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*Review*

# Micrografting for fruit crop improvement

**Hussain, G.[1]*, Wani, M. S.[1], Mir, M. A.[1], Rather Z. A.[2] and Bhat, K. M.[1]**

[1]Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar - Srinagar (J&K), India.
[2]Division of Floriculture, Medicinal and Aromatic Plants, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar - Srinagar (J&K), India.

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**Micrografting is an *in vitro* grafting technique which involves the placement of a meristem or shoot tip explant onto a decapitated rootstock that has been grown aseptically from seed or micropropagated cultures. Following early experiments of micrografting in ivy and chrysanthemum, the technique has been used in woody species, especially fruit trees. Major work was carried out in different *Citrus* species for the elimination of various viral diseases. *In vitro* micrografting has been used for improvement and multiplication of fruit trees as the technique has potential to combine the advantages of rapid *in vitro* multiplication with the increased productivity that results from grafting superior rootstock and scion combinations. Successful micrografting protocols have been developed for various fruit crops including almond, apple, cherry, chestnut, *Citrus*, grapes, mulberry, olive, peach, pear, pistacio, walnut, etc. Special techniques have been used for increasing the percentage of successful micrografts with the use of growth regulators, etiolation treatments, antioxidants, higher sucrose levels, silicon tubes, etc. The technique has great potential for improvement and large scale multiplication of fruit plants. It has been used on commercial scale for production of virus-free plants in fruit crops and viroid free plants in *Citrus.* Micrografting has also been used in prediction of incompatibility between the grafting partners, histological studies, disease indexing, production of disease-free plants particularly resistant to soil borne pathogens and multiplication of difficult to root plants.**

**Key words:** Fruit crops, graft incompatibility, crop improvement, micrografting, propagation, shoot tip grafting.

## INTRODUCTION

Micrografting is a relatively new technique for propagation of plants. According to Hartmann et al. (2002), micrografting is an *in vitro* grafting technique which involves the placement of a meristem or shoots tip explant onto a decapitated rootstock that has been grown aseptically from seed or micropropagated cultures.

*Corresponding author. E-mail: h_gousia@rediffmail.com.



**Abbreviations: PVP,** Polyvinyl pyrrolidine; **DIECA,** sodium diethyl-dithiocarbamate; **NAA,** naphthalene acetic acid; **BAP,** benzylaminopurine; **MS,** Murashige and Skoog; **STG,** shoot tip grafting.

Following early experiments by Doorenbos in 1953 in ivy and later by Holmes (1956) on chrysanthemum, micro-grafting technique have been used in particular on woody species and especially on fruit trees, where work was carried out on different species of citrus with a view to eliminate viral diseases. The technique was modified and improved for increasing the graft success by Murashige et al. (1972) and Navarro et al. (1975). The technique has great potential for improvement and large scale multiplication of fruit plants. It has been used for the production of virus and viroid-free plants in fruit crops. Micrografting has also been used in prediction of incompatibility between the grafting partners, histological studies, virus indexing, production of disease-free plants particularly resistant to soil borne pathogens, safe germplasm exchange between countries and multi-plication of difficult to root plants. Reviews on micrografting have been published by Jonard et al. (1983), Jonard (1986), Roistacher et al. (1976), Parkinson et al. (1990) and Monteuuis (2012). The present review aims at examining the published literature related to micrografting to increase the application of this technique at commercial level for the improvement of fruit crops.

## STAGES OF MICROGRAFTING

Micro-propagation protocol for scion as well as rootstock needs to be standardized separately before performing the micrografting operation under *in vitro* conditions. Thus, micrografting can be divided into three main stages:

### Establishment and multiplication of scion

Shoot or meristem tips intended for grafting can be taken from actively growing shoots in greenhouse, chambers, field or *in vitro*. Generally, apical shoot tips or nodal cuttings are used as explants for the establishment of *in vitro* cultures. Following establishment, microshoots are transferred to shoot proliferation medium where shoot number increases by the development of new axillary shoots. Microshoots of desired thickness, age and length are used as scions for *in vitro* grafting operations.

### Establishment and multiplication of rootstock

Rootstocks used for micrografting are *in vitro* or *in vivo* germinated seedlings and rooted or unrooted micropropagated shoots. When seedling rootstocks are used and all stages of grafting are conducted *in vitro*, seeds are surface sterilized and germinated aseptically in vessels containing nutrient salts. The seedlings may be supported on agar medium. Seedlings can also be on a porous substrate, such as sterile vermiculite, which allows the growth of a branched root system.

### Preparation of rootstock and scion for micrografting

Micrografting is affected by cutting off the top of the seedling rootstocks usually just above the cotyledons or top of the micro-propagated shoot and placing small shoot apices of scion onto the exposed surface of decapitated rootstock in such a way that the cambium layer or vascular ring of the cut surfaces coincides with each other. This is called surface placement method. Wedge or cleft grafting is performed, incase thickness of rootstock and scion material is large enough to allow making of wedge on the scion material. Firm contact between rootstock and scion is extremely important at the graft junction for proper union of partners and callus formation (Canan et al., 2006). Several techniques have been developed for holding grafts together until fusion takes place such as translucent silicon tubing (Gebhardt and Goldbach, 1988), elastic strip (Jonard et al., 1983), filter paper bridge (Huang and Millikan, 1980), and glass tubing, nylon bands, aluminum foil tubes, dual layer apparatus of aluminum foil and absorbent paper (Obeidy and Smith, 1991). When grafts are successful, rootstock and scion grow together to produce a plant. It is usually necessary to examine freshly grafted seedlings on a regular basis and remove any adventitious shoot arising on or below the graft union.

## GRAFTING SUCCESS DURING MICROGRAFTING

Grafting is a traditional method for production of composite plants but is season dependent. Failure of grafting means loss of one year for the production of grafted plants. This problem has been overcome through the use of micrografting which is done under controlled environmental conditions throughout the year and production can be planned according to market demand. Micrografting has particular utility in fruit tree production and protocol have been developed in many fruit crops including almond (Yıldırım et al., 2013; Isıkalan et al., 2011), apple (Huang and Millikan, 1980), apricot (Piagnani et al., 2006), avocado (Simon and Richard, 2005), cashew (Mneney and Mantell, 2001), cherry (Amiri, 2006, 2007), grapes (Tangolar et al., 2003; Aazami and Bagher, 2010), pear (Faggioli et al., 1997), pistachio (Abousalim and Mantell, 1992), walnut (Wang et al., 2010), etc. For commercialization of micrografting, protocol should be perfect to give higher percentage of successful micrografts. Good micrografting protocols have been developed for large scale production of micrografted plants in many fruit trees with high percentage of graft success (Table 1).

**Table 1.** Micrografting success in fruit crops.

| S/N | Fruit crop | Scion cultivar | Rootstock | Successful micrografts (%) | Source |
|---|---|---|---|---|---|
| 1 | Pistachio | *Pistacia vera* cv. Siirt | Seedling raised rootstocks | 79.25 | Onay et al. (2004) |
| 2 | Pistachio | *Pistacia vera* cv. Mateur | Seedling raised rootstocks | 94-100 | Abousalim and Mantell (1992) |
| 3 | Mulberry | (*Morus alba*) cv. 707 | Seedling raised rootstocks | >90 | Fengtong et al. (1996) |
| 4 | Olive | *Olea europea* cv. Zard | *In vitro* raised seedlings of olive | 45-83 | Farah et al. (2011) |
| 5 | Grape | *Vitis venifera* cvs. Sahebi, Soltanin, Fakhri | 41 B | 50.1 -60.6 | Aazami and Bagher (2010) |
| 6 | Grape | *Vitis venifera* cv. Early Cardinal | 41 B, Salt Creek | 71.4 - 80.0 | Tangolar et al. (2003) |
| 7 | Apple | *Malus domestica* cv. Lal Ambri | M-9 rootstock | 42.25 | Khalid Mushtaq (2009) |
| 8 | Hazelnut | E-295-S (hazelnut) | G-029-N | 72.00 | Nas and Read (2003) |
| 9 | Chestnut | 907 (chestnut) | 711 | 80.00 | Nas and Read (2003) |
| 10 | Walnut | *Juglans regia* cvs. Jinlong No 1, Xiangling | Seedling raised rootstock | 56.70 - 73.30 | Wang et al. (2010) |
| 11 | Almond | *Amygdalus Communis* cvs. Ferragnes, Ferraduel | *In vitro* germinated wild almond seedlings | 90-100 | Yildirim et al. (2010) |
| 12 | Almond | *Amygdalus Communis* cv. Nonpareil | *In vitro* germinated wild almond seedlings | 90.00 | Isıkalan et al. (2011) |
| 13 | Almond | *Prunus dulcis* cvs. Texas, Ferrastar, Nonpareil | *In vitro* germinated almond seedlings | 83.33 - 100 | Yıldırım et al. (2013) |
| 14 | Citrus | I: Kinnow mandarin<br>II Succari sweet orange | *In vitro* germinated seedlings of rough lemon | I 36<br>II 33.3 | Naz et al. (2007) |
| 15 | Pear | Pyrus communis cv. Le-Cont | *In vitro* shoots of *Pyrus betulaefolia* | 83.00 | Hassanen (2013) |

## IMPROVEMENT IN MICROGRAFTING TECHNIQUE

Micrografting procedures are difficult and generally results in a low rate of successful grafts, which makes it an expensive and time-consuming production technique. It is due to the fact that more technical expertise is required in preparing successful grafts on small-scale material and handling difficulties associated with preserving the delicate graft unions. In many experiments, failure rate for micrografts was higher than desired. *In vitro* grafts of fruit plants often fail due to incompatibility reaction, poor contact between stock and scion and phenolic browning of cut surfaces (Ramanayake and Kovoor, 1999). In order to alleviate some of these limitations, different techniques have been developed to make micrografting a successful and superior technology for the benefit of technicians, researchers, nursery operators and commercial tissue culture laboratories.

### Browning and tissue blackening

Exudation of phenolic compounds from the cut surfaces and their oxidation by polyphenoloxidase and peroxidase enzymes cause discolouration of the tissues which results in poor micrografting (Martinez et al., 1979). Browning of the cut surfaces inhibits the growth and development of new cells and results in poor graft union. To block the oxidation phenomena and prevent tissue browning, various substances have been used which include thiourea, cysteine, chlorhydrate (Jonard, 1986), citric and ascorbic acid (He et al., 2005), Phytagel (Zhang and Luo, 2006), PVP (Rather et al., 2011), DIECA (Martinez et al., 1979). Tissue blackening, which commonly results in the death of very small scions, has been reduced by soaking explants in an anti-oxidant solution, and/or placing a drop of solution onto the severed rootstock immediately before inserting the scion (Jonard et al., 1990; Ramanayake and Kovoor, 1999).

### Sucrose concentration of the medium

Sucrose concentration of nutrient medium had a significant effect on the percentage of successful grafts. Navarro et al. (1975) reported that sucrose concentration of medium of grafted plants played a significant role and that the highest rate of successful grafts in citrus species was obtained with 7.5% sucrose. Generally *in vitro*

growth and development increases with increased sugar concentration (Pierik, 1987). Naz et al. (2007) used 14 days old seedlings of rough lemon (*Citrus jambheri* Lash) grown under *in vitro* etiolated conditions as rootstock and microshoots of Kinnow mandarin/Succari sweet orange as scion. Micrograft success improved with increase in sugar levels in both cultivars from 20-22% with 3% sucrose to 36-38% with 7% sucrose. Hamaraie et al. (2003) also reported improvement in the micrograft success from 30 to 60% and scion growth from 8.7 to 13.8 mm with the increase in sucrose concentration from 2.5 to 7.5%, respectively during his studies with micrografting of grapefruit (*Citrus paradisi*) on sour orange seedlings germinated *in vitro*.

**Light/dark incubation treatments**

Significant variations have been reported in the percentage of successful grafts according to exposure of seedlings to light. Hamaraie et al. (2003) reported higher frequency of successful grafts (50%) in grapefruit (*Citrus paradisi*) cv. "Miami, when rootstock seedlings (sour orange) were obtained from seeds germinated under continuous darkness for two weeks as compared to only 5% successful grafts with seedlings which developed under light. Navarro et al. (1975) reported a very low frequency of successful grafts using Troyer citrange seedlings grown under continuous light as compared to seedlings grown in continuous darkness. Ewa and Monika (2006) found high percentage of successful micrografts in cherry under dark conditions.

**Use of growth regulators**

Usually growth regulators are not used in traditional grafting for increasing the graft success. However, under *in vitro* conditions, growth regulators particularly cytokinins and auxins have been found effective for improving the graft success rate. These growth regulators increase the rate of cell division and improve callus formation, which in turn help in increasing the percentage of successful graft unions. At the time of performing micrografting operation, prepared micro-scion is given a quick dip (5-10 s) in sterilized growth regulator solution of desired concentration and then inserted into or placed on the rootstock. Wang et al. (2010) found NAA effective in improving the micrograft success in walnut. Rafail and Mosleh (2010) reported increase in micrograft success from 30 to 90% in pear (cv. Aly-sur on Calleryana pear) and 40 to 90% in apple (cv. Anna on MM106) with increasing BAP concentration from 0-2.0 mg/L. Triatrniningsih et al. (1989) obtained a 24% increase in the frequency of successful grafts over untreated controls in *Citrus* by the use of BAP at 0.5 mg/L.

**Nature of the supporting medium**

Agar solidified medium and liquid medium have been used for the growth of micrograted plants. Rafail and Mosleh (2010) observed that number of successful micrografts increased from 10% in agar-solidified medium to 60% in apple and 70% in pear with use of liquid medium. There is usually more up take of nutrients and growth regulators by the microshoots in liquid media, which makes it more effective than solidified medium for micrografting success. MS liquid medium with vermiculite was found best for further development of the micrografts, because liquid medium alone or with agar forms asphyxic conditions, which prevents formation of lateral roots (Mosella-Chancel et al., 1979).

**Preventing desiccation of the graft**

Desiccation of graft or surfaces of the grafting partners is one of the major causes of graft union failure (Pliego and Murashige, 1987; George et al., 2008). To prevent this phenomenon, Pliego and Murashige (1987) applied a layer of moist nutrient agar gel to connect the grafting partners and obtained better graft success. Different chemicals have been tried to prevent graft desiccation so as to enhance the graft union. Rafail and Mosleh (2010) used an agar drop from the solidified culture medium and placed it on the cut area of the rootstock. Micrografts in which an agar drop was added to their grafted area were highly successful (70% in apple and 60% in pear) as compared to those without an agar drop (10%). Adding an agar drop usually prevents scion drying and makes the transport of different materials possible and holds the graft units together until the fusion takes place. Addition of agar drop supplemented with minerals and/or phytohormones further improved graft success. Amiri (2007) obtained 65% successful grafts in cherry using homoplastic grafting method (adding two drops of agar solution around the fitting site of micrograft) as compared to 41% through heteroplastic method (without application of agar drops).

**Pretreatment of shoot apex**

A technique which pretreats the apex allowing the selection of the viable apex and helping their development greatly improves the micrografting success. This is particularly effective when very small sized shoot apices are used. Excised apex is placed into a hemolyse tube on filter paper moistened by mineral solution of Murashige and Skoog (1962), supplemented with auxins and cytokinins. This treatment modified the physiological state of the excised apex and led to rapid development of leafy shoots even from smallest apices of 0.1 – 0.2 mm, the direct grafting of which is generally difficult and

ineffective (Jonard et al., 1983). Following proper development, the apex is micrografted on the rootstock. Mosella-Chancel et al. (1979) reported 64% successful micrografts in peach when pretreated with zeatin (0.1 mg/L) for 48 h as compared to 21.7% without any pretreatment.

### Suitability of rootstock

Micrograft success varies with the rootstocks because of the compatibility reactions between the grafting partners**.** Evaluating the rootstocks for higher graft success with a particular scion will definitely help in commercializing micrografting technique for mass multiplication of fruit crops. Tangolar et al. (2003) studied micrografting success in two cultivars of grape (Early Cardinal and Yalova incisi) when grafted on four different rootstocks (Dogridge, Salt Creek, 1613 C and 41 B) and reported different rates of graft success which varied from 26.1% (Yalova incise on 1613 C)) to 80% (Early Cardinal on 41 B).

## APPLICATIONS OF MICROGRAFTING

Micrografting has been used for the improvement and multiplication of various fruit crops and several papers have been published (Jonard et al., 1983; Jonard, 1986; Bhat et al., 2010). Some of the main applications of micrografting in fruit crops are discussed below:

### Virus and viroid elimination

The production of high-quality plants which can be certified genetically and virus-free is considered problematic and very challenging. An innovative technique of micrografting was developed by Murashige et al*.* (1972) for production of uniform virus-free plants on commercial scale in a controlled environment. They grafted small apical shoot of citrus to the top of a decapitated seedling grown *in vitro*. A few of these grafted plants, when indexed, were found freed of exocortis and stubborn pathogens. Navarro et al. (1975) improved the technique by testing various media formulations, different ways of placing the scion tip on the decapitated epicotyl, different rootstocks, light intensities, different sources and sizes of the scion shoot-tip and reported maximum survival of micrografted plants when transplanted from the test tube to soil. Roistacher et al. (1976) used the shoot tip grafting (STG) technique for production of virus-free planting material in various selections of citrus including sweet orange, mandarin, grapefruit, lemon, lime, citron and tangor. Each source plant was infected with 1 or 2 viruses including virus of tristeza (TV), seedling yellows-tristeza (SYTV), psorosis-A (PSV-A), concave gum (CGV), yellow vein (WV), infectious variegation (IVV), cachexia, and tatterleaf (TLV), citrus exocortis viroid (CEV) and Spiro*plasma* citri (stubborn). Virus indexing of micrografted plants was carried out over a 2-year period using different indicator plants for different viruses. Out of 33 different cultivars of citrus, they were able to develop virus-free mother block of 31 cultivars through shoot tip grafting.

Since then, micrografting technique has been widely used for elimination of viruses, phytoplasma, systemic pathogens in fruit crops and a large number of fruit plants have been made virus-free (Jonard et al., 1983; Burger, 1985; Navarro et al., 1976, 1980, 1982; Navarro and Juarez, 1977; Deogratias et al., 1986; Navarro, 1988; Jarausch et al., 2000; Zilka et al., 2002). *In vitro* grafting was used in Spain to produce virus-free plants of citrus and is considered a major factor in improving the Spanish citrus industry (Navarro et al., 1975). The technique has been used since 1998 for elimination of *Citrus psorosis virus* (CPsV), Citrus cachexia viroid (CCaVd), *Citrus exocortis viroid* (CEVd) and other related viroids in the local Arakapas mandarin of Cyprus (Kapari-Isaia et al., 2002). Hartl-Musinov et al. (2006) succeeded in elimination of *Citrus tristeza* virus (CTV) in Satsuma mandarin in Croatia. Abbas et al. (2008) succeeded in producing 91-95% *C. tristeza virus* (CTV) free plants of Kinnow mandarin (*Citrus reticulata)* and sweet orange (*Citrus sinensis*) cultivars through this technique.

Micrografting exploits two concepts- meristems are relatively virus-free and meristems from mature plants retain the mature phase. Meristematic tissues in the shoot tips and axillary buds normally remain virus-free because the growth of the meristem is quicker than the systemic spread of the virus within the plant. Using micro shoot tips (less than 0.5 mm) as scions, STG produces plants that are virus-free and reproductively mature. Production of virus-free plants from nucellar seedlings or by thermotherapy has certain limitations. Although nucellar seedlings of citrus are both clonal and virus-free, the seedlings are juvenile and take many years to flower. In the case of thermotherapy, many viruses and viroids, such as exocortis viroid and stubborn virus, are difficult to clean up with this process (Roistacher, 2004). Thermotherapy has failed to eliminate citrus exocortis viroid, yellow vein virus (YVV), cachexia virus and Dweet mottle virus (Calavan et al., 1972; Roistacher and Calavan, 1972). These problems have been overcome through STG technique. High temperatures inactivate many viruses, thus *in vitro* propagation can be used in combination with heat treatment to produce virus-free material. A massive project was launched in Morocco and Israel to develop virus-free plants of commercial almond cultivars through *in vitro* micrografting in combination with thermotherapy during 1997-2001. The project resulted in successful sanitation of almonds and permitted recovery of virus-free plants from various varieties infected with PNRSV (Prunus necrotic ringspot virus), PDV (prune

dwarf virus, CLSV (chlorotic leafspot virus). Thermotherapy treatment at 30- 35°C for 14 days was applied to *in vitro* shoot cultures prior to excising shoot tips for performing shoot tip grafting. Size of shoot apex had a paramount influence on elimination of virus from the plants. Singh et al. (2008) reported low recovery of ICRSV-free plants (20%) from an infected plant of kinnow mandarin with shoot tip size of 0.3 mm through STG which increased to 100% with shoot tip size of 0.2 mm. Manganaris et al. (2003) developed an efficient micrografting protocol for production of nectarine plants free from PPV and PNRSV. Conejero et al. (2013) sucessfully used micrografting in stone fruits for elimination of not only graft-transmissible viruses but also viroids, like PLMVd affecting *Prunus* species worldwide. Once bud is obtained, micrografted plants were placed in a cold chamber at 4°C and then forced for 15 days at 35°C. This resulted in the elimination of not only viroids but also viruses in higher percentage than the previous protocols. In short, micrografting is the only technique to purify the horticultural crops from viral diseases without the spray of harmful pesticides.

## Production of plants resistant to pests and diseases

Micrografting can be used as a means of elimination of pathogens in fruit crops. It has been successfully used in a wide range of horticultural plants as an effective method for the acquisition of plants resistant to soil borne pathogens. Grape phylloxera (*Daktulosphaira vitifoliae*) is considered as the most destructive insect pest of cultivated grapes worldwide, which feeds on the sap of grape roots, causing damage and often death of vines (Makee et al., 2004). An efficient and robust micrografting system was developed for production of phylloxera resistant plants in grapes by Kim et al. (2005) using pest resistant cultivars as rootstocks (Millardet et de Grasset 101-14, Couderc 3309, Rupestris du Lot and Kober 5 BB) and commercial favorable table grapes as scions (Kyoho, Campbell Early, Tamnara and Schuyler).

## Assessment of graft incompatibility

The inability of two different plants when grafted together to produce a successful union and also to develop satisfactorily into one composited plant is termed as graft incompatibility. Graft incompatibility in fruit trees has been classified by Mosse (1962) into translocated incompatibility and localized incompatibility. Translocated incompatibility is often associated with the movement of some labile factors between the grafting partners and is not overcome by the insertion of a mutually compatible inter-stock. An example of this category is the combination of 'Nonpareli' almond on 'Mariana 2624' plum. Localized incompatibility depends upon actual contact between stock and scion. Separation of the components by insertion of a mutually compatible inter-stock overcomes the incompatibility. Bartlett pear grafted directly on quince rootstock shows this type of incompatibility. Another example is grafting of certain apricot cultivars with peach which is associated with a clear break of the trunk at the point of graft following strong winds even after several years of normal growth (Jonard et al., 1990).

Prediction of incompatible graft combinations is a very important area of study for preventing economic loss due to graft incompatibility. Signs of graft incompatibility are often detected after several years in the field but can be identified early using micrografting and *in vitro* callus fusion technique (Jonard et al., 1990; Errea et al., 2001). Micrografting has been used for assessment of graft compatibility/incompatibility between the grafting partners (Burger, 1985; Navarro, 1988). The technique facilitates early diagnosis of grafting incompatibilities and may provide a model for in-depth analysis of the incompatibility phenomenon (Chimot-Schall et al., 1986; Jonard, 1986; Jonard et al., 1990; Hossein et al., 2008; Errea et al., 1994; Espen et al., 2005). It has been used for studying histological, histochemical and physiological aspects of graft incompatibility between scions and rootstocks (Richardson et al., 1996; Ermel, 1999). Histological examination of the graft union revealed callus formation, cytodifferentiation and xylogenesis leading to the formation of vascular connections in successful micrografts (Gebhardt and Goldbach, 1988). Anatomical studies of incompatible grafts demonstrated a poor vascular connection, vascular discontinuity and phloem degeneration at the union area, which might be detected as early as few weeks after a graft establishment (Darikova et al., 2011).

In the case of incompatible associations, Martinez et al. (1979, 1981) used *in vitro* micrografting to analyse localized incompatibilities of apricot/myrobalan and translocated incompatibilities of peach/apricot or peach/myrobalan. In the case of localized incompatibility, the percentage of success was very good during first three weeks but from the 14$^{th}$ day, signs of incompatibility appeared around the graft. After 60 days, all the grafts perish leaving no visible plants. Translocated incompatibility also called delayed incompatibility resulted in the development of whole plants *in vitro*, but the early symptoms of incompatibility still appeared on the young plants in pots 2 months after grafting (Martinez et al., 1981). During this experiment, 80% homografts of peach/peach and apricot/apricot provided viable plants. However, the percentage of surviving plants after 60 days was very low under incompatible combination of *Prunus persica*/ *Prunus armeniaca* (6.0%) and *Prunus persica* / *Prunus cerasifera* (1.25%). Though *in vitro* grafting techniques did not give viable plants but gave a prediction of incompatibility. Signs of this type of incompatibility often develop 5-10 years later after branch

grafts are made in the orchard (Rodgers and Beakbane, 1957).

Micrografting was used to study the compatible and incompatible combinations of grape varieties, using survival rate as an index. The higher survival rate of grafting (>85%) was achieved in compatible combinations of RizamatV/Baixiangjiao and Canepubu/Muscat Hamburg. Under incompatible combinations of Canepubu/Baixiangjiao and Carignane/Baixiangjiao, the survival rates were only 3.33 and 13.33%. Both translocated and localized incompatibilities exist in the Canepubu/Baixiangjiao, while Carignane/Baixiangjiao had only translocated incompatibility. At the late stage of grafting union, necrotic layer (isolation layer) of compatible combinations became thinner and finally disappeared, conducting tissue of rootstock-scion connected and the graft plants survived. To incompatible combinations, the necrotic layer always existed or disappeared partly, and the grafting failed, vascular disconnection contributes to the failure of grafting (JiLing, 2001).

## Improvement of plant regeneration

Micrografting provides an alternative production technique for mass multiplication of plants which are difficult to root (Preece et al., 1989) or propagation of difficult-to-root novel plants created in tissue cultures (Barros et al., 2005). This is done by micrografting micro shoots of difficult to-root plants/cultivars on seedling rootstocks grown *in vitro*. Micrografting has been used to rejuvenate cashew cultivars which were found difficult to root (Thimmappaiah et al., 2002). The technique has been successfully used to multiply difficult to root plants including walnut (Pei et al., 1998; Wang et al., 2010), pistachio (Onay et al., 2007; Abousalim and Mantell, 1992) cashew (Ramanayake and Kovoor, 1999) and almond (Martinez-gomez and Gradziel, 2001; Ghorbel et al., 1998; Channuntapipat et al., 2003).

## Mass multiplication

Micrografting is a technique that potentially can combine the advantages of rapid *in vitro* multiplication with the increased productivity that results from grafting superior rootstock and scion combinations (Gebhardt and Goldbach, 1988). Mass production of superior plants through micrografting can be achieved throughout the year under controlled conditions in the tissue culture laboratories, by grafting elite scions onto desirable rootstocks. Generally, micro-propagation of woody trees is difficult due to low regeneration capacity, especially mature plant tissues. A major limitation is root regeneration rather than shoot multiplication (Hartmann et al., 2002). *In vitro* micro-grafting is often used where rooting capacity of micro-cuttings is poor. It has been used in the propagation of novel plants created in tissue cultures through transgenic or of novel plants created in tissue cultures that are difficult-to-root (Barros et al., 2005). Genetically transformed shoots of Avocado from somatic embryos were rescued by micrografting them onto the *in vitro* germinated rootstock seedlings with 70% success (Simon and Richard, 2003).

## Indexing viral diseases

Grafting is used to determine the presence of latent (unseen) viral diseases in plants. A plant (the indicator plant) that is known to be susceptible to the disease of interest may be grafted onto the suspect plant. If the plant is in question is infected, typical symptoms induced by the specific virus are expressed on the indicators after the virus has been moved into the indicator plants. This type of testing is regularly carried out on plants imported to the country including grapes and roses. This test does not require the formation of a permanent, compatible graft union. Tanne et al. (1993) used micrografting system which increased the speed of viral detection. They reported the detection of corky-bark virus 8–12 weeks after grafting. Pathirana and McKenzie (2005) reported that micrografting of leaf roll infected scion material on to virus-free indicator rootstock (*Cabernet sauvignon*) resulted in the development of symptoms within 2-3 week. Valat et al. (2003) demonstrated that grapevine fan leaf virus is transmitted from infected rootstock to the uninfected indicator 41B variety used as scion within 45 days. Kapari-Isaia et al. (2002) used Madam Vinous or pineapple, sweet orange as indicator plants for indexing of CPsV in Local Arakapas Mandarin in Cyprus. This type of microindexing can be used for post-entry quarantine of imported materials (Sivapalan et al., 2001; MAF, 2004).

## Safe germplasm exchange

Small micrografted trees are a convenient way to exchange germplasm between countries (Navarro et al., 1975). The exchange of fruit tree propagation material between countries is a major cause of spread of new pests and pathogens, particularly graft-transmissible viruses and viroids. The expansion of Prunus breeding worldwide, mainly in peach, has resulted in more than 20 new breeding programs producing hundreds of new varieties yearly. The associated exchange of plant material has increased notably the risk of introduction of new pathogens and pests (Llacer, 2009; Llacer et al., 2009). Imports of fruit budwood lacking effective phytosanitary control measures present the highest risks. More than 100 virus or virus-like diseases have been reported to affect Prunus species worldwide. For approxi-

mately half of these diseases, nothing is known about the causal agent except that it is graft-transmissible (Cambra et al., 2008). Moreover, traditional quarantine procedures are often ineffective, prompting the search for alternative procedures including those based on tissue culture techniques. An improved STG procedure based on the protocol described by Navarro et al. (1982) which is effective for virus and viroids elimination is a prerequisite for safe peach and Japanese plum budwood exchange (Conejero et al., 2013). It is a minimum risk method for importing plant material through quarantine.

## CONCLUSION

Micrografting has great potential for improvement of fruit plants and has been used for the production of virus and viroid-free plants in horticultural crops without the application of harmful pesticides. Besides, it has also been used in prediction of incompatibility between the grafting partners, histological studies, virus indexing, production of disease-free plants particularly resistant to soil borne pathogens, safe germplasm exchange between countries and multiplication of difficult to root plants. It is a safe *in vitro* technique, which can be utilized for commercial production of virus-free grafted plants with desired cultivars and suitable rootstock throughout the year under controlled conditions.

## Conflict of Interests

The author(s) have not declared any conflict of interests.

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