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TISSUE CULTURE OF PEPPER AND TREE SPICES: POTENTIAL, PRIORITIES AND APPLICATIONS

M.K. NAIR and P.N. RAVINDRAN
Central Plantation Crops Research Institute
Regional Station, Calicut, Kerala

Abstract

The paper highlights the problems in the propagation and improvement of pepper and tree spices and the potentialities offered by the tissue and cell culture technology in solving some of these problems. In pepper, tissue culture will be useful in meeting the expected demand for rooted cuttings of improved lines during the rehabilitation and developmental programmes visualized for the future. It will be especially useful in accelerating the screening process against pathogens like *Phytophthora palmivora* and nematodes. In nutmeg the tissue culturing of elite female trees will be useful in overcoming the dioecy problem as well as in the multiplication of elite lines. Tissue culture technique will be useful in the maintenance of germplasm of pepper and tree spices, in inducing more variability for breeding purposes, in the production of pure lines by anther culture and in the incorporation of new genetic characters through protoplast fusion.

INTRODUCTION

PEPPER (*Piper nigrum* L., Piperaceae) is a plant of the humid tropics requiring adequate rainfall and warmth for its growth. Over 75 varieties of pepper are in cultivation in India and most of them are monoecious, though dioecious forms also exist. Within the last quarter of a century, though world production of pepper has increased from 35,000 to 1,42,820 tonnes, it remained almost static at about 30,000 tonnes in India. The present average yield of pepper in India is only 250 kg/ha, which is the lowest in the world.

Nutmeg (*Myristica fragrans* Houtt., Myristicaceae) is indigenous to the eastern islands of Moluccas, and the tree produces two separate spices, nutmeg (dry-shelled seeds) and mace (dry aril). The annual production in the world is around 7,000 tonnes of nutmeg and 1,150 tonnes of mace. Indonesia is the major producer of nutmeg accounting for about 60% of the world production. In India its production is only around 180 tonnes of nutmeg and 15 tonnes of mace. The species is dioecious and obligatorily cross fertilized.

Clove (*Syzygium aromaticum* syn. *Eugenia caryophyllata* Thunb., Myrtaceae) is a medium-sized evergreen tree indigenous to Moluccas Islands. It was introduced in India only about 150 years ago. While no reliable

statistics are available regarding the production, the requirement of nutmeg in India is about 100 to 150 tonnes per year. Tanzania (Zanzibar and Pemba) is the largest producer of clove followed by Indonesia. The world production of clove varies from 20 to 30 thousand tonnes per year.

Cinnamon of commerce is the dried inner bark of two species of *Cinnamomum* (Lauraceae), namely, *C. verum* (Bercht. & Presl.) and *C. cassia* (Bercht. & Presl.). The former gives the true cinnamon or 'Ceylon cinnamon' and the latter the cassia cinnamon or 'Chinese cinnamon'. They differ in their aroma and pungency. 'Ceylon cinnamon' is having a more pleasant and milder aroma than 'Chinese cinnamon', which is more pungent. *Cinnamomum verum* is indigenous to Sri Lanka and South India. The bark and leaf of both the species yield the cinnamon oil, used extensively in perfumery, foodstuffs and pharmaceuticals. Preliminary studies have shown that considerable variability exists in the germplasm collection of *C. verum*, with respect to taste, pungency and aroma. *C. cassia* is an introduced plant, and only a few trees exist in India.

PROBLEMS IN PROPAGATION AND IMPROVEMENT

Scarcity of Planting Materials and Clonal Multiplication

In pepper, about 50% of the vines in the existing gardens require urgent replanting and gap filling with better cultivars, which means that the replanting programme should cover 56,000 hectares. At the rate of 2,200 vines per hectare the number of rooted cuttings required will be 12.3 crores. Even if this requirement is to be met in a phased programme, the existing pepper multiplication plots will be able to supply only 50% of the anticipated requirements. Hence measures will have to be taken to produce about six to seven crores of cuttings of the popular cultivars. The conventional method of planting two- to three-noded cuttings has limitation, since a lot of planting material is wasted and the field establishment has been found to be low due to poor root development. Even the rapid method of propagating pepper using single-noded cuttings, developed by Bavappa and Gurusinghe (1978), which incidentally involves higher initial cost, will not help much to meet the anticipated demand of planting materials.

In clove, the economically useful part is the unopened flower-bud, and hence seed materials from high-yielding elite trees may not be easy to obtain as the production of 'mother clove' (clove seed) is believed to reduce the tree yield considerably. Seeds are also highly priced, and they are not easy to procure. Moreover, the variability existing in the germplasm collection, available in the country, is very much limited due to the very limited number of initia introductions. The existing types are also reported to be alternate bearers.

The problem of procuring planting material is very acute in *C. cassia* (cassia cinnamon)and allspice. Only a few of these trees are known to exist in

South India, that too in private gardens. There is much scope for this spice for export to all the Asian and Middle-East countries. Popularization of the cassia cinnamon depends upon the availability of a rapid multiplication method of the limited materials now available. To ensure the retention of the delicate quality factors involved in the final aroma, it is essential to resort to clonal multiplication, as seed propagation may affect quality drastically.

Susceptibility to Diseases and Pests

In India, the production of pepper is threatened by the rapid spread of quick wilt disease caused by *Phytophthora palmivora*. All the cultivars of pepper now being grown are susceptible to the pathogen, though some cultivars such as 'Kalluvally', 'Narayakodi' and 'Balancotta' show relative tolerance.

Even maintenance of the pepper germplasm in the field has become difficult, not only because of the *Phytophthora* wilt but also due to infection by the nematode *Radopholus sinilis*. Efforts were made to breed for resistance using selection in segregating population by hybridization and by induction of mutations. Thus for improving pepper production in India what is needed is the genetic upgrading of cultivars in respect of yield and disease and pest resistance combined with a technology for rapid multiplication of such elite resistant clones.

Dioecy in Nutmeg

The major problem facing the propagation of nutmeg is the segregation of seedling progenies into unproductive males and productive females in almost equal proportions. Though determination of sex at seedling stage based on leaf form and venation, the colour of sprouts and shape of calcium oxalate crystals in leaf epidermis have been claimed, but none of these is reliable. The classical methods of vegetative propagation such as grafting and budding, though successful, are costly and time-consuming. There is also dearth of scion materials since tropophysis exists in nutmeg, and only orthotropic shoots are useful for grafting because trees developing from plagiotropic scions tend to be of spreading habit.

During surveys conducted in the past we could identify a few very high-yielding trees, but the non-availability or proven 'elite male combiners' makes it rather difficult to use the conventional crop improvement methods in nutmeg. Segregation of sexes, yield and quality factors make the condition more complex.

Long Juvenile Phase in Nutmeg, Cloves and Allspice

Nutmeg and clove have long juvenile periods, taking 7-10 years for the first flowering. Doorenbos (1965) and Borchert (1976) studied the juvenility problem in trees. As the tree grows, it passes through different stages from

juvenile to mature. The transition from one to the other stage manifest first in the most recently formed upper parts of the tree. In other words, the upper parts are more physiologically advanced than the lower parts, which often retain the juvenile characteristics. Along with juvenility in nutmeg, the orthotropic and plagiotropic branches differ in their subsequent growing habit when propagated. A tissue culturist has to encounter these problems while taking up work on these crops, though their expression persists under some conditions, and is unstable under certain other conditions, as is noted in *Hedera helix* (Banks *et al.*, 1979).

Retention of Quality in Cinnamon

The retention of the delicate quality factors is an important aspect in cinnamon production. In seedling progenies owing to outcrossing and subsequent segregation, much variability occurs. Cuttings do root, but this is not useful in large-scale multiplication.

POSSIBILITIES OF TISSUE CULTURE IN PEPPER AND TREE SPICES

Rapid Multiplication of Improved Cultivars

Tissue and cell culture technique would open up new vistas in the rapid propagation of elite selections of pepper and tree spices either directly by meristem culture or by differentiation of either root or shoot callus. Pierik (1975) suggested that tissue culture propagation would be useful when the existing methods of vegetative propagation are too slow and/or non-profitable; when classical methods of vegetative propagation are not successful; when plant breeders need several plants of a selected genotype without delay; and when plant materials free from pathogens are desired. A very rapid and less expensive method of multiplication such as *in vitro* culturing is the only recourse to meet the heavy demand for rooted pepper cuttings. In *Cinnamomum cassia* development of an *in vitro* multiplication is all the more important in view of the extremely lower number of live plants available for multiplication. Such *in vitro* methods will also cut down the expenses on seedling production considerably, especially in the case of cloves and nutmeg.

The axillary bud micropropagation method developed for *Populus* and *Eucalyptus* (Cresswell and Nitsch, 1975) seems to be an ideal method for the large-scale propagation of tree spices. It was calculated that a million plants could be produced from one bud in one year by micropropagation. In the case of nutmeg, excising the terminal bud (because of the existing tropophysis) from an elite female tree and culturing to develop plant propagules could overcome the present problem of segregation of sex as well as the limited availability of planting materials.

Tissue Culture for Maintenance of Germplasm

Maintenance of germplasm in pepper is besieged with a lot of problems such as diseases, pests and sensitivity to drought. Tissue culture, combined with cryo-preservation, would be an ideal, risk-free and cheap method for maintenance of germplasm (Bajaj and Reinert, 1977) in pepper, nutmeg and clove.

Multiplication of Female Trees in Nutmeg

Perhaps the most important application of tissue culture in nutmeg would be for the rapid multiplication of high-yielding female trees. This will be immensely useful to the farmer, as he need not spend his time and money in nursing unproductive male trees, which at present in the seedling population could be identified only after 7-8 years of planting.

***In Vitro* Screening for Resistance and Quality**

One of the important applications of pepper tissue culture would be the *in vitro* screening of cultivars and clones for disease tolerance against *Phytophthora*, the pathogen causing pepper wilt. The toxin produced by *Phytophthora* has been isolated, and probably it would be feasible to use suspension cultures to screen for resistance to *Phytophthora* using a known concentration of zoospore suspension or even purified toxin. Drought susceptibility is a major problem in the cultivation of pepper, nutmeg and cloves. Extensive damages were recently reported in major pepper-growing areas due to moisture stress. The extended drought period (November to May) makes it almost essential to breed drought-tolerant types in these crops. The suspension culture method would also be useful in testing for drought tolerance by the incorporation of stress inducing substances such as mannitol or PEG.

The *in vitro* selection technique would be very useful in screening *Cinnamomum* germplasm for quality factors. It will be possible to identify the strains ('Chemovars') having high eugenol and cinnamaldehyde contents by *in vitro* screening.

Induction of Variability through Tissue Culture

Variability and not uniformity seems to be the rule with nearly all plants cultured *in vitro*, the resulting plants exhibiting some sort of variations (Skirvin, 1978). The variability associated with clones derived from callus cultures ('calliclones') can profitably be used for crop improvement through suitable selection procedures. Variability in callus and calliclones may also be increased by ageing, repeated transfer through the incorporation of mutagens into the medium, and irradiation of cultures. The association of polyploidy with tissue culture may be utilized as a technique to obtain either increased or reduced chromosome numbers as reported in lemon callus cultures

(Murashige *et al.*, 1967).

The usefulness of induced mutations in the improvement of tree crops is limited, as they tend to produce mostly chimeras, which often get sieved off by the strong forces of intrasomatic selection operating in the plant. This situation can be avoided by growing plantlets from single cells, which then ensure a completely mutant plant. This was advocated as an important method of fruit tree improvement, as the number of mutated plants produced would be much higher and the mutation spectrum much wider (Skirvin, 1981).

Production of Compounds in Culture

Production of secondary metabolic products in tissue culture is well known in crops like cocoa and coffee (Townsley, 1974 a,b). By culturing tissues of tree spices in suspension cultures, it may be possible to produce valuable chemical compounds such as myristic acid, myristicin and sterols (from nutmeg), eugenol, cinnamaldehyde, safrole and camphor (from *Cinnamomum*), eugenol from cloves and even essential oils of all these spices.

Anther Culture

Anther culture will be useful in pepper and tree spices for producing completely homozygous plants. It will be interesting in nutmeg as it may give 'pure males and females'. Such females and males from appropriate parentage may give us a perfect hybrid with maximum hybrid vigour. This will also be useful in analysing the nature of sex in nutmeg and allspice.

Incorporation of New Genetic Characters

One of the most significant developments in plant tissue culture is the isolation culture, and fusion of protoplasts which has got far-reaching significance in crop improvement through somatic hybridization. This method may be useful in future for introducing genes for resistance from resistant to susceptible parents, across incompatibility barriers. For instance the gene for *Phytophthora* resistance from *Piper colubrinum* could possibly be transferred to cultivated pepper.

Genetic engineering in higher plants is expected to make rapid strides in the year to come. Then it may be possible to transfer resistance or other desirable genes (such as the gene for nitrogen fixation) from other sources through the incorporation and integration of genes—either by direct DNA uptake or via agents such as *Agrobacterium tumefaciens*. This is a difficult proposition with the present level of technology, but let us hope that the revolutionary advances in the field of genetic engineering and protoplast culture will make such gene transfers possible in the near future.

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DISCUSSION

Y.R. Sarma (*Comment*) : The toxins of *Phytophthora* are non-specific and can be given a low priority for screening, whereas direct zoospore inoculation will be ideal method to test for resistance in callus variants.

V. Helena Mathews : Preliminary experiments on these tree spices were carried out. Pepper shoot-tips were found to give multiple shoots *in vitro.* Infection in subcultures was a serious problem.

H.C. Chaturvedi : The proposition of production of secondary metabolites of nutmeg and clove by tissue culture will merit investigation if the active principles looked for are very precious/costly.

M.K. Nair : The compounds suggested are active principles which determine the quality characteristics of these spices.

K.V.A. Bavappa : For the information of tissue culture scientists it is pointed out that cinnamon has 'camphor' at the root, 'cinnamic aldehyde' in stem (bark) and 'eugenol' in the leaf. In breeding varieties for the cinnamic aldehyde and eugenol, combining these two are difficult. Explants from leaf and stem and their behaviour as main plants is of considerable importance.

V.N. Madhava Rao : The establishment of nutmeg is also a problem that requires investigation. Tissue culture programmes may do better because of initial hormonal treatment.