Journal of Horticulture
ISSN: 2376-0354

Rehman and Gill, J Horticulture 2015, 2:3
DOI: 10.4172/2376-0354.1000151

Research Article Open Access

Micrografting of Fruit Crops-A Review

Rehman HU* **and Gill MIS**
Department of Fruit Science, PAU, Ludhiana-141004, India

***Corresponding author:** Rehman HU, Department of Fruit Science, PAU, Ludhiana-141004, India, Tel: 9996457556; E-mail: haseebpom@gmail.com

Rec date: Apr 17, 2015; **Acc date:** Jul 20, 2015; **Pub date:** Jul 23, 2015

Abstract

Micrografting was developed in 1980s and consists of the placement in aseptic conditions of a maintained scion onto an *in vitro* grown rootstock. The results of *in vitro* micrografting and the plant material derived from it can be further multiplied in tissue culture conditions or acclimatized to outdoor conditions. Micrografting is a technique that potentially can combine the advantages of rapid *in vitro* multiplication with increased productivity that results from grafting, superior rootstock and scion combinations. Among various methods of micrografting, slit or wedge grafting has been found most suitable in case of fruit crops. *In vitro* shoot tips are better as compared to *in vivo* shoot tips for carrying out micrografting resulting in higher graft success, less contamination, less vitrification, lower shoot tip necrosis and good vigour of micrografts. Media used for growing micrografted plantlets also affects grafting success, vitrification, contamination and vigour. Use of appropriate medium depends on genotype and growing conditions. Modification of basal medium formulation has been an effective means for achieving good graft success. The sucrose concentration of the nutrient medium of grafted plants has been found to play a significant role. Besides these, scion length and rootstock development stage also influences graft success, vitrification, shoot tip necrosis, vigour and contamination. The use of pre-treated shoot apices not only markedly increases the grafting success but also overcome problems encountered during the handling of *in vitro* micrografting that results in browning and drying of apices.

Keywords: Micrografting; *In vitro; In vivo;* Scion, Graft success; Vitrification; Vigour; Scion length; Shoot tip necrosis

Introduction

The necessity to modernize the planting material production technologies of fruit crops has been stimulated by many considerations such as the trends towards increasing the planting densities in field grown trees and the transition to intensive growing systems, which includes the selection of new parents for breeding programmes, the creation and introduction of new cultivars and the modernization of tree habit and pruning. All of these changes have created a demand for more and more quantities of quality planting material. The conventional system of propagating this material is not only time consuming but the material raised is neither uniform nor healthy. Application of *in vitro* techniques in fruit growing can, therefore, be a viable alternative to circumvent these problems. One such application is shoot tip grafting or micrografting. Micrografting was developed in 1980s and consists of the placement of meristem tip or shoots tip explant onto a decapitated rootstock that has been grown aseptically from seed or micropropagated [1]. The results of *in vitro* micrografting and the plant material derived from it can be further multiplied in tissue culture conditions or acclimatized to outdoor conditions. Micrografting is a technique that potentially can combine the advantages of rapid *in vitro* multiplication with increased productivity that results from grafting, superior rootstock and scion combinations [2]. The juvenile phase can be circumvented by micrografting to a rootstock that induces early maturity. Besides to the benefits of traditional grafting, micrografting shoot tips can be an efficient means of regenerating plant material free of endogenous contaminants [3] with enhanced potential for true to type cloning mature plants [4]. Micrografting technique appears to be useful in early screening of graft compatibility in fruit crops as the overlapping of different stress response induced by the graft itself at the interphase between the partners makes identification of the mechanism underlying localized incompatibility difficult [5]. Micrografting has application for physiological analysis of the rejuvenation of mature phase plants [6]. It is possible to graft at any time of the year and to transplant the grafted plants when desired [7].

Grafting under *in vitro* conditions has several advantages both for production and research. *In vitro* shoot tip grafting has often been applied for the (i) improvement and rejuvenation of several tree species [8,9] (ii) virus elimination [10,11] (iii) study of physiological connections between rootstocks and scions such as (in)compatibility, root to shoot communication or transport [12,13] and (iv) use in quarantine as this method has a minimum risk for importing plants [14]. Due to the multiple uses and advantages of shoot tip grafting, this technology may be of interest or of potential practical value to technicians, researchers and nursery operators. Professionals in each of these areas would benefit from the introduction of a simpler and more efficient micrografting procedure that is less dependent on mastery of complex techniques and thus will contribute to the practical utility of micrografting as a tool in fruit tree biotechnology.

Review of Literature

The literature relevant to micrografting in fruit crops is reviewed under the following headings.

Scion origin

The origin of the scion can greatly affect the micrografting success. Deogratias et al. [15] studied *in vitro* micrografting of apricots by using actively growing or dormant shoot tips (0.5-1.0 mm) from

different sources: (a) dormant buds collected from November to February (b) vegetative flushes from plants growing in the field (c) *in vitro* derived vegetative shoots. The effects of rootstock cultivar, position of the shoot tip on the rootstock, shoot tip size, light and temperature, composition of the medium and growth regulator treatments on the success of grafting on rootstock seedlings growing *in vitro* were studied. The best source of shoot tips was found to be *in vitro* derived micro shoots while, dormant buds collected in November proved inferior one. Many of the grafts became quiescent after initial growth.

Hassanen [16] also reported the highest grafting success while using *in vitro* scion source and obtained grafting success of 83 per cent by using *in vitro* scion (>0.5 cm but <1 cm) of Le Conte pear on *in vitro* decapitated *P betulaefolia* as rootstock.

Bhatt [17] also reported that the best source of shoot tips was from *in vitro* derived shoots and the poorest source was from *in vivo* forced shoot tips in apple. Higher contaminated cultures (39.04%), browning/ necrotic cultures (57.15%) and browning/ necrosis intensity (3.38) was observed when scions derived from *in vivo* forced tips were used for grafting which decreased to 10.80 per cent, 39.06 per cent and 2.20, respectively when *in vitro* derived scion tips were used.

Dziedzic and Malodobry [18] carried out micrografting using cherry cultivar Regina on rootstocks Damil and Gisela 5 and cv. Van on rootstock Damil. Shoot tip explants of Regina and Van were 0.2-0.3 and 0.4-0.5 cm in length, respectively. Different pre-treatments of scions and stocks, ways of grafting and light conditions after grafting influenced the graft success. Before grafting, the upper part of the rootstocks and the lower end of scions were dipped for one minute in different antioxidant and growth regulator combinations. There was no browning of scion and stocks cut surface following these treatment combinations. Treatment combination of 150 mgl^{-1} citric acid+0.1 mgl^{-1} GA3 +0.5 mgl^{-1} IBA resulted in higher graft success.

Baydar and Celik [19] used *in vitro* micrografting technique in grapes. Shoot tip meristems of KalecikKarasi, Emir, Uslu, Hafizali and Razaki grape cultivars were micrografted onto Kober 5BB seedlings. Shoot tip meristems were obtained from *in vivo* (shoots from actively growing vines in the vineyard or shoots from one-year-old cuttings in the greenhouse) or *in vitro* (shoots obtained by shoot tip culture in aseptic conditions) sources. The micrografted plantlets were cultured on solid MS medium for 2 months. The micrografting success rate was highest using *in vitro* shoot tips, varying from 40.6 per cent in cv. Hafizali to 68.3 per cent in cv. Emir.

Thimmappaiah et al. [20] performed *in vitro* grafting of almond cv. Achak on aseptic rootstocks in order to estimate the success of micrografting in relation to the mode of grafting, the scion origin and the physiological state of the grafts. Both active and dormant small shoots, buds and meristematic apices were collected from field plants. In this study, 4-6 week old shoots and small apical buds (3.0-5.0 mm) formed *in vitro* were also used. These shoots and buds grafted on top or in T-grafts gave a higher percentage (82.1% and 79.2%) respectively of successful grafts. Contamination occurred more often in material taken from the field and necrosis was observed frequently when physiologically active meristematic apices were used. Similarly, Onay et al. [21] reported that the best growth of microscion in Pistachio was obtained with the *in vitro* shoot tips rather than with shoot tips excised from a field grown tree.

Sanjaya et al. [22] were successful in achieving *in vitro* micrografts of Sandal wood (*Santalum album L.*) by placing 1-2 cm long scions derived from nodal segments onto the hypocotyls of 45 days old *in vitro* rootstocks. Use of *in vitro* grown shoots as a source of scion gave better graft success (60%) than scions collected directly from field grown trees. *In vitro* grafting was also influenced by scion size and root age.

Scion length

Hassanen [16] reported successful micrografting of pear cv. Le Conte on *in vitro* decapitated P betulaefolia as rootstock. Shoot tips consisted of two different sizes, <0.5 cm long and >0.5 cm but <1 cm long were examined. The highest percentage of successful grafts, axillary shoots development percentage, micrograft length increase and number of new buds formation (83%, 80%, 3.5 cm and 4 cm, respectively) were obtained with scion length greater than 0.5 cm, while the scion of smaller than 0.5 cm was achieved 37% of successful grafting.

Rehman and Gill [23] also reported maximum graft success using 5-10 mm scions as compared to <5 mm and 10-15 mm in case of Patharnakh pear when grafted on Kainth rootstock. Besides, graft success per cent, they also reported that scion length have significant influence on necrosis and vigour of micrografts.

It is necessary to choose a shoot tip of size that gives a realistic degree of grafting success. Onay et al. [21] carried out *in vitro* grafting in Pistachio and studied different variables including different size of microscion, grafting methods, effect of culture medium and effect of time of the year at which shoot tips were used. The results indicate that the easiest and most successful method for grafting was slit micrografting. Higher levels of micrograft take were achieved with 2-4 mm (56.76%) and 4-6 mm (79.25%) long scions obtained from the regenerated shoot tips.

Bhatt [17], while working on micrografting of Lal Ambri apple on M-9 rootstock, observed that graft success increased with the size of scions up to 10 mm, then decreased with increase in size. Graft success of 12.92 per cent was obtained when scion tips of 10 mm were used for grafting which decreased to 10.53 per cent and 8.94 per cent when scion tips of 5 mm and 15 mm were used, respectively.

Amiri [24] studied the effect of grafting method, scion size and its origin on grafting success. Grafting success was significantly dependent on the method of grafting and size of the scion. One month old *in vitro* cultured meristematic apices with length of 5-15 mm were used as microscion. The highest percentage (65%) of successful grafts was obtained for homoplastic apex graft (shoot tip), with apical bud scion length greater than 6 mm. Whereas, the lowest percentage (16%) of success was observed in the heteroplastic side bud apices (wedge) grafts. As the size of the microscion increased (from 3 to 15 mm), the success rate of the grafts improved and reached 34 to 65 per cent. Khalafalla and Daffalla [25] noticed that rate of successfully grafted shoots in Gum Arabic tree was influenced by both scion length and rootstock age. Success rate was 100 per cent with scion length of 3.0 cm and rootstock of 14 days.

Thimmappaiah et al. [20] observed that length of scion shoot had significant effect on micrografting success. Graft success was higher (79.5%) when the scion length was greater than 5 mm and it was lesser (0.5%) when size of scion was smaller (3-5 mm) in cashew.

Rootstock age

Deogratias et al. [26] carried investigations to recover virus free Prunus plants by *in vitro* shoot tip grafting and reported that rootstock age is involved in the success of micrografting. The optimal period to produce meristem resumption is between 7 to 10 days. Earlier than 7 days, rootstock callus invaded the meristem tips and after 10 days, rapid desiccation of rootstock section and scion necrosis occurred.

Dong et al. [27] carried experiments to examine the relationship between the physiological state of the rootstock/scion and grafting success. He revealed that fast-growing tender shoots of walnut had high levels of cytokinin, auxin, glucose and laevulose and formed strong graft unions than older and longer shoots.

Sanjaya et al. [22] carried micrografting of *Santalum album* using *in vitro* and *in vivo* methods that consisted of grafting under aseptic conditions of the miniaturized scion onto a grown rootstock; was conducted and compared the success of the two methods. The frequency of success in shoot apex grafting of *Santalum album* was highest with rootstock and shoots derived from *in vitro* method. The probability of successful graft unions of both *in vitro* and *in vivo* methods increased with increase in length of scion material. However, the success of grafting declined as the age of the seedlings increased, since it was observed that the hypocotyls region became narrower and harder with age. Khafalla and Daffalla [25] observed that the rate of successfully grafted shoots in gum Arabic tree (*Acacia senegal*) was influenced by both scion length and rootstock age. Fourteen days old rootstock was found to be more efficient than seven day old one.

Media used

Modification of basal medium formulation has been an effective means for achieving graft success. The sucrose concentration of the nutrient medium of grafted plants has been found to play a significant role.

Hosoi et al. [28] reported that sucrose concentration in the liquid medium significantly affected the percentage success in citrus and best results were obtained with 45 gl^{-1} sucrose. Gebhardt and Goldbach [2] described micrografting technique of Prunus shoots derived by shoot tip culture. Autografts of plum cv. Hauszwetsche as well as heterografts of several sour cherry cultivars (Schattenmorelle, Weiroot 158 and Koroser) were established. A mechanically strong graft union was formed during the course of a three week subculture of micrografts in a liquid medium without the addition of growth regulators. Deogratias et al. [15] obtained best results in micrografting of apricot when cultured in medium comprising MS salts with 3 per cent sucrose.

Bhatt et al. [29] while investigating effect of different media formulations on graft success reported highest micrografting success rate using MS semisolid media followed by MS liquid media and MS liquid plus vermiculite media. Higher graft success (24.55% and 21.89%) was obtained when micrografts were cultured on MS semi-solid media at 3 and 6 per cent sucrose, respectively.

Rehman and Gill [23] reported that medium composition and its phase has significant effect on graft success, necrosis, vigour and vitrification of Patharnakh micrografts. Maximum graft success was observed on Murashige and Skoog liquid medium containing 20 g/l sucrose.

Abousalim and Mantell [30] carried out study on micrografting in Pistachio cv. Mateur, on the rootstock of the same cultivar. The scion bases of cv. Mateur were cut into gently sloping wedges and were inserted into vertical splits in the cut surface of the epicotyls. Micrografted seedlings were cultured on liquid MS medium using a filter paper bridge. In second experiment, surface disinfected seeds were cultured directly on top of a Milcap-synthetic (polypropylene fibre) support and after 12 days, the seedlings were micrografted in situ with scions. The best results were obtained using the later method and vascular connections were established across grafts within 21 days. Axillary shoots were produced by 60 per cent of the scions. The Milcap -polypropylene supports allowed good growth and branching of root system and prevented root damage during grafting.

Grewal et al. [31] successfully cultured shoot tip grafted plants of various cultivars of citrus in a liquid medium containing the salts of MS medium, the vitamins of White's medium and sucrose (7.5%). Jonard [6] micrografted several grape cultivars onto Kober 5BB seedlings. These micrografted plantlets were cultured on Murashige and Skoog (MS) medium for two months. Banerjee et al. [32] developed an efficient and highly reproducible *in vitro* micrografting protocol for microshoots of cotton (*Gossypiumhirsutum L.*). Culture of grafted shoots in Murashige and Skoog's basal liquid medium devoid of growth regulators showed maximum rate of survival (95%), elongation of scions and number of nodes per plant

Thimmappaiah et al. [20] developed a successful micrografting technique in cashew using *in vitro* germinated seedlings as rootstocks and axenic shoot cultures (shoot tip and nodal cultures) established from mature tree as microscions. *In vitro* germinated seedlings which emerged 20-25 days after inoculation on absorbent cotton were decapitated and used as rootstock. Mature tree explants initiated on hormone-free [33] modified medium were prepared as scions of 3-15 mm length for grafting. Micrografts were successfully cultured on hormone-free liquid half MS medium and potted out after 10-12 weeks of culture growth.

Dziedzic et al. [33] carried out micrografting of cv. Regina on Gisela 5 rootstock on two types of MS media prepared as liquid and semi-solid with 0.7 per cent agar. High per cent of successful micrografts were achieved on semi-solid MS medium. Higher contaminated cultures were observed in liquid media. However, vigorous plantlets developed in this liquid media. Naz et al. [34] carried study to assess the potential use and applicability of micrografting technique for the development of virus free nursery in citrus. Some techniques that tend to increase the grafting success were employed. MS medium fortified with 3, 5 and 7 per cent sugar was used in combination with two grafting methods viz. inverted-T incision and surface placement in Kinnow mandarin and Succari sweet orange. Grafting was carried out under aseptic conditions by using 15 day old etiolated seedlings of rough lemon as rootstock. Shoot tips (1-2 mm) and 3 leaf primordia (0.3-0.5 mm) were taken from the fresh shoot flushes and grafted *in vitro*. Higher grafting success of 34.7 per cent was recorded with inverted-T incision than surface placement which gave 26.7 per cent successful micrografts. A total of 21 per cent successful micrografts were achieved at 3 per cent sugar level which increased significantly to 33 per cent with increase in sugar level to 5 per cent in both cultivars.

Graft method

The method of placement of the excised shoot tip onto the rootstock significantly influences the grafting success. Various

methods of placement of scion tip onto rootstock have been described. Abreu et al. [35] studied micrografting of tissue cultured apple plants. Scions were cleft-grafted on the rootstocks of Maluspumila and Malusprunifolia. Graft development resulted in the generation of meristem tissue with parenchymatous cells originating at the graft interface and cambial tissue proliferation from the scion. This allowed a vascular system connection between the scion and rootstock, resulting in micrograft viability.

Bhatt et al. [17] and Bhatt et al. [29] observed that vertical slit grafting as best method for carrying out micrografting in apple. He reported highest graft success (23.83%) and lowest browning/ necrotic cultures (29.33%) were obtained with vertical slit method. The graft success decreased to 21.00 per cent and 13.10 per cent and browning/ necrosis incidence per cent increased to 34.68 per cent and 40.36 per cent when scion was grafted by wedge and horizontal placement, respectively.

Rehman and Gill et al. [23] observed wedge grafting to be better than horizontal grafting in terms of graft success, incidence of necrosis and vitrification. Graft success reached upto 33.71% when Patharnakh scions of 5-10 mm were micrografted using wedge grafting technique allowed to grow in Murashige and Skoog liquid medium. Bhatt et al. [32] reported the highest vigorous plantlets resulted by carrying vertical slit grafting techniques compared to wedge and horizontal grafting.

Onay et al. [21] studied the influence of different grafting methods on graft success in Pistachio. He observed that the slit method was easiest and most successful one giving success of 80 per cent whereas 60 per cent graft success was obtained under wedge micrografting. In vertical slit micrografting no problem was observed during the union formation as long as the contact surfaces were perfectly smooth.

Dobranszki et al. [36] described the requirements for *in vitro* micrografting in apple cv. Royal Gala which served as source of both rootstock and scion. Oxidative browning of cut surface was inhibited by the use of antioxidant mixture during grafting process. Scion base of Royal Gala cut in V-shape was dipped in 1.0 per cent agar-agar solution and stuck into the vertical slit of rootstock. There was no displacement and the rate of fused and further developed grafts were 95 per cent.

Naz et al. [34] investigated the applicability of micrografting technique for the development of virus free nursery in citrus and reported that success in micrografting varies with the method and plant species or genotype. Graft success of 34.7 per cent was recorded with inverted-T incision whereas; surface placement gave 26.7 per cent successful micrografts. Succari sweet orange responded maximum with surface placement method whereas, Kinnow mandarin showed better response with inverted T incision [37].

Toth et al. [38] studied the efficiency of *in vitro* grafting method of six plant species-pepper, melon, apricot, peach, tobacco and chrysanthemum. Plants with short roots were removed from the media and the stem of the scion and the stock (at the four to five leaf stage) were cut at right angles, each with 2-3 leaves remaining on the stem. The stem of the scion was cut in a wedge with scalpel in Petri-dish and the tapered end was fitted into a cleft made in the cut end of stock. The graft was firmed with silicon rubber clip hauled up with dentist's forceps. The survival rate of the grafted plants was excellent in case of herbaceous plants (90-100%) whereas it was 30 per cent for woody species.

Dziedzic and Malodobry [18] carried out micrografting using cherry cv. Van and Regina on Damil rootstock using different methods of grafting. The wedge shaped end of scion 'Van' was inserted into 0.5-0.6 cm slit in rootstock Damil, whereas, even end of shoot tip of Regina was placed on Damil rootstock. The grafts were placed individually into test tubes on WPM medium, with 30 gl^{-1} sucrose and agar 7gl^{-1}. High per cent of successful grafts were obtained for cleft grafting method than shoot tip grafting. The scions and stocks were attached to each other closely and the per cent of graft unit displacement was low.

Wu et al. [39] developed shoot tip micrografting technique in Proteacynaroides wherein, seedlings were decapitated and a vertical incision made from the top end. The axenic microshoots were cut into a wedge (V) shape and placed them into the vertical incision of the rootstock. Best results were obtained by placing the microscions directly onto the rootstocks without any pre-treatment. Hassanen et al. [16] also reported that grafting success was significantly influenced by grafting method employed during micrograting of pear cv. Le Conte on *P betulaefolia*.

Vitrification

Micropropagation of woody plants is often hampered by the phenomenon of hyperhydricity or vitrification [40]. Hyperhydricity, which is characterized by a glassy or swollen appearance to the tissue, usually results in reduced multiplication rates, poor quality shoots and tissue necrosis [41]. The so-called vitrified, vitreous or hyperhydric shoots appear turgid, watery at their surface and hypolignified. Their organs are translucent and in some cases less green and easily breakable.

Occurrence of hyperhydricity remains unpredictable and many factors appear to induce hyperhydricity [42]. Stress is believed to be the major factor underlying the phenomenon. Stress during *in vitro* culture could arise as a result of wounding, the properties of the tissue culture medium, infiltration of tissue by the culture medium which is generally of a higher ionic strength and rich in nitrogen or environmental conditions during *in vitro* culture in sealed containers [42]. This stress could mediate a rapid endogenous ethylene burst. One of the reasons of poor success rate in micrografting is vitrification.

Bhatt [17] reported that media formulations significantly influences vitrification percentage of apple. Lowest vitrified cultures (4.94%) were obtained in MS semi- solid media with 6 per cent sucrose which increased non-significantly to (6.29%) in the same media but with 3 per cent sucrose. Highest vitrified cultures (16.32% and 12.58%) were observed in liquid medium at 3 per cent and 6 per cent, sucrose respectively. MS liquid medium plus vermiculite also showed significantly higher vitrified cultures than obtained in MS semi solid media.

Amiri [24] reported that initial percentage of successful grafts of cherry (Prunusavium L.) var. Seeyane Mashad was about 75 per cent which decreased at the end of experiment. The poor success rate was mainly due to excessive collogenesis observed on more than 50 per cent of the apices and vitrification on more than 30 per cent of the explants.

Al-Maarri and Al-Ghamdi [43] noticed that the frequency of vitrification increased on medium containing a low concentration of sucrose. Ghorbel et al. [37] studied *in vitro* grafting of almond cv. Achak performed on asceptic roots in order to estimate the success of micrografting in relation to the mode of grafting, the scion origin and

physiological state of grafts. He achieved low success rates due to vitrification in more than 70 per cent of explants. Dziedzic [33] carried out micrograting experiments in cherry cv. Regina on Gisela-5 rootstock on two types of Murashige and Skoog medium: liquid medium and semi-solid agar medium. High per cent of successful micrografts were obtained on MS semi-solid medium. Use of liquid media resulted in maximum vitrified plantlets.

Pretreatment of growth regulators

The success of micrografting to obtain disease free plant material depends on various factors; among the many other factors pre-treatment with growth regulators have resulted in speeding up the healing of tissue at graft union, resulting in higher survival of grafts. The use of pre-treated apices not only markedly increases the grafting success but also overcome problems encountered during the handling of *in vitro* micrografting that results in browning and drying of apices. Together with auxins, cytokinins stimulate both apex initiation and the union between rootstock and scion by increasing vascular bundle formation [44]. Cytokinins rejuvenate plant cells and stimulate cell proliferation of graft union tissue with auxins [45].

Bhatt [17] reported treatment of LalAmbrimicroscions with combination of Kinetin and 2,4-D at 0.5 mgl^{-1} and 5 mgl^{-1}, respectively before grafting on M-9 showed highest graft success (42.25%) and lowest browning/ necrosis incidence (19.00%) and browning/necrosis intensity (0.20).

Starrantino et al. [46] reported that best results in citrus were obtained when both components of graft were pre-treated with 0.5 ppm BA, 0.5 ppm Kinetin or 10 ppm 2, 4-D. Lemons gave the highest percentage of successful grafts (88-92%) and clementines the lowest (63-73 per cent).

Edriss and Burger [47] studied the various factors affecting the success of shoot tip grafting. Growth regulator pre-treatment of scions with 2, 4-D and Kinetin was accomplished by dipping the shoot apex of 'Mexican' lime, 'Valencia' orange and 'Star Ruby' grape fruit in growth regulator solutions just prior to excision of the apical dome and primordial leaves. Dipping the shoot tip in 2, 4-D (10 mgl^{-1}) or Kinetin (1 mgl^{-1}) before grafting nearly doubled the success rate compared to control. High concentration of Kinetin (10 mgl^{-1}) and/or low concentration of 2, 4-D (1 mgl^{-1}) were no more effective than the control. There were no differences among the 3 rootstocks in their response to pre-treatments.

Jonard et al. [48] reported that inserting an agar block containing different plant growth regulators resulted in 100 per cent successful *in vitro* grafts of Eureka lemon on Troyer Citrange. Apex pre-treatment on [35] medium plus 3 per cent sucrose with GA3 at 2.9 m moles/litre, BA at 44 mmoles/litre and 2, 4-D at 0.009 mmoles/litre.

Starrantino and Caruso [46] carried out micrografting experiments in citrus species with several growth substances and observed that dipping the apex and the decapitated seedlings for 10 minutes before micrograting in a solution of BA (0.5 ppm) increased the percentage rate of sprouting from 73 to 91 per cent.

Parthasarathy et al. [49] conducted experiments to standardize procedures for *in vitro* grafting of Khasi mandarin on various rootstocks. The immersion of Khasi mandarin shoot tips in 2,4-D (10 μgl^{-1}) for five minutes before grafting improved the grafting success on Cleoptara mandarin, Rangpur lime, Kagzi lime and Khashi mandarin. Histological studies using a scanning electron microscope showed callus development between rootstock and scion. Good graft union and complete vascular connection between the shoot tips and the rootstocks were achieved.

Kumarin et al. [50] reported the influence of pre-treatments of growth regulators on the success of graft take in Nagpur mandarin cultivar on rough lemon rootstock. Just prior to grafting, both rootstocks and scions were dipped in growth regulator solutions for 10 minutes. The solutions tested were BA (0.5, 1.0, 2.0 and 5.0 mgl^{-1}), Kinetin (0.5, 1.0, 2.0 and 5.0 mgl^{-1}) and 2,4-D (1.0, 2.0, 5.0 and 10.0 mgl^{-1}). Grafting success was highest with Kinetin (1.0 mgl^{-1}) or 2,4-D (10.0 mgl^{-1}).

Nunes et al. [51] studied the micrografting technique in apple by using *in vitro* plants and verifying the influence of application of growth regulators IBA and BAP in Gala/M-9 and Gala/Marubakaido as scion rootstock combinations. The percentage of viable micrografts for the connection of vascular tissues, varied from 73 to 93.2 per cent. The percentage of viability observed highest in Gala/M-9 (93.2%) on ½ MS medium supplemented with 2.2 mM IBA, followed by ½ MS medium pre-treatment with IBA (4 mM) in both combinations showed (86.4%) viability. Results showed that treatment with growth regulators at the point of grafting significantly increased the viability of the graft.

Conclusion

Micrografting is a technique that potentially can combine the advantages of rapid *in vitro* multiplication with increased productivity that results from grafting, superior rootstock and scion combinations. Graft success has been reported to be influenced by large number of factor individually and as well as in interactive manner such as scion length, scion origin, graft method, media used and rootstock development stage. Grafting under *in vitro* conditions has several advantages both for production and research. *In vitro* shoot tip grafting has often been applied for the improvement and rejuvenation of several fruit tree species, virus elimination, study of physiological connections between rootstocks and scions such as (in) compatibility root to shoot communication or transport and use in quarantine as this method has a minimum risk for importing plants. Micrografting has application for physiological analysis of the rejuvenation of mature phase plants. It is possible to graft at any time of the year and to transplant the grafted plants when desired. Due to the multiple uses and advantages of micrografting, this technology may be of interest or of potential practical value to technicians, researchers and nursery operators.

References

1. Gebhardt K, Goldbach H (1988) Establishment, graft union characteristics and growth of Prunusmicrografts. Physio Plant 72: 153-159.
2. Zilka SE, Faingersh E, Rotbaum A, Tam Y, Spiegel S, et al. (2002) *In vitro* production of virus free pear plants. Acta Hort 596: 477-479.
3. Franclet A (1983) Rejuvenation : theory and practical experiences in clonal silviculture. In :Clonal Forests : Its Impact on Tree Improvement and Our Future Forests (Eds. L. Suffa, R.L. Rauter, W.W. Yeatman). Toronto: Canadian Tree Improvement Association, pp 96-134.
4. Pirovano L, Sacchi GA, Aburuzzese A, Cocucci M (2002) Study on gene expression in compatible and incompatible pear/pear and pear/quince grafts by mRNA differential display. Acta Hort 596: 393-395.

5. Jonard R (1986) Micrografting and its application to tree improvement. In: Biotechnology in Agriculture and Forestry (Ed) YPS Bajaj. Trees I-Springer-Verlag, Berlin, Heidelberg, New York.
6. Musacchi S, Ganino T, Grani S, Fabbri A (2004) Grafting *in vitro* shoots on acclimating plant : Morphological and anatomical aspects of compatible and incompatible pear/quince combinations. Acta Hort 658: 559-63.
7. Parthasarathy VA, Nagaraju V, Rahman SAS (1997) *In vitro* grafting of Citrus reticulate Blanco. Folia Hort 9: 87-90.
8. Canon C, Mehmet O, Hakan T, Kamil S, Elman I (2006) *In vitro* micrografting of pistachio (Pistaciavera L.) var. Siirt on wild pistachio rootstocks. Journal of Cell and Molecular Biology 5: 25-31.
9. Bandeira SF, Xavier A, Otoni WC, Days JMM (2006) *In vitro* grafting in the propagation of Eucalyptus urophylla x Eucalyptus grandis clones. Agropecuaira research Brazilian 41: 223-232.
10. Bisognin C, Ciccotti AM, Moser M, Grando MS, Jarausch W (2008) Establishment of an *in vitro* screening system of apple proliferation–resistant rootstock genotypes based on micrografting. Acta Hort 781: 375-380.
11. Ribeiro LM, Peixoto JR, Andrade SRM, Fonseca RS, Vieira LM, et al. (2008) Ex vitro micrografting aiming the CABMV virus elimination in passion fruit plant. Pesquisa. AgropecuariaBrasileira 43: 589-594.
12. Nelson T (2004) Plant signalling: notes from the underground. Current Biology 14: 929-930.
13. Bortolotti C, Murillo L, Fontanet P, Coca M, Segundo BS (2005) Long-distance transport of the maize pathogenesis-related PRms protein through the phloem in transgenic tobacco plants. Plant Science 168: 813-821.
14. George EF (1993) Micrografting. In: George, E.F. (ed.). Plant propagation by tissue culture. Part-1. Exegetics Ltd. Edington Wilts., UK, pp. 64-66.
15. Deogratias JM, Castellani V, Dosba F, Jaurez, J, Arregui JM, et al. (1991) Study of growth parameters on apricot shoot-tip grafting *in vitro* (STG). ActaHorticulturae 293: 363-371.
16. Hassanen SA (2013) *In vitro* grafting of pear (Pyrus spp.) World Applied Sciences Journal 21: 705-709.
17. Bhatt KM (2008) *In vitro* micrografting of apple (Malusdomestica Borkh.) cv. LalAmbri on dwarfing M-9 rootstock. Ph.D thesis submitted to Sher-e-Kashmir University of Agricultural Sciences and Technology, Shalimar, Srinagar, J&K.
18. Dziedzic E, Malodobry M (2006) Vegetative cherry rootstocks in tissue culture. SodininkysteIr Darzininkyste 25: 77-84.
19. Baydar N G and Celik H (1999) The effect of shoot tip source on the success of *in vitro* micrografting in grapevine (VitisviniferaL.). Turkish Journal of Agriculture and Forestry 23: 741-747.
20. Thimmappaiah, Puthra GT, Anil SR (2002) *In vitro* grafting of cashew (Anacardiumoccidentale L.) SciHort 92: 177-182.
21. Onay A, Pirinc V, Adiyaman F, Isikalen C, Tilkat E and Basaren D (2004) *In vivo* and *In vitro* micrografting of pistachio (Pistaciavera L. cv. 'Siirt'). Turkish J Bio 27: 95-100.
22. Sanjaya, Ananthapadmanabho HS, Ravishankar R, Rai R (2003) In-vitro and in-vivo micrografting of (Santalum album L.) shoot tips. Journal of Tropical Forest Science 15: 234-236.
23. Rehman HU and Gill MIS (2014) *In vitro* shoot tip grafting of Patharnakh [Pyruspyrifolia (Burm F.) Nakai] pear on Kainth rootstock. Vegetos 27: 363-369.
24. Amiri ME (2006) *In vitro* techniques to study the shoot tip grafting of PrunusaviumL. (cherry) var. SeeyaheMashad. Journal of Food Agriculture and Environment 4: 151-154.
25. Khafalla MM, Dafalla HM (2008) *In vitro* micropropagation and micrografting of gum Arabic tree (Acacia Senegal L.) wild. InterJ Sustainable Crop Prod 3: 19-27.
26. Deogratias JM, Lutz A and Dosba F (1986) *In vitro* micrografting of shoot tips from juvenile and adult Prunusavium L. and Prunuspersica L. Batsch to produce virus free plants. ActaHorticulturae 193: 139-145.
27. Dong P, ShengKe X, Dong-F, Pei D, Xi-SK, Dong FX (1998) Studies on some scion-rootstock physiological indexes related to micrografting survival of walnut. Forest Research 11: 119-23.
28. Hosoi T, Machida H, Ooishi A, Miura T (1979) Studies on shoot tip grafting of citrus. Bulletin of the Faculty of Agriculture, Shizuoka University 29: 11-17.
29. Bhatt KM, Banday FA, Mir MA, Rather ZA,Hussain G (2013) *In vitro* grafting in apple(Malusdomestica. Borkh) cv. LalAmbri. Karnataka Journal of Agricultural Sciences26: 399-402.
30. Abousalim A, Mantell SH (1992) Micrografting of pistachio (Pistaciavera L. cv. Mateur). Plant Cell Tissue and Organ Culture 29: 231-34.
31. Grewal HS, Dhatt AS, Gosal SS (1994) *In vitro* shoot tip grafting in citrus. Annals of Biology, Ludhiana 10: 1-6.
32. Banerjee AK, Agrawal DC, Nalawade SM, Krishnamurthy K V (2000) Recovery of *in vitro* cotton shoots through micrografting. Current Science 78: 623-26.
33. Dziedzic E (2004) Preliminary studies on vaccination cherry (Prunus avium L.) *in vitro*. The film Universitatis Agriculturae Stetinensis, cheese. Agricultura 240: 41-44.
34. Naz AA, Jaskani MJ, Haider A, Qasim M (2007) *In vitro* studies on micrografting technique in two cultivars of citrus to produce virus free plants. Pakistan Journal of Botany 39: 1773-78.
35. Abreu MF, Nunes JCO, Santos M, Pedrotti EL (2003) Preliminaries histological studies from the micrografting of tissue cultured apple. Revista-Brasileira-de-Fruticultura 25: 195-96.
36. Dobranszki J, Magyar TK, Jambor BE, Lazanyi J (2000) New *in vitro* micrografting method for apple by sticking. International Journal of Horticultural Sciences 6: 79-83.
37. Ghorbel A, Chatibi A, Thaminy S, Kchouk ML, et al (1998) Micrografting of almond [Prunusdulcis (Miller) D. A. Webb] cv. Achak. ActaHort 470: 429-33.
38. Toth EK, Kriston E, Csillery G, Csanyi ME (2006) Application of silicon rubber grafting clips for *in vitro* phytopathological studies. ActaHort 725: 373-78.
39. Wu HC, Toit ESD, Reinhardt CF (2007) Micrografting of Proteacynaroides. Plant Cell Tissue and Organ Culture 89: 23-28.
40. Chakrabarty D, Park SY, Ali MB, Shin KS, Paek KY (2005) Hyperhydricity in apple: ultra structural and physiological aspects. Tree Physiology 26: 377-88.
41. Ziv M (1991) Vitrification: morphological and physiological disorders of *in vitro* plants. In: Micropropagation Technology and Applications. Eds. P.C. Debergh and R.H. Zimmerman, Kluwer Academic Publisher, Dordrecht, Netherlands, pp. 71-93.
42. Kevers C, Coumans M, Coumans GMF, Gaspar T(1984) Physiological and biochemical events leading to vitrification of plants cultured *in vitro*. PhysiologiaPlantarum 61: 69-74.
43. Al-Maarri K , Al-Ghamdi, AS (1996) Factors affecting the incidence of vitrification of *in vitro* propagated fruit trees. Journal of King Saud. University 8: 139-49.
44. Quoirin M, Lepoivre P (1977) Improved media for *in vitro* culture of Prunussp. ActaHort 78 : 437-42 (Original not seen. Cited by Bell R L and Reed B M, 2002, ActaHort 596: 412-18.
45. Bessis R (1986) Grapevine Physiology: the contribution of culture *in vitro*. Cellular and Molecular Life Sciences: 927-33
46. Starrantino A and Caruso A (1988)The shoot-tip grafting technique applied in citriculture. ActaHort 227: 101-03.
47. Edriss MH, Burger DW (1984) Micrografting shoot tip culture of citrus on three trifoliate rootstocks. ScientiaHort 23: 255-59.
48. Jonard R, Soedharma I, Villemur P (1987) Analysis of the influence of various factors on the improvement of successful micrografts in citrus. Comptes-Rendus-de-l'Academie-des-Sciences, III Sciences-de-la-Vie 305: 45-49.
49. Parthasarathy VA, Nagaraju V and Rahman SAS(1997)*In vitro* grafting of Citrus reticulataBlanco. Folia Hort 9: 87-90.

50. Kumarin V, Shyam S, Kumarin V, Singh S (2000) Shoot tip grafting with growth regulators for virus elimination in Nagpur mandarin (Citrus reticulata). Indian Journal of Agricultural Sciences 70: 396-97.

51. Nunes JCO, Abren MF, Dantas ACM, Pereira AJ, Pedrotti EL (2005) Morphologic characterization in apple micrografts. RevistaBarasileira de Fruticultura 27: 80-83.