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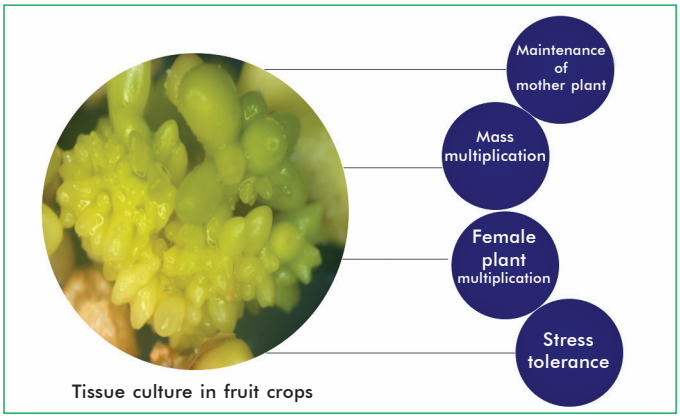
Tissue culture based clean planting material: A niche and profitable venture

The availability of quality and true-to-type planting material is prerequisite for any orchard establishment. In this context, the Government's initiative on establishment of "Atmanirbhar Clean Plant Program" in high value horticultural crops, particularly fruit crops are boon to the orchardist. In vegetatively propagated perennial fruit crops, it is relatively difficult to obtain disease-free quality planting materials through conventional propagation methods, which however, can be achieved through the tissue culture techniques retaining the benefit of vegetative propagation and reduced juvenility. *In vitro* culture techniques are indispensable for the production of uniform, season independent, disease-free and genuine planting material in less time and space. The innovative cost-effective tissue culture technique like bioreactors and bio-immune can enhance the production of immune planting materials of high quality. In near future, production of tissue culture based clean planting material has the potential not only to alter social organization of nursery industry but can also be a proficient occupation for women and young entrepreneurs.

FRUITS are universally promoted healthy as it serves as a primary source of dietary essentials. India's diverse climate favours the cultivation of variety of fruit crops. India is the second largest producer of fruits in the world, after China with a production of 108.34 million metric tonnes during 2022-23. However, productivity of fruit crops during the last one decade has been witnessing a declining trend from 17.6 Mt/ha (2012-2013) to 15.45 Mt/ha (2022-2023) thus resulting in a decrease of almost 12.22% which is thought provoking (MoA&FW 2023). This could be attributed to the several factors such as rapid urbanization resulting in smaller land holdings, indiscriminate use of irrigation water and subsequent soil related problems, abiotic stress such as drought, salinity, thermal stress, recurrent frost, flood and particularly the non-availability of quality and disease-free planting material. Supply of clean planting material still continues to be one of the major challenges in fruit crops like citrus, grape, pomegranate, apple etc. Even though an array of rootstocks are available for diverse agro-ecological conditions (biotic and abiotic stresses), canopy architecture including dwarfing rootstock for high density orcharding (HDO), most of the fruit crops even today are propagated on rootstocks of an unknown origin. Thus, the government recent initiative on production and supply of clean planting material program in fruit crops like almond, apple, avocado, berries, citrus, grapes, guava, litchi, mango, pomegranate, walnut etc. with a budget outlay of ₹2,200 crores for the next seven years is a welcome step for the nursery industry, unemployed youth and entrepreneurs. Since most of the fruit crops are commercially propagated by vegetative means such as grafting, budding, layering, cuttings etc., there are immense possibilities for the propagated material being infected by microbial endophytes (fungus, bacteria, virus and phytoplasma) depending on the source of the mother plant. Pomegranate is a classic example where devastating disease like bacterial blight has spread through contaminated planting material and has now become a national problem and difficult to eradicate. Similarly, Citrus greening (HLB leaf yellowing) and guava wilt are the serious threat to these fruit crops. Although technologies such as thermotherapy and nucellar seedlings find solutions to some of the above-mentioned problems, tissue culture is the only technique which can go a long



Tissue culture in fruit crops

way in overcoming such problems for the production of clean planting material due to its added advantage of mass multiplication under aseptic environment in less space and time. Unknown pedigree of mother plants, relatively less multiplication rate/plant and more time requirement on attaining desirable size as well as multiple sources of mother plants are some of the other problems related with conventional propagation techniques. Exchange of clean planting material from or to other countries is facing tough quarantine issues. To overcome these challenges, tissue culture (TC) can be used as supplementary as well as complementary alternative tool to fulfill the objective of clean planting material. The opportunities on tissue culture for production of clean, genuine and high-quality planting material in fruit crops are discussed below.

Maintenance of mother plant

Tissue culture has supplementary role in maintenance of mother plant. Among the biological threats, viral disease and endophytes are the most dangerous and hard to eliminate. However, tissue culture offer chance for complete elimination of viruses and other microbes through meristem culture using <0.2 mm meristematic region as an explant. In fruit crops, the possibility of meristem culture for virus elimination has been demonstrated in apple, banana, peach, strawberry etc. Candidate or identified plus tree can be regenerated *in vitro* via meristem culture and can be maintained under insect proof net as source of mother plant. Further mother plant blocks can be established by cloning the virus free plant via conventional propagation techniques.

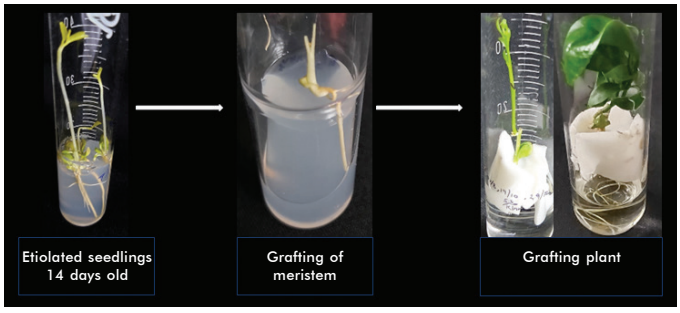
Recalcitrance in meristem culture due to high secondary metabolite synthesis is a major problem in some of the perennial fruit crops like citrus, avocado, almond, peach, sweet cherry and passion fruit. To overcome the problem, shoot tip grafting can be used as an alternative tool. In this technique, meristem of <0.2 mm obtained from scion cultivar are grafted on *in vitro* germinated seedlings in inverted 'T' shaped cuts. To reduce the establishment time and increase the *ex vitro* survival rate, the shoot tip grafts are double grafted on *ex vitro* maintained rootstock under insect proof net house. The established shoot tip grafted mother plant can then be disseminated to stakeholders.

Mass multiplication

Micropropagation is the complimentary process of producing plants vegetatively through tissue culture techniques. Through this technique, although millions of plants can be generated in less space *via* indirect regeneration, direct mode of regeneration is suggested due to the occurrence of somaclonal variations in former techniques. The multiplication of plants through direct organogenesis is commonly followed in monocot fruit crops like banana, coconut and pineapple using shoot tip culture, while in dicots such as grape, guava, pomegranate, pome and stone fruits, axillary node culture is normally used. Besides organogenesis, direct somatic embryogenesis from inflorescence and floral organs other than zygotic embryo also has immense potential in *in vitro* recalcitrant fruit crops like mango, date palm, citrus, coconut, avocado etc. High frequency multiplication rate in aseptic environment provides genetically stable clean planting material, irrespective of the season. Collection of explants from diagnosed *in vitro* meristem culture/shoot tip grafting regenerated mother plant of known cultivar should be given priority. For maximizing *ex vitro* survival, step wise hardening should be followed with regular management practices. For getting better establishment, field planting should be done during congenial environment preferably September-October or during early spring (February).

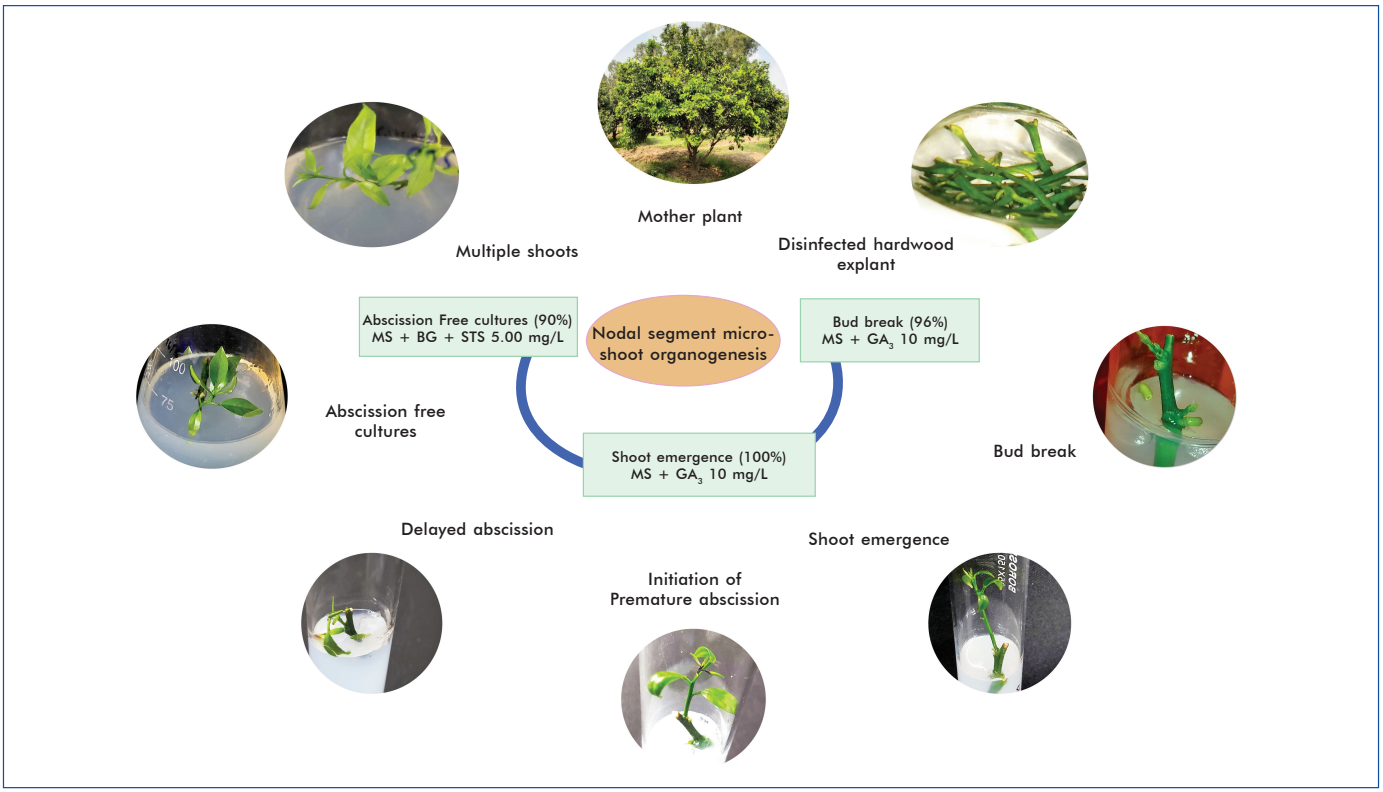
Multiplication of dioecious fruit trees

In dioecious fruit crops like papaya, date palm, pistachio, kiwi fruit etc., selective multiplication of male and female tree is relatively difficult. In these fruit species, tissue culture can play a pivotal role in overcoming the constraints faced in the traditional methods of propagation via somatic tissues. For female plant multiplication, the explants of somatic tissues collected from the clean female mother tree at bearing stage can be used for mass multiplication through standard protocols. The success story of date palm cultivation using imported tissue cultured plants from UAE of cultivars Barahi, Khuneizi, Khalas and Medjool from Rajasthan, Gujarat, Tamil Nadu etc., have opened avenues to multiply the *in vitro* plants in our country as well. Although some of the private firms are producing the *in vitro* plants, efforts to multiply tissue cultured, clean plant at lower cost should be prioritized so as to reduce the import cost.



In vitro grafting in citrus

Direct somatic embryogenesis in citrus



Silver supplementation to overcome ethylene induced premature abscission in citrus

Stress tolerance

The recent innovations on banana bio-immune technology for eradicating deadly disease of Fusarium wilt caused by TR4 using polypeptide bio-engineering during tissue culture organogenesis phase in banana give insights about the similar technological innovations in other fruit crops. Moreover, under changing climate, mitigation of crop specific biotic and abiotic stresses using tissue culture strategies needs to be developed.

Basic problems and solution

In vitro recalcitrance such as browning of inoculated explant tissues due to high ethylene evolution, secondary metabolite exudation, hyperhydricity and low multiplication rate are the some of the major problem encountered during the multiplication process of woody perennial fruit crops. To overcome the problems, modification in the medium composition, i.e. inclusion of ethylene absorbents like silver compounds, activated charcoal (secondary metabolite absorption) etc., in the media can be used in crops like mango, citrus, pomegranate, date palm etc. Similarly, to alter the growing environment, particularly for improved aeration and nutrient replenishment, bioreactor based temporary immersion systems with automation can be used and has been optimized in crops like banana, date palm, papaya, pineapple, strawberry, grapes and citrus. In India, low cost temporary immersion system (TIS) based banana micropropagation technique has been designed and validated for cv. Rasthali. This innovative bioreactor technique can also enhance the multiplication efficiency, reduce the labour and ensure the *ex vitro* recovery in difficult to regenerate woody perennials. High initial establishment cost for sophisticated laboratories is also a limitation. The financial support by the centrally sponsored subsidies and schemes such as MIDH, RKVY and NABARD can be utilized for the establishment of tissue culture laboratories.

Certification system

National certification system for tissue culture raised plants (NCS-TCP) was implemented during 2006 by Department of Biotechnology (DBT), Government of India. It is a comprehensive system to ensure that the tissue culture raised plants are free from viruses and fastidious pathogen and are also genetically uniform. To provide authenticity and traceability, the tissue culture material needs to be tested and certified by sending the samples to accredited testing laboratories (ATL). In fruit crops the standards on Indian NCS-TCP certification is available for banana and apple, while recently during 2021, citrus has also been included in the system. The major activities of Indian NCS-TCP system are testing and certification of tissue culture plants, accreditation of test laboratories, advisory services and updation of guidelines/SOPs etc.

Conclusively, tissue culture has immense potential in producing quality and clean planting materials in fruit crops. The current support rendered by the Government of India may be taken as an opportunity by rural and urban unemployed youth and self-help groups to multiply clean planting material and make it a profitable venture.

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