In-Vitro Micrografting Technique For Crop Improvement

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Introduction

Micrografting, an *in-vitro* grafting approach, is comparatively a recently developed vegetative propagation technique for propagating plants by grafting miniaturized scions under axenic conditions of culture. Micrografting involves the fusion of small pieces of tissue, either in vivo or *in vitro*. It is a cost-effective and economical technique which facilitates the growers to distinguish incompatibility in a couple of weeks instead of years which are often required to observe incompatibility in vivo, disease indexing, histological studies, producing plants resistant to soil borne pathogens and multiplication of plants difficult to root.

Micrografting is a technique which involves the placement of meristem or shoots tip explant on a decapitating rootstock grown aseptically from seed or micropropagated culture. The progress and shortcomings of the technique is largely dependent upon the compatibility responses that occur between grafting tissues. It pools the benefits of grafting and shoot tip culture thus overcoming certain limitations. It comprehends grafting inability and physiological rejuvenation as an essential requirement for mass clonally propagating mature selected genotypes.

Special techniques have been exploited to increase the rate of successful micrografts by using by using growth regulators, increasing sucrose level, etiolation treatments, silicon tubes etc as it has an enormous potential for improvement and multiplication of horticultural crops at a large scale.

The small shoot apices are transferred onto rootstocks either *in vitro* or in vivo. Navarro in 1988 listed four uses of this technique found to be profound:

Production of virus and viroid free plants

- A method to separate virus and virus-like organisms in mixed infections
- Predictions of incompatibility between scions and rootstocks, and the histological study and physiological aspects of grafting
- Germplasm exchange with a minimum risk for importing plant material through quarantine between countries.

Micrografting stages

The protocols have been standardised separately for the scion and rootstock to grow under *in vitro* conditions.

1) Multiplication and establishment of scion

The meristem tips are collected from actively growing shoots from field, greenhouse or *in vitro* chambers. Explants such as apical shoot tips or nodal cuttings are established in aseptic conditions. The microshoots are transferred to shoot proliferation media to increase the development of auxillary shoots. The microshoots possessing desired thickness, age and length are used as scion for *in vitro* grafting operation.

2) Multiplication and establishment of rootstock

Newly germinated seedlings, rooted cuttings or micropropagated shoots can be utilized as rootstocks. While using the seedling rootstock, all the various stages of grafting, are conducted under aseptic conditions (*in vitro*) and the surface sterilised seeds germinate aseptically in vessels comprising of nutrient solutions such as MS media. To encourage the growth of the branched root system, the seedlings are grown on agar media or on a porous substrate with sterile vermiculite.

3) Development of rootstock and scion for micrografting

Surface placement method is adopted where the top of the seedling rootstocks are cut just above the cotyledon or the micropropagated shoot and placed on small shoot apices over the exposed surface of decapitated rootstock in a manner such that the cambium layers of cut surfaces coincides with each other. For an appropriate union and callus formation, a firm contact between rootstock and scion is vital. To hold the grafts together, several sophisticated techniques such as translucent silicon tubing, elastic strip, filter paper bridge, and glass tubing, nylon bands, aluminium foil tubes, dual layer apparatus of aluminium foil and absorbent paper. When the graft union is successful then rootstock and scion grows together to produce desired plants which are examined regularly and the adventitious shoots arising in the graft reunion are removed. (Hussain *et al*., 2014)

In an experiment, conducted by Zang *et al.,* 2015 watermelon inbred line A7 were used. The Seedlings were cultured for 3 weeks and hypocotyls used as rootstocks. The regenerated shoots of watermelon (D66) cotyledon were used as scions. When incubated for over 45 days, adventitious shoots were cut for grafting. The number of successfully grafting seedlings was counted after 10 days of culture. Shoot tips excised from *in vitro*-raised seedlings were micro-grafted onto rootstocks. Twenty micrografted seeldings were decapitated in each treatment using almost uniform microscions and rootstocks and the graft union was done by using cleft grafting and top hole insertion grafting. It was reported that the survival rate of top hole insertion grafting was higher compared to cleft grafting. The wound healed three days later and the micrografted seedlings survived after 10 days since being grafted.

Advancement in micrografting techniques

Micrografting is a time consuming technique of producing plants and predominantly results into low rate of successful grafts. Technical expertise is essential to device successful grafts using small-scale materials by handling the challenges by preserving delicate graft unions. The failure of the grafts prepared *in vitro* is due to-

a) Browning or blackening of tissue due to exudation of phenolic compounds from cut surface which inhibits growth and development of new cells resulting into death of new scions.

b) Sucrose concentration of the medium

c) Light and dark incubation treatment which leads to significant variation in percentage of successful graft formation.

d) Poor contact between stock and scion

These limitations can be alleviated by using superior technologies along with the use of growth regulators such as auxin and cytokinin for a higher survival rate of grafts, nature of supporting media such as liquid media and vermiculite, preventing desiccation of grafts by using moist agar gel to establish a better connection between grafting partners, proper selection and treatment of the viable apex and suitability of rootstock for mass multiplication.

Implementation of micrografting technique

a) To eliminate virus and viroids: Genetically certified and uniform virus free

plants could be produced easily on a commercial scale under controlled environment through micrografting. Micro-shoot tips of size less than 05mm as scions produced virus free plants and were found to be reproductively mature.

b) **Producing plants resistant to pest and diseases:** Micrografting can be used successfully as an efficient method for procurement of plants free from soil borne pathogens.

c) **Assessment of graft incompatibility :** This procedure helps to determine histological, histochemical and other physiological parameters of graft incompatibility between scion and rootstock. Histological study exhibits callus formation and differentiation along with vascular connections. The an atomical study depicts poor vascular connection, phloem disintegration and discontinuity after the establishment of grafts. (Darilova *et al.,* 2011)

d) **Mass multiplication of superior plants:** Micrografting helps in enhancing the productivity by the formation of successful grafts due to superior rootstock and scion combination which can be achieved in tissue culture laboratories.

e) **Safer exchange of germplasm:** The propagated plants could be easily and conveniently exchanged between the countries due to production of virus free plants and those free from pest and diseases.

Study of compatible graft

1) Vascular redifferentiation was observed in *Lycopersicon esculentum* where indole-3-acetic acid (IAA) was applied on the apical end of the grafted internode to produce callus tissue and initiate the formation of vascular tissue.

2) Grating of unwounded callus mass of *Sedum telephoides* and *Solanum pennellii* were allowed to grow in the culture media to promote callus mass interdigitation.

Conclusion

In vitro micrografting system are of utmost importance as it would lessen the time interval and space required to grow horticultural crops free from diseases and pests and thus improving productivity by adopting efforts to determine graft compatibility. The technique would help to examine numerous botanical problems, issues related to nitrogen fixation, flowering, fruiting and thus the growth. Other tissue culture techniques so incorporated would help to determine some aspects related to graft compatibility.

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