



## Biotechnological Intervention in Spice Crop Improvement

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Biotechnological approaches have increasingly become a valuable tool, assisting breeders to release new species and cultivars into the market more rapidly. Conventional breeding methodologies have not led to any improvement in any crops, and alternative procedures like biotechnological, molecular, and biological interventions for enhancing yield and tolerance to biotic and abiotic stresses have to be explored. These technologies open up many novel avenues for enhancing productivity, profitability, sustainability, and stability in many cropping systems. Biotechnology, if used wisely to ensure biosafety and equitable benefit sharing, offers opportunities for converting biological wealth into economic wealth and new employment opportunities on an environmentally and socially sustainable basis.

### Important Areas of Biotechnological Intervention

#### Micropropagation technology

The development of convenient protocols and their standardization not only help in the mass multiplication of elite disease-free clones but also open new areas for the application of recombinant DNA technologies for the development of transgenic crops. Micropropagation is a powerful tool for large-scale propagation, especially where there is a great demand for quality, disease-free planting material. Tissue culture is a preferred method for many plantation crops like cardamom, vanilla, black pepper, betel vine, cumin, curry leaf, celery, coriander, camphor, fenugreek, ginger, mint, safed musli, turmeric, ashwagandha, and large cardamom.

#### Plant regeneration and somatic embryogenesis

Somatic embryogenesis can become a very useful technique for automation and large-scale propagation, provided the somatic embryos are genetically stable. Many efficient protocols are being standardized for efficient cloning of plants through somatic embryogenesis in cinnamon, ginger, turmeric, black pepper, and vanilla.

#### Somaclonal variation and *in vitro* selection

Variation generated *in vitro* can be a good source of variability, especially in crops with limited genetic variability. In addition, highly unstable callus culture and plant regeneration systems are ideally suited for *in vitro* selection for biotic and abiotic stresses. A few useful somaclonal variants have been selected for further use in breeding programs in cardamom, coriander, ginger, turmeric, and vanilla. A few clones tolerant to Katte were identified (Peter et al. 2001) in cardamom.

#### *In vitro* flowering, pollination and embryo rescue

There are reports of *in vitro* flowering and seed setting in spice crops like coriander and cumin. *In vitro* culture and embryo rescue technique could be successfully used for germination of seeds and selection of useful genotypes.

### **Development of haploids**

Haploids are regenerated from anthers and pollens through the tissue culture method. Through anther and pollen culture, homozygous diploid or isogenic diploid plants can be produced within a very short period as compared to the long inbreeding method. The main advantage of the *in vitro* production of haploid over the conventional plant breeding method is the saving of time. Attempts at anther and microspore culture were made by Ravindran and Madhusoodanan (2002) in cardamom.

### **Protoplast culture and development of somatic hybrids**

The protoplast is a naked cell, and the absence of a cell wall makes the protoplast suitable for a variety of manipulations that are not normally possible with intact cells. Hence, the protoplast is an important tool for the sexual modification of the genetic content of cells. Successful isolation and culture of protoplasts are being reported in mint, cardamom, ginger, fennel, turmeric, vanilla, nutmeg, garlic, fenugreek, peppermint, and saffron. Successful isolation and culture of protoplasts are reported in *P. nigrum* (Sim *et al.* 1998). Cardamom and ginger protoplasts could be successfully plated on culture media and made to develop to the microcalli stage (Geetha *et al.* 2000).

### ***In vitro* conservation and cryopreservation of germplasm**

The genetic resources of most of the crop species are conserved either in seed gene banks or in field repositories. Storage of germplasm in seed banks is not practical in some crops as they are vegetatively propagated and seeds are recalcitrant and heterozygous. Conservation of germplasm in *in vitro* gene banks and cryobanks is a viable and safe alternative. Cryopreservation of black pepper and cardamom seeds in liquid nitrogen was reported by Choudhary and Chandel in 1994.

### **Production of secondary metabolites**

The use of biotechnology for the biosynthesis of secondary metabolites, particularly in plants of pharmaceutical significance, holds an interesting alternative to the production of plant constituents. Biotechnological approaches are presently mainly used as a tool to facilitate a better understanding of the biochemical synthesis of saffron secondary products (Ahmad *et al.*, 2014). *In vitro* proliferation of nutmeg mace and synthesis of flavor components in culture were reported by Nirmal Babu *et al.* (1992).

### **Diagnostics for biotic stresses**

Although the availability of planting materials and creative agro-techniques has expanded in the horticulture industry, biotic variables remain the bane of horticultural output. Horticultural crops are affected by a broad range of diseases, causing moderate to major crop losses. Many diagnostic methods like ELISA, radio immunoassay, immunofluorescence, immunoblotting, PCR, RTPCR, LAMP, Nested/TAIL PCR, ITS-PCR, real-time PCR, multiplex PCR, etc. have been developed for plantation crops and spices, which are effectively used in the detection and management of diseases, viruses, and pests.

### **Marker assisted selection**

For a better comprehension of and ability to manipulate genome architecture, it is now crucial to have a molecular-level grasp of biology and genetics. DNA markers have quickly replaced the previous methods and have a number of benefits over conventional phenotypic markers. This aided in the creation of marker-based gene tags, phylogenetic analysis, map-based cloning of crucial genes for agriculture, studies of variability, marker-assisted genotype selection, synteny mapping, etc. In betelvines, genetic variation was carried out by using the RAPD method to distinguish between male and female betelvines as well as between different land races.

## Conclusion

Reasonable progress has been made in developing protocols for genetic improvement for most of the spice crops. These improved technologies are to be adopted for commercial multiplication and genetic enhancement of these crops wherever necessary. The use of molecular markers for genetic characterization of germplasm as well as plant varieties needs to be given priority. Additionally, this aids in the development of diagnostic markers for varietal identification and finger prints, both of which are crucial for the certification of planting materials.

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