APPLICATIONS OF PLANT CELL AND TISSUE CULTURE IN SPICES

Biotechnology with its apparently unlimited potential has opened up many exciting possibilities in the field of agriculture. It besides offering excellent opening for research in plant developmental biology, biochemistry and molecular biology, also offers the possibility of improved crop plants and commerical production of planting materials as well as plant products. Some of the most promising applications of biotechnology are in the field of agriculture.

In spices, the major bottle necks in increasing the production and productivity are the inadequate supply of disease-free planting material of 'elite' lines, the absence of resistant genotypes for various pests and pathogens and the absence of variability in the germplasm in certain introduced crops like clove, nutmeg etc. The methods of biotechnology can effectively be used to solve many of these. Some of the important applications of biotechnology in spices are:

(1) Rapid multiplication of 'elite' genotypes by meristem culture for production of planting material which are free of viruses.

(2) Generating and exploiting somaclonal variation for crop improvement.

(3) In vitro selection for resistance to biotic (pests & pathogens) and abiotic stresses (drought etc.)

(4) Wide hybridisation through somatic cell fusion.

(5) Production of flavour compounds and volatile constituents in vitro and

(6) In vitro conservation of germplasm and exchange of germplasm using tissue cultures.

(1) Rapid multiplication:

Plant tissue cultures can be used for mass propagation of plants. The most successful examples are orchids and ornamentals. In spices too, this technique has been standardised in cardamom, turmeric, ginger, black pepper, clove and cinnamon. Now it is possible to multiply cardamom on commercial scale as is being done by A.V. Thomas & Co., Cochin, Indo American Hybrid Seeds, Bangalore, Hindustan Leaver, Bombay, Unicorn Biotech, Hyderabad etc. In other crops though the techniques are available, they have to be improved further for their commerical exploitation. At the NRCS, Meristem culture is being used to multiply high yielding cardamom clones free of 'Katte' virus. Tissue culture technique once standardised will help in multiplying the desirable female plants of nutmeg thus overcoming the seggregation of sex in its seedling progenies. In cassia and allspice, this technique could be used to multiply the very few plants available in India thus saving these precious genotypes from being lost.

(2) Somaclonal variation:

It is now an established fact that plants regnerated from callus cultures exhibit variations to some extent when compared to the mother plants. The extent of variation depends on culture medium explant used, hormones used, length of culture, rate of multiplication etc.

This variation could be used for selecting plants with good agronomic traits. In turmeric, varieties with high curcumin have been isolated from tissue cultured plants by Nadgauda et al. (1982) at national Chemical Laboratory. Similarly, this technique could be used in ginger, clove, nutmeg etc. for creating variation and utilising the resultant variation in crop improvement. The range of somaclonal variation could be further increased by incorporating the mutagens like EMS and Colchicinc in the culture media or by irradiating the callus/tissue cultures. These techniques are of great utility in crop improvement programme of ginger where the lack of seed set hampers conventional crop improvement programmes.

(3) In vitro selection for biotic and abiotic stresses:

One of the major constraints in increasing the production of spices is the prevalence of diseases and pests. This is further aggreveated by the periodic drought conditions. Tissue culture techniques were successfully exploited in many crops (eg. Sugarcane, Potato etc.) in isolating disease resistant types both from somaclones as well as utilising *in vitro* selection techniques using patho toxins as selecting agents. In Ginger *Pythium* (causal organism of soft rot) tolerant types have been isolated by Kulkarni *et al.* (1984) using *in vitro* selection techniques using culture filtrate of *Pythium* as selecting agent. These techniques are being used at the NRCS to develop resistant types of ginger for rhizome rot.

(4) Somatic cell fusion:

Somatic cell fusion forms an alternative to sexual fusion and can be employed in producing cell hybrids. This technique could be used more effectively between the plant species which are sexually incompatible. In black pepper, *Piper nigrum* (the cultivated black pepper) is not crossable with *P. colubrium* a distantly related species which is immune to *Phytophthora capsici* the causal organism of 'Phytophthora foot rot'. By employing the technique of somatic cell hybridisation and gene transfer it is possible to bring the resistance gene from *P. colubrium* to cultivated black pepper. It is also possible to produce transgeneric hybrids of black pepper with resistance to nematode pests also.

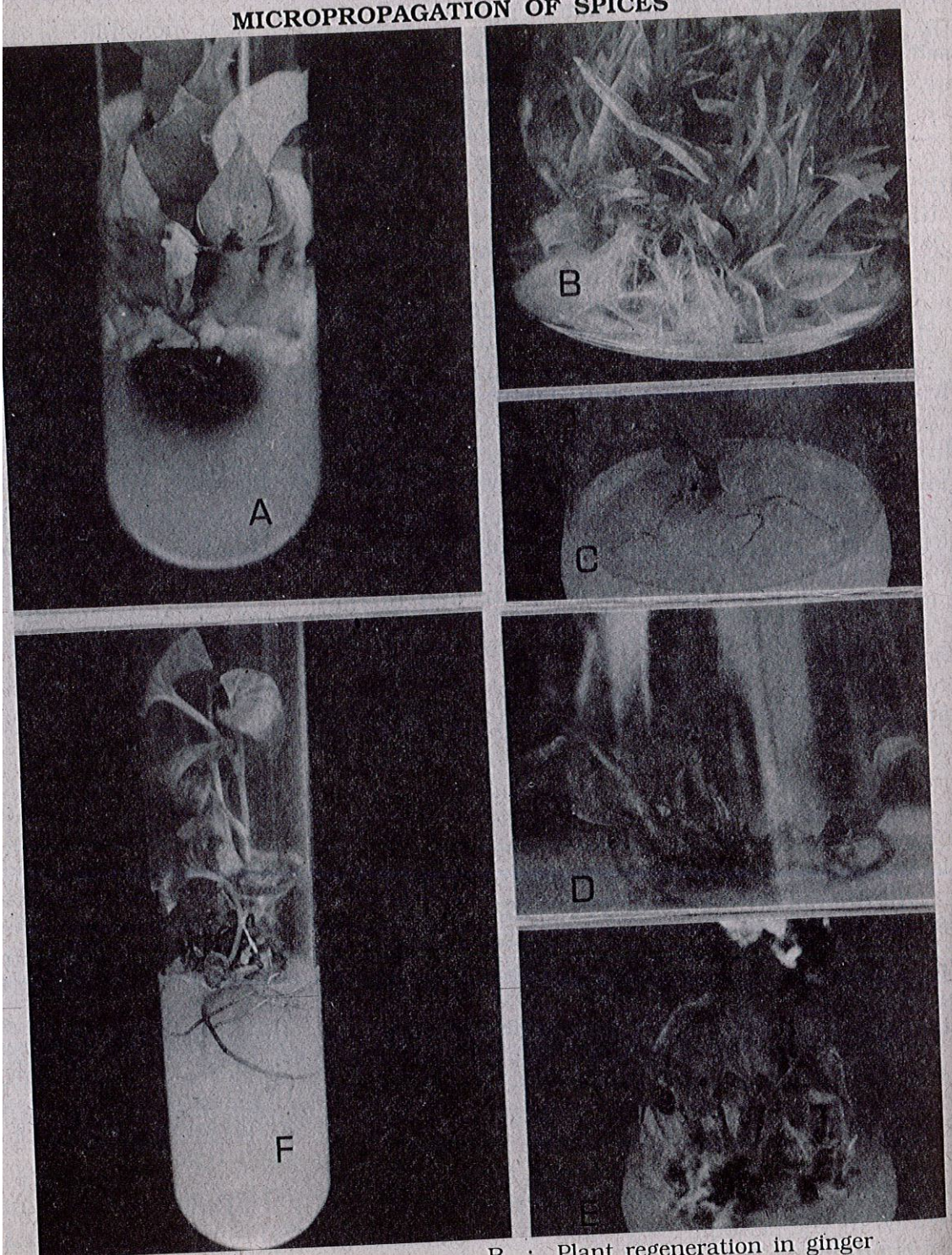
(5) Production of flavours and volatile constituents:

Another major advantage of plant cell and tissue cultures is the possibility of producing the flavours and volatile constituents directly in cultures. It is now possible to produce essence like the vannilla in tissue cultures as claimed by Escagen Corporation of the USA. This product will be substantially below the current natural vanillins price.

Production of 'Anethole' from cell cultures of *Foeniculum Vulgare* (Hunault *et al.* 1989), products of Anise oil from tissue cultures *Pimpinella anisum* (Earnst, 1989) and induction of Crocin, crocetin, Picrocrocin and small saffranal synthesis in callus cultures of Saffron, *Crocus sativus* (Ravisankar and Venkataraman, 1990) have been reported.

Direct proliferation of Saffron (stigma tic obes) in culture was also reported by Japaneso workers Sano & Himeno (1987). It is

MICROPROPAGATION OF SPICES



A : Multiple shoots in black pepper
B : Plant regeneration in ginger
C : Micropropagated plantlet of cinnamon
D : Multiple shoots in vanilla
E : Plant regeneration in turmeric
F : Plant regeneration in piper longum

possible, in the near future, to bypass the plants, in production of these flavouring compounds.

(6) In vitro conservation of germplasm:

Tissue culture technique are currently being used in the conservation and exchange of germplasm especially in vegetatively propagates crops. These techniques are being standardised for spice crops like Ginger, Turmeric, Black Pepper, Cardamom etc. making conservation of germplasm easier. It is also possible to keep them free from pests and pathogens by utilising in vitro techniques.

Thus tissue and cell culture technology has then opened up entirely new horizons and has given considerable hope to the spice Plant Breeders, Plant Pathologists and industrials alike. But the possibilities are yet to be realised in real terms.

REFERENCES:

1. Earnest, D. (1989) *Pimpinella anisum* L. (Anise): Cell culture, Somatic Embryog-enesis and Production of Anise oil in Biotechnology in Agriculture and Forestry. Vol. 7 (Medicinal & Aromatic Plants II) ed. Y P S Bajaj. Springer-Verlag, Berlin.

2. Hunault, G., P Desmarest and J. Du Manoir. (1989) *Foeniculum vulgare* Miller: Cell culture, Regeneration and Production of Anethole in Biotechnology in Agriculture and Forestry. Vol. 7 (Medicinal & Aromatic Plants II) ed. Y P S Bajaj. Springer - Verlag, Berlin.

3. Kulkarni, D. D., Khuspe, S S., Mascarenhas, A F. (1984) Isolation of *Pythium* tolerant ginger by tissue culture. Proc. VI Sym Plantation Crops (ed) S N Potty pp.3-13 Rubber Res. Inst. India, Kottayam.

4. Nedgeada, Khuspe, S S., Mascarenhas, A F. (1982) Isolation of high curcumin varieties of turmeric from tissue culture. In Proc. V Ann. Symp. Plantation Crops (ed) R D Iyer pp. 143-147 CPCRI Kasaragod.

5. Ravishankar, V S and Venkataraman, I V. (1990) Induction of Crocin, Crocetin,. Picrocrocin and Safranal Synthesis in callus cultures of Saffron - *Crocus sativus* L. Biotechnology and Applied Biochemistry 12, 336-340.

6. Sano, K and Hyouta, H. (1987) In vitro proliferation of Saffron (*Crocus sativus* L) stigma. Plant Cell Tissue and Organ Culture 11: 159-166.

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