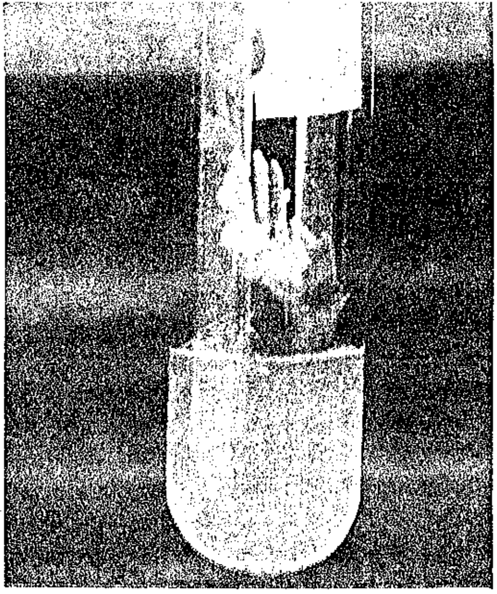


In ginger: The vegetative bud *in vitro* can be used for multiplication of planting material (*left*). Such plants are capable of producing rhizome *in vitro* (*right*).

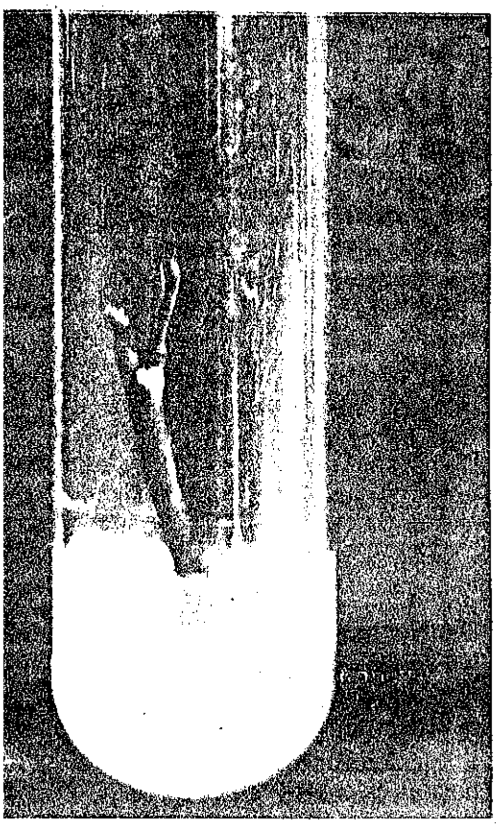
Single flower culture in ginger: The ovary is capable of producing a complete plantlet directly. The breeders could obtain permanent variants for improving the vegetatively propagated ginger.

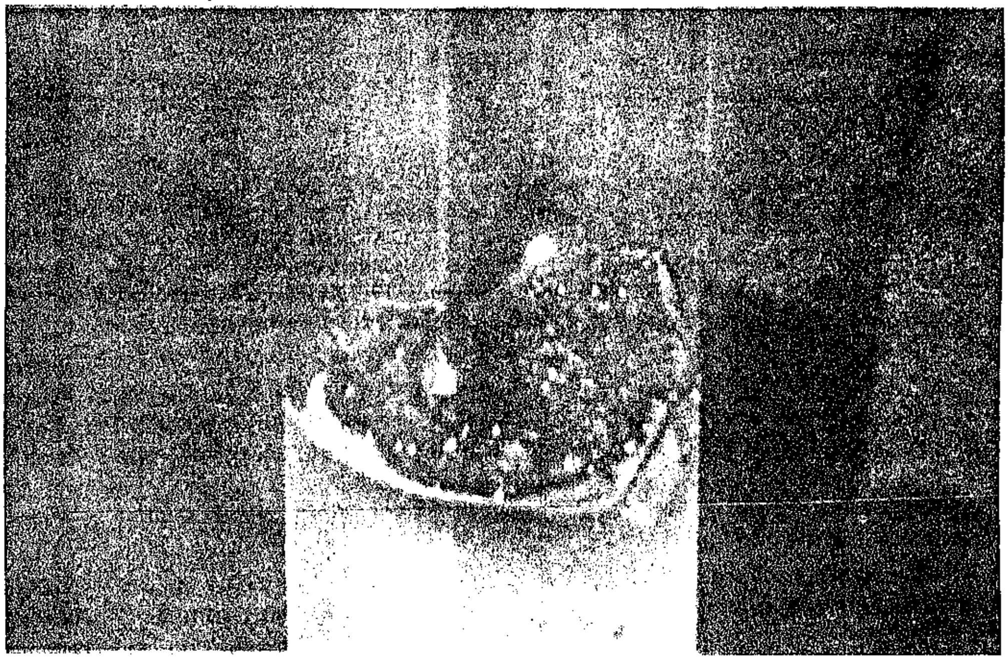
The floral buds of immature inflorescence are converted into the vegetative ones in ginger *in vitro*.

Vegetative bud culture is now a practice for mass production of planting material in cardamom.



Floral buds of the immature inflorescence can be readily converted into vegetative buds in cardamom.





Establishment and growth of nutmeg shoots *in vitro* (*left*). Proliferation of mace (the spice from nutmeg) also in culture (*above*).

Shoot-tip of cinnamon establishes in culture. Only a few explants showed rooting.

disease-free-nucleus-planting material.

The field experiments conducted at the NRCS indicate that the tissue cultured plantlets derived from vegetative buds and the suckers produce equal yield.

Immature inflorescences could also be used to multiply cardamom clones, by converting the floral buds into vegetative buds.

Regeneration of plantlets from callus: The protocol for organogenesis and plant regenerations from vegetative-bud-derived-callus cultures is endowed with an excellent regeneration system, that at present is used for large-scale production of somaclones and isolation of useful types from them.

Black pepper

'King of spices', the black pepper is the dried mature fruit of a perennial climber *Piper nigrum* Linn. We are the major producer and exporter of this spice.

Micropropagation: Using shoot explants from both mature and juvenile plants micropropagation of black pepper can now be done. The technique was perfected by the NRC. Though up to 6 suckers have been obtained in some cultures, the multiplication rate is still low (average 3) commercially.

Establishment of callus cultures and regeneration of plantlets: Callus cultures, using leaf or stem tissues, could easily be established in black pepper and its related species *Piper colubrinum* and *P. longum*. But regeneration of plantlets from callus appears to be hampered by phenolic exudations. Sporadically though, plantlets have been observed in a few cultures where the leaf tissue was used as explant. A high-frequency regeneration system once standardized will help in developing techniques for transfer of resistance to *Phytophthora* (the casual organism of *Phytophthora* foot-rot) from *Piper colubrinum* into the cultivated black pepper.

Micropropagation of other species of piper and in-vitro conservation of germplasm: Related species *P. longum, P. colubrinum, P. chaba* and *P. barberi*, may also be multiplied from shoot tips. *P. longum* can also be micropropagated using internal explants that results in direct organogenesis. The growth in *Piper* species is slow with no necrosis symptoms. These cultures could be used for *in-vitro* conservation of germplasm. The subculture intervals should be extended substantially for any meaningful *in-vitro*-conservation programme.

Ginger

Ginger, the underground rhizomes of the perennial herb *Zingiber officinale* Rosc., is produced and exported in largest quantity from India. There is no seed propagation, therefore variability is limited. Somaclonal variation can be exploited for evolving high-yielding, high-quality lines and for developing resistant types to major diseases, rhizome-rot and bacterial-wilt.

Rapid clonal multiplication: Ginger being a vegetatively-propagated crop responds readily to the tissue culture propagation method. An average of ten adventitious shoots could be obtained using the protocol standardized at the NRCS. Since major diseases spread through the contaminated seed rhizomes in ginger, using tissue culture pathogen-free planting material can be produced. The tissue cultured plants cannot be directly planted in the field. It takes around two seasons for the tissue cultured plants to develop rhizomes of sufficient size.

In vitro rhizome formation has been observed in some cultures.

Regeneration of plantlets: Plants have been regenerated from leaf, ovary and vegetative bud tissues via the callus phase. These techniques are used in the production of somaclones screened for disease resistance and other useful characters.

In-vitro *selection:* The high-frequency regeneration system developed at the NRCS is being used for *in-vitro* selection of resistant types to *Pythium aphanidermatum* and *Pseudomonas solanacearum*, the causal agents of dreaded rhizome-rot and bacterial-wilt. The crude culture filtrates extracted from these pathogens are being used as selecting agents.

Inflorescence cultures and in-vitro development of 'fruit': In ginger there is no report of fruit set. However, by supplying the required nutrients to the immature inflorescence in culture, it was possible to make the ovary develop into 'fruit'. By culturing immature inflorescences in a suitable medium, direct development of clonal plantlets could be achieved by the conversion of floral buds into vegetative buds and their subsequent development into complete plants. In single flower cultures also, complete plantlets could be developed directly from the ovary tissues.

Turmeric

Turmeric of commerce is the dried rhizome of *Curcuma longa* Linn. India is one of the major producers and exporters of this spice.

Young vegetative buds from the germinating rhizomes as explants were used for micropropagating turmeric. These buds readily respond to the culture condition putting out 8–10 adventitious shoots in 40 days of culture. This technique may be used for the production of disease-free planting material.

Callus differentiation and plantlet formation has been achieved in turmeric.

Tree spices

Nutmeg (*Myristica fragrans* Houtt.), cinnamon (*Cinnamomum verum*

Presl.), clove [*Syzygium aromaticum* (Linn.) Merr. & Perry] and allspice [*Pimenta dioica* (Linn.) Merr.] are the tree spices of relevance in the Indian context. At present the demand for these spices in the domestic market exceeds production. Segregation of sex in the seedling progenies of nutmeg, sharp fall of seed viability within 2 days of harvest in clove, lack of genetic variability in clove, nutmeg and allspice are the major production constraints. In all these crops which are perennial, identification and clonal multiplication of high-yielding genotypes to be used as planting material, gains importance. The existing vegetative propagation methods are inadequate to meet the demand. Micropropagation in rapid multiplication of 'elite' planting material in these crops is a priority.

Micropropagation: Shoot-tip cultures of nutmeg, cinnamon and allspice have been established. Growth of these cultures was satisfactory in allspice, but slow in both nutmeg and cinnamon. In cinnamon, rooting of shoot-tip explants was observed in a few cultures.

Callus cultures from leaf and shoot explants were established in cinnamon, nutmeg and allspice.

In-vitro *proliferation of mace:* Nutmeg and mace are the two important spices obtained from the nutmeg tree. Nutmeg is the kernel of seed while mace is the dried aril that surrounds the seed.

Protocol for *in-vitro* proliferation of mace tissue has been perfected. The proliferated tissue not only retains the red colour but also has the flavour of the original mace. This technique if refined further has tremendous potential for industrial production of mace tissue.

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