Use of Plant Tissue Culture and Transgenicsa in Crop Improvement in India

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INTRODUCTION

In order to fight a number of biotic and abiotic stresses, several approaches have been followed by the scientists to improve the existing cultivars of the crop plants. Some of the important approaches are, production of transgenic cultivars, manipulation of the culture media to develop salt, drought and disease resistant cultivars making use of somaclonal variation, micropropagation of elite species, another culture, development of isogenic lines etc. Plant tissue culture is a very useful technique in all these approaches. The type of tissue culture and transgenic research that is being done in India is summarized here.

Micropropagation

Tissue culture is now a very powerful mean for the mass production of plants of desired varieties at a minimum cost, and in a limited space. The technique which is known as micropropagation is very effective and economical for cloning elite varieties of plants. In India, a lot of micropropagation work has been done on several plants including horticultural or ornamental plants (e.g. orchids, gerbera, chrysanthemum, rose, carnation), cash crops (sugarcane, potato), fruit plants (banana, grapes, pineapple, papaya, mulberry, guava), vegetable (tomato), spices (Turmeric, cardamom, ginger, tamarind), plantation crops (tea, coffee, vanilla), medicinal plants (Neem, ashwagandha , Aloe, Taxus, Gloriosa) and forest trees including angiosperms (Acacia nilotica, Albizia lebbeck, Albizia procera, Azadirachta indica, Bauhinia purpurea, Butea monosperma, Dalbergia sp., Dendrocalamus strictus, Eucalyptus sp., Ficus religiosa, Morus sp., Populus sp., Shorea robusta, Tectona grandis), gymnosperms (Biota orientalis, Cedrus deodara, Cryptomeria japonica, Picea smithiana, Pinus sp.). Woody ornamentals like Tabebuia bear colorful and showy blossoms and play a pivotal role in the bioaesthetic planning. Successfu) micropropagation to Tabebuia has been achieved in the Botany Department of Mysore University. Work is underway to establish and standardize a protocol for the mass propagation of Tabebuia on a commercial scale.

Jackfruit is a source of an important vegetable that has been successfully cloned in vitro by the Department of Horticulture, Mysore University. Attempts are now being made to standardize the protocol for the mass clonal propagation part and success has been achieved in the tissue culture of aonla. A number of Himalayan multipurpose trees such as Bauhinia, Diploknema and Sapium have been selected for in vitro.

Commercially propagated plants through micro-propagation in India

1. **Fruits-** Banana, Pineapple, Strawberry,

2. **Cash crops-** Sugarcane, Potato

3. **Spices-** Turmeric, Ginger, Vanilla, Large cardamom, Small Cardamom

4. **Medicinal plants-** *Aloe vera*, *Geranium*, *Stevia*, Patchouli, Neem

5. **Ornamentals-** Gerbera, Carnation, *Anthurium*, Lily, *Syngonium*, *Cymbidium*

6. **Woody plants-** Teak, Bamboo, Eucalyptus, *Populus*

7. **Bio fuel-** *Jatropha*, *Pongamia*

Indian jasmine is a tree noted for its aroma. Micropropagation of jasmine has been achieved. Chrysanthemum root suckers can now be obtained from the tissue culture generated plants. Micropropagation of Dieffenbachia and Gerbera, two of the beautiful ornamental plants, has resulted in the protocols using which large number of plantlets can be generated in the laboratory. The micropropagation of rice cultivars, banana, pigeonpea, sunflower and potato has been achieved and protocols are being developed to generate these plants in large number, and to improve the existing cultivars.

Delonix reoia is a tropical legume ornamental tree. Micropropagation of this ornamental tree has achieved. Likewise, protocols are now being standardized for the tissue culture of such important forest tree species as sisam and teak in a bid to improve the quality of their wood and for the mass propagation of these trees.

The tissue culture of mango has not been very successful so far. In a major headway in mango tissue culture some commercially important varieties such as Dasheri, Chausa, Langra and Safeda have been cultured *in vitro* at the National Botanical Research Institute, Lucknow. Further, studies are on the way to improve the protocol for tissue culture. Clonal propagation of peach and aonla is now a distinct possibility. Studies on the micropropagation of mulberry varieties at Mysore University have yielded protocols for their mass propagation.

Micropropagation work in India

Keeping in the view, the need for large scale production of planting material of elite plant species through micropropagation, Department of Biotechnology, Government of India established two pilot plants, one at TERI, New Delhi and other at NCL, Pune. Consortium on Micropropagation Research and Technology Development (CMRTD) was also established by DBT that includes 'Micropropagation Technology Parks', Regional Hardening facilities and National Facility for Virus Diagnosis and Quality Control of Tissue Culture Raised Plants in different parts of the country. National Facility for Plant Tissue Culture Repository (NFPTCR) was also established at NBPGR, New Delhi for the slow growth or short-term conservation of germplasm of economically important crops like- tuber and bulbous crops, spices, fruit crops, medicinal and aromatic plants and other endangered plant species. Micropropagation work for quality seed production in some sugarcane varieties including CoC 671, CoS 8436, CoS 96268, CoH 56, CoH 99 Co 89003, CoS 96275, Co 0238, Co 0239, CoJ 64 and Co 86032 is also going on at Navsari Agricultural University, Gujarat, Vasantdada Sugar Institute, Pune, Sugarcane Breeding Institute, Coimbatore, Uttar Pradesh Council of Sugarcane Research (UPCSR), Shahjahanpur, Indian Institute of Sugarcane Research, Lucknow and Department of Agriculture

Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, Uttar Pradesh. In India, the micropropagation work is also going on in more than 100 private laboratories for the commercial propagation of various plant species including Cash crops, fruit crops, medicinal plants, spices and forest trees. The first Indian commercial tissue culture plant was set up in 1987-88 by A.V. Thomas & Company in Cochin.

Synthetic seeds or artificial seeds

T. Murashige of USA for the first time gave the concept of artificial seeds at a symposium in Belgium in 1977. Artificial seeds are the somatic embryos covered with a protecting gel. These seeds are compared to the true seeds. In these seeds, the gel acts as seed coat and artificial endosperm providing nutrients as in true seeds. Synthetic seeds can be efficiently used for the management of transgenic and seedless plant species, polyploid plants with elite traits and plant lines that are difficult to propagate through conventional propagation methods and for long term conservation of valuable germplasm and its easy exchange across countries. The production of artificial seeds in Eucalyptus sp. has been reported by Muralidharan and Mascarenhas in 1987 by encapsulating somatic embryos in Na/Ca alginate beads. In India, this technique of synthetic seeds was standardized and practiced at Bhabha Atomic Research Centre (BARC), Bombay for sandalwood and mulberry. In several other plants including *Ocimum americanum* (hoary basil), *O. basilicum* (sweet basil), *O. gratissimum* (shrubby basil), *O. sanctum* (sacred basil), *Morus alba, M. australis, M. bombycis, M. cathyana, M. latifolia* and *M. nigra* (mulberry), *Musa spp*. AAB group (banana cv. 'Rasthali'), *Adhatoda vasica* (vasaka), *Dendrobium, Oncidium* and *Cattleya* (orchids), *Dalbergia sissoo* (sissoo), *Oryza sativa* (hybrid rice), *Rotula aquatic* (takad), *Ananus comosus* (pineapple), *Punica granatum* (pomegranate), *Vanilla planifolia* (vanilla), *Phyllanthus amarus* (bahupatra), *Citrus nobilis×C. deliciosa* (Kinnow mandarin), *Coleus forskohlii* (makandi), *Psidium guajava* (guava), *Cannabis sativa* (marijuana), *Curculigo orchioides* (black musali), *Spilanthes mauritiana* & Spilanthes acmella (toothache plant), *Vitex negundo* (five-leaved chaste tree), *Vitis vinifera* (grape), *Cucumis sativus* (cucumber) and *Picrorhiza kurrooa* (katuka) synthetic seed development also recorded in the last years.

Tissue culture of herbal plants and production of Secondary metabolites

The production of useful compounds (pharmaceuticals, essential oils, food flavorings and colourings) by *in vitro* cultured plant cells has become a field of special interest in various biotechnological programs. Several secondary metabolites including alkaloids, terpenoids, phenolics etc. have also been produced through *in vitro* cultured plant cells. In India, for the enhancement of secondary metabolites production, systems have been developed at BARC, Bombay, Regional Research Laboratory, Jammu and Botany Department, University of Jaipur, Rajasthan.

Attempts are being made to generate know how for mass propagation and genetic improvement of rare and threatened plants of Thar Desert and Arawali. Tissue culture methods have been defined for such plants as *Carallulma, Ceropegia, Dipcadi, Psoralea* etc.

Tea is a major plantation crop of India. With the support of Department of Biotechnology, Government of India, tea tissue culture is being pursued in the G. B. Pant Institute of Himalayan Environmental and Development, Almora.

Micro-tuber formation for mass cultivation of Kalazeera, an important medicinal plant, and micropropagation of other medicinal plants such as fennel, cumin, ocimum, *Achyranthes* etc. is being done in M. S. University of Baroda. These plants produce high yields of desired compounds. Two other medicinally important plants, namely, *Ammi majus* and *Datura*, for the production of hyoscyamine and furocoumarin have been successfully culture *in vitro* in Hamdard University, New Delhi.

Alpha-bisabalol is an important raw material for cosmetic industry. Studies to produce this compound by the callus of popular generated from the tissue culture are in progress in Pantnagar. Further, studies are in progress to standardize the protocol in the Department of Molecular Biology and Genetic Engineering in Pantnagar.

Somaclonal variations

In 1981, P.J. Larkin and Scowcroft at the Division of Plant Industry, C.S.I.R.O., Australia gave the term somaclonal variation to genetic variability generated during tissue culture. The mass occurrence of somaclonal variants, increase in the resistance, productivity and vital force of the plant (heterotic effects) has been explained by Carlson (1983). Most significant achievement of somaclonal variation was made by Sheppard and co-workers in old varieties of potatoes. Moreover, somaclonal variations are applicable for seed propagated plants only *e.g.* rice, wheat, maize, tobacco *etc.* not for vegetatively propagated plants. Thus, somaclonal variations have been proved alternative tool to plant breeding for generating new varieties that can exhibit disease resistance and improvement in quality and yield in plants such as cereals, legumes, oilseeds, tuber crops, fruit crops, *etc*. In India, somaclonal variations have been utilized to produce new cultivars of sugarcane and salt tolerant safflower. Another culture has been used to produce fine grain type rice tolerant to submergence. Sugarcane Breeding Institute, Coimbatore has released sugarcane variety Co 94012 through the process of somaclonal variation for commercial cultivation in Maharashtra and is found to give high sugar recovery. A somaclonal variant of lemon grass (*Citronella java*), a medicinal plant has been released as 'Bio-13' for commercial cultivation by Central Institute for Medicinal and Aromatic Plants (CIMAP), Lucknow, Uttar Pradesh. Bio-13 yields 37% more oil and 39% more citronellon than control variant. However, some other new cultivars through this approach have been obtained in rice (IARI, New Delhi), turmeric and custard apple (NCL, Pune), Pusa Jaikisan (Bio-902) variety of *Brassica Juncea* and 'Ratan' a low neurotoxin somaclonal variant of Khesari (*Lathyrus sativus*) has been developed by IARI, New Delhi for commercial cultivation

Using somaclonal variation the genetic improvement of finger millets has been achieved, similarly somaclonal variation has been used to select Basmati Rice- 370 tolerant to high salt. Again somaclonal variation has been used to develop lines of wheat tolerant to leaf blight. Attempts are also being made to produce transgenic wheat lines with high lysine content in their seed proteins using biolistic methods. Similarly genetic transformation has been attempted in chickpea to develop resistant lines to pod borer (*Helicoverpa armigera*) using *Agrobacterium* mediated transformation.

The storage proteins of mungbean and pigeonpea generated through tissue culture are being studied. Gamma radiation has been used to generate variations in sugarcane during the tissue culture by the scientists of Indian Institute of Sugarcane Research, Lucknow.

The technique is being used to enhance somaclonal variation in sugarcane. In an interesting study the ploidy level of plants generated through tissue culture of cotton has been studied to improve the existing cultivars of cotton. Safflower is an important oilseed bearing plants. Studies are in progress in agriculture and food engineering department of IIT Kharagpur to improve the present cultivars of safflower through tissue culture. In a bid to develop new varieties of grass used for fodder, studies are underway in the Indian Grassland and Fodder Research Institute in Jhansi on marble grass and buffelgrass and considerable success has been achieved in the tissue culture of these grasses.

Neem is a panacea for all maladies. Dabur research foundation has achieved success in somatic embryogenesis studies in neem and complete plantlets have been generated *in vitro*.

Anther culture

In India, work on anther culture was started during 1950 at the Department of Botany, University of Delhi, by Panchanan Maheswari who is regarded as father of embryology in India. Different tissue culture methodologies were involved for morphogenic studies involving ovary, embryo, endosperm, ovules etc. At the same university, Supra Guha Mukherjee and S.C. Maheswari (1964-67) for the first time developed the haploid through anther and pollen culture of an angiospermic plant *Datura innoxia*. Discovery of haploid production was a landmark in the development of plant tissue culture. At present, more than 250 plant species and hybrids belonging to 40 genera and 36 families of dicots and monocots have been regenerated through anther culture technique. Till date, in India, no variety in any crop has been developed through anther culture or haploid breeding.

Ovary culture derived rice plants in Pantnagar are being tested for their performance. In an interesting study done in the department of Plant Physiology in Pantnagar, it has been possible to produce miniature cobs in maize in tissue-culture generated plants. It is now possible to generate these miniature cobs at will and the system is being explored further to locate the genes and their regulator sequences responsible for the development of cobs. Using tissue culture it has been possible to develop haploids and dihaploids in *Brassica juncea*, the major oilseed crop of India. In an interesting study done in the Department of Molecular Biology and Genetic Engineering in Pantnagar, production of *in vitro* flowering plants regenerated from the *Brassica capestris* (yellow sarson protoplasts have been achieved). The system is now being used to introduce genes into the *Brassica campestris* plants to make them resistant to early blight.

Embryo rescue and triploid plants

Embryo rescue technique is very useful in wide hybridization, complete growth of embryo in plant, breaking dormancy of certain seeds where dormancy period is very long. By using embryo rescue technique wild varieties can be crossed with cultivars. At International Centre for Research in Semi Arid Tropics (ICRISAT, Hyderabad) has been used embryo rescue technique to improve groundnut, chickpea and pigeonpea. A new hybrid variety of pigeonpea (ICPH8) has been developed at ICRISAT that matures in 100 days instead of 200 days and have resistance against pathogen and pest and yields 20% more yield than original cultivars.

Triploid plants are of great importance because these are self-sterile and seedless. These characteristics increase edibility of fruits and desirable in fruit plants such as apple, banana, grape, mulberry, mango, watermelon etc. The triploids of poplar have better quality pulpwood. In India, first time B.M. Johri and S.S. Bhojwani (1965) at the University of Delhi reported the endosperm culture for triploid production. Some examples of triploid plants raised from endosperm culture are, *Asparagus officinalis*, Barley, rice, maize, *Prunus persica, Citrus grandis,* sandalwood etc. Till date, Solanaceae is the most common family for the development of somatic hybrids. In India, a collaborative research programme in family Brassicaceae was undertaken at IARI, TERI and Delhi University for the development somatic hybrids.

In Vitro screening for biotic and abiotic stress tolerance

Tissue culture techniques can be used utilized for the production of virus-free plants either through meristems culture or chemotherapy or selective chemotherapy of larger explants from donor plants or dormant propagules or a combination of two. In India, Sood and Palni (1992) have developed various steps for the production virus-free plants in Easter lily plant (*Lilium longoflorum*). Rao (1989) raised a cell line of pearl millet (*Penisetum americanum*) which was resistant to downy mildew caused by *Sclerospora graminicola.* Rao and Palni also reviewed *in vitro* selection of cell lines for disease resistant in plants. *In vitro* screening for various biotic and abiotic stress tolerances was also done in plants including *Chrysanthemum morifolium* (Chrysanthemum), *Triticum aestivum* (wheat), *Vigna radiate* (mungbean), *Brassica juncea* (Indian mustard), *Cymbopogon martinii* (palma rosa), *Dendrocalamus strictus* (bamboo), *Morus* sp. (mulberry), *Oryza sativa* (rice) for salt tolerance, in *Arachis hypogaea* (groundnut), *Brassica juncea* (Indian mustard), *Cocos nucifera* (Coconut), *Oryza sativa* (rice) for drought tolerance, *Nicotiana tabacum* (Tobacco) for Copper tolerance, *Setaria italic* (foxtail millet), *Brassica* sp. (Indian mustard) for Zinc and Manganese tolerance. *Arachis hypogaea* (groundnut) for resistance against *Cercosporidium personatum*, in *Carthamus tinctorius* (safflower) against *Alternaria carthami*, in *Curcuma longa* (turmeric) against *Pythium graminicolum*, in *Gossypium hirsutum* (Cotton) against *Fusarium oxysporum and Alternaria macrospora, in Hordeum vulgare* against *Fusaric acid Fusarium sp.,* in *Mangifera indica* against *Colletotrichum gloeosporioides* in *Oryza sativa* (rice) against *Helminthosporium oryzae*, in *Saccharum* sp. (sugarcane) against *Colletotrichum falcatum* and in *Vitis vinifera* (grapes) against *Elsinoe ampelina.* In India, a lot of research works have also done for the development of *Citrus tristeza* virus and Indian citrus ringspot virus (ICRSV) free plants through plant tissue culture techniques.

Developmental Biology

Tissue culture is a powerful tool in the studies in the field of developmental biology. Transgenic plants of soybean have been generated and the enzyme glutamine synthetase has been successfully used as a marker of ammonia assimilation. Tissue culture is also a means to induce somatic embryogenesis in winged bean. Studies on the polyamine biosynthesis in relation to stress tolerance in egg plant, and the effects of polyamines in somatic embryogenesis of egg plants are underway in the Department of Genetics in the Delhi University.

National certification system for tissue culture raised plants (NCS-TCP)

Micropropagation, a technique of plant tissue culture is effectively used for producing contamination and diseases free quality planting material for directly using either by farmers or researchers for their purposes. But there may be some chances of virus presence in those plants and their inadvertent propagation will not only result in poor performance of the crops but also tend to spread hazardous viruses. Beside this, there also may be the chances of deleterious variations in these plants that can affect yield and carry viruses and other pathogens that are difficult to diagnose. This area is of great concern so, there is need to develop a well structured and organized system to support the tissue culture industry to ensure virus-free quality planting material for commercial

Therapeutic Ingredients Based Whey Beverages

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INTRODUCTION

Milk is a complete food with nine major nutrients viz. Calcium, Protein, Potassium, Phosphorus, Vitamin D, Vitamin B12, Vitamin A, Riboflavin (B2) and Niacin ((National Dairy Council). Children need the milk for their healthy growth good health, but they usually avoid natural taste or flavour of regularmilk and milk products. To overcome this issue, flavours should be added to milk and other dairy products. As we know, India is the largest producer of milk with annual milk production around 187.7 MT (NDDB 2020). So, this value addition to milk and milk products will increase its value in the market. There are alreadymany flavoured milk products in the market, but most of them contain artificial flavours and colours that may be quite unhealthy for us, especially children.

On the other hand, people are becoming more health-conscious day by day.Also, in India, there are many spices and herbs, which our ancestors used to add in their food, but now, these spices and herbs are being replaced byartificial and unhealthy flavours. But now, people are moving back to healthy and nutrition-rich food. If we talk about milk products, whey beverages are one of them.

Whey is the watery part and a byproduct that remains after the separation of coagulated milk products like cheese. *In manufacturing these products, only 10% to 20% milk portion is recovered as the desired end product and rest 80% to 90% remaining liquid is whey where most of it is disposed as a waste, which is very sad.* Whey can be sweet or acid depending upon the type of casein coagulation. Whey protein contains less than 1% of dry matter.

Whey can be used as a very healthybeverage. Whey liquid has protein, calcium, magnesium, iron, sodium, potassium, carbohydrates and low fat (0.4 g per 100 g whey). This excellent nutrition value makes it healthy for the consumption of children to old age persons. The old ages and health-conscious people who avoid milk due to the risk of fat and cholesterol can drink whey drinks without thinking twice.

Production of whey-based beverages was started in 1970, and the mixing of native, acid or sweet whey with different additives like fruits, vanilla, cocoa, chocolates etc. develops a variety of whey-based beverages. There are also fortified whey beverages in which vitamins and other nutrients are fortified to enhance its nutrition value. Instead of artificial flavours, colours and additives, the addition of natural healthy flavours or spices can improve the nutrition and taste of whey drinks, which increases its market valueresulting in economic benefits. Many healthy ingredients like cardamom, fennel, cinnamon, basil, CuminCumin, coriander, jaggery, and honey should be added to make sweet and salted whey beverages which not only give a good taste but also have therapeutic properties to cure after suitable modification from *Bacillus thuringiensis* has been successfully transferred to the plants of tobacco using *Agrobacterium* mediated transformation. Genetic improvement of aromatic crops such as *Mentha arvensis, M. piperita, M. pulegium* and *Melissa officinalis* has been achieved through the transfer of foreign DNA using *Agrobacterium.*

At Delhi University South Campus, transgenic *Brassica juncea* was developed for herbicide resistance by transferring bar, barnase and barstar genes and the plants were used for the production of hybrid seeds. A collaborative research project was launched between TERI, India and Monsanto and Michigan State University, USA to develop golden mustard. It yields cooking oil with high content of beta-carotene (provitamin A).

In India, in the year 2002 for the first time *Bt*-cotton was approved for commercial cultivation that revolutionized Indian cotton industry. Till date, India has occupied largest area (about 11.0 mha) in the world under Bt-cotton cultivation which comprises more than 87% of the total cotton area. After that, in 2008, *Bt*-brinjal was approved for large-scale seed multiplication and in 2009, it was also approved for GEAC for release to farmers but unfortunately in 2009 an indefinite moratorium imposed by Ministry of Environment and Forest, Govt. of India.

Table-1: Major Indian developments/field trials in transgenic research

Institute/University	Crop plant	Transgene inserted	Purpose of the development
Bose Institute, Kolkata (W. B.)	Rice	5-adenosylmethionine decarboxylase	To generate stress tolerant plants
CICR, Nagpur	Cotton	*Bt Cry* genes	To generate insect resistant plants
CPRI, Shimla (H. P.)	Potato	*Bt Cry* IA (b)	To generate insect resistant plants
CRRI, Cuttack (Orissa)	Rice	*Bt Cry* IA (b), *Xa*21	To develop plants resistant to pests and bacteria
Delhi University (South Campus)	Mustard/ Rapeseed	*bar, barnase, barstar*	To generate herbicide tolerant plants
	Brinjal	Chitinase, Glucanase	To develop disease resistant plants
	Basmati rice	Pusa *cod*A, *cor*47	To develop abiotic and biotic stress tolerant plants
IARI, New Delhi	Brinjal,	*Bt Cry* IA (a) and *Cry* IA (abc)	To generate insect resistant plants
	Cauliflower Cabbage	*Bt Cry* IA (b)	
	Tomato	Antisense replicase gene of tomato leaf curl virus	To generate virus resistant plants

Institute/University	Crop plant	Transgene inserted	Purpose of the development
ICGEB, New Delhi	Tobacco	*Bt Cry* IIa5	To generate insect resistant plants
ICRISAT, Hyderabad	Groundnut	Chitinase gene from rice (Rchit)	To develop fungus resistant plants
Madurai Kamraj University, Madurai	Coffee	Chitinase, beta-1,3-glucanase and osmatin genes	To develop fungus resistant plants
NBRI, Lucknow (U. P.)	Rice	*Bt Cry* (b) genes	To generate plants resistant to bacteria and fungi
PAU, Ludhiana (Punjab)	Rice	*Bt Cry* IA (b), *Bt Cry* I A (c)	To develop plants resistant to pests
TNAU, Coimbatore (Tamilnadu)	Rice	GNA genes, rice chitinase (chi11) or tobacco osmotin gene	To develop plants resistant to pest gall midge
UAS, Dharwad	Muskmelon	Rabies glycoprotein genes	To develop edible vaccine
Mahyco, Mumbai	Rice	*Cry*1Ac, *Cry*2Ab	To develop plants resistant to insect and pests
	Tomato	*Cry*1Ac	
	Brinjal	*Cry*1Ac	
	Okra	*Cry*1Ac, *Cry*2Ab	
Monsanto, Mumbai	Corn	*Cry*1Ab gene	(Mon 810 event)
Sungro Seeds Ltd, New Delhi	Brinjal	*Cry*1Ac	
	Cauliflower	*Cry*1Ac, *Cry*1Ba and *Cry*1Ca	
M/s. Nunhems India Pvt. Ltd., Gurgaon	Cabbage	*Cry*1Ba and *Cry*1Ca	
	Cauliflower	*Cry*1Ac, *Cry*1Ba and *Cry*1Ca	

Conclusion

Plant tissue culture is now a well established technology which has made significant contributions to the propagation and improvement of agricultural crops in general.Greater contribution is envisaged from this technology in years to come, both in its own right and as an adjunct to the application of molecular biology.

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