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Grafting horizons: A review on exploring micrografting for enhanced plant performance

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Abstract

In horticulture, micrografting has become a potent method for modifying plant physiology and enhancing crop production. The success of micrografting can be augmented through the application of plant growth regulators, etiolation therapies, antioxidants, elevated sucrose levels, and silicon tubes. Micrografting may be used to commercially generate plants in fruit crops free of viruses and viroids, as well as to multiply plants on a large scale. This review explores the steps involved in micrografting, the anatomical and molecular aspects of graft unions, the effects of varying durations on the development of graft union tissues and the physiological mechanisms underlying micrografting with a focus on changes in gene expression, protein expression and the movement of mRNA molecules. It also explores the processes involved in tissue fusion, vascular reconnection and long-distance signaling. Furthermore, we examine the various uses of micrografting in horticultural crops, emphasizing how it may improve quality, yield and stress tolerance. Through an explanation of these complex procedures and their real-world applications, this review provides insightful information about using micrografting for crop enhancement and sustainable agriculture.

1. Introduction

Recently horticulture crops have been known for their commercial purpose besides being cultivated widely. The cultivation of these crops asexually includes different practices along with the traditional practices such as cuttings, grafting, layering, budding, *etc*. An old method of vegetative, asexual plant multiplication is called grafting. The most popular method for achieving this is joining two plant segments: “scion” (the shoot portion) and “rootstock” (the root portion) (stock). Recently, a summary of seedling micrografting procedures was provided (Turnbull, 2010). The process of grafting a tiny meristem or a segment of a micro shoot onto the top of a rootstock is known as micrografting. There are two kinds of micrografting techniques: *in vivo* and *in vitro*. *In vivo* micrografting involves young plants cultivated in greenhouses or nurseries using recognized grafting methods. A severed young plant cultivated from a seedling in aseptic circumstances is used in *in vitro* micrografting, as is micro-cutting derived from *in vitro* vegetative multiplication. The first study to use micrografting was to remove viruses from sick citrus *in vitro* cells (Navarro *et al.,* 1975). A technique of shoot tip grafting for obtaining virus-free citrus propagative structures to lower the economic loss was developed in Citrus (Murashige *et al*., 1975). The *in vitro* grafting technique appears promising and has been successfully used to many species of fruit crops (Cardoso *et al.*, 2012; Miguelez-Sierra *et al.,* 2017). It consists of younger tissues in a growing chamber with regulated environmental parameters. Grafting *in vitro* has several advantages in industry and research. *In vitro* shoot tip grafting is frequently used for; (i) The rejuvenation and improvement of certain tree species; (ii) Killing viruses; (iii) Studying the physiological interactions between scions and rootstocks, such as incompatibility and root-to-shoot transmission or communication and (iv) Using them in quarantine because they provide the least risk of spreading new plants. Due to its versatility and benefits, shoot tip grafting could be valuable for technicians, researchers, and nursery operators with potential applications in practical settings.

2. Factors affecting *in vitro* micrografting

Significant variables that impact the pace at which *in vitro* micrografted plantlets develop include grafting devices, culture medium type, plant growth regulators (PGRs), and naturally occurring organic compounds that can encourage cell division and cause the formation of calluses to make micrografting easier (Conejero *et al.,* 2013; Khierallah and Hussein, 2013; Yýldirim *et al.,* 2013; Ribeiro *et al*., 2015; Amer *et al.,* 2017; Gentile *et al.,* 2017). More specifically, the effectiveness of micrografting is largely dependent on how well the grafted union is attached using an appropriate, readily detachable method that causes the least amount of damage (Obeidy and Smith, 1991). Specialized tools have been strategically utilized to optimize micrografting outcomes. For instance, to enhance apple micrografting efficiency, use of filter paper bridge and the best method of maintaining the grafted zone in peaches was to use an elastic strip (Huang and Millikan, 1980; Jonard, 1986). The scion and rootstock of prunus explants were kept in contact using transparent silicon tubing (Gebhardt and Goldbach,1988).

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2.1 Application of growth regulators

Pre-treatment with growth regulators has accelerated tissue healing at graft union, among many other benefits, raising the graft survival rate. Micrografting is a successful method of obtaining plant material free of disease. By using pre-treated apices, grafting success is increased significantly and issues with browning and drying of the apices during *in vitro* micrografting handling are resolved. Cytokinin, in conjunction with auxins, promotes the development of vascular bundles, this encourages the union of rootstock and scion as well as apex initiation. Cytokinin revitalizes plant cells. Growth regulators are often not utilized in conventional grafting in order to improve graft success. By speeding up cell division and enhancing callus development, these growth regulators contribute to a higher rate of successful graft unions. During the process of micrografting, the generated microscion is briefly (5-10s) immersed in a sterile solution of growth regulators of proper concentration before being inserted into or placed onto the rootstock. The success in micrograft increased from 30 to 90% in pears (cv. Aly-sur on Calleryana pear) (Rafail and Mosleh, 2010). From 40-90% in apples of cultivars such as Anna on MM106 with increasing BAP (0-2.0 mg/l). In apple and pear, the number of effective micrografts grew from 10% in agar solidified media to 60% and 70% in liquid medium. Because the micro-shoots tend to take more nutrients and growth regulators from liquid media than from solidified medium, liquid media is more effective for successful micrografting.

3. Phases of micrografting

Before carrying out the micrografting procedure *in vitro*, the micropropagation protocols for the scion and rootstock must be established independently.

3.1 The origin of scion

The results underscore the significance of carefully choosing the source of shoot tips, with the best success rates in micrografting being seen with shoots generated *in vitro*. To achieve excellent grafting results, it is also necessary to minimize browning/necrosis and manage contamination. In the study of apricot (Hussain *et al.,* 2014) micrografting, different sources of shoot tips were evaluated for their effectiveness in achieving successful grafting onto rootstock seedlings growing *in vitro*. Grafts using *in vitro* derived shoot tips exhibited the highest success rate of 83%. On the other hand, grafts using shoot tips derived from *in vivo* forced shoots in apple (Wang *et al*., 2019) and in *Citrus macroptera* (Singh and Khawale, 2008) showed poorer success rates and higher rates of contamination and browning/necrosis. The study also investigated other factors affecting grafting success, includes the kind of rootstock, the size and location of the shoot tip, the light and temperature conditions, the medium's composition, and the use of growth regulators. The recommended duration for meristem resumption is seven to ten days. The meristem tips were invaded by rootstock callus earlier than seven days, and after ten days, the rootstock portion dried out quickly, and the scion suffered tissue necrosis. A positive correlation was observed between scion length and the success rate of graft union formation in both *in vitro* and *in vivo* environments. But when the seedlings grew older, it was found that the hypocotyl area became firmer and narrower, which made grafting less successful.

3.2 Rootstock formation and multiplication

Micro-propagated shoots that are rooted or unrooted, as well as *in vitro* or *in vivo* germinated seedlings, are the rootstocks utilized in micrografting. As all grafting steps are carried out *in vitro* and seedling rootstocks are utilized, in nutrient-salted jars, seeds undergo surface sterilization and germinate aseptically. To promote the development of a branching root system, seedlings may also be placed on a porous medium such as sterile vermiculite.

3.3 Rootstock and scion preparation

Micrografting involves truncating the top of seedling rootstocks, generally above the cotyledons or at the top of micropropagated shoots. The scion's little shoot apices are then positioned onto the exposed rootstock surface, making sure that the vascular ring or cambium layer of the sliced surfaces line up with one another. We call this technique the surface positioning approach (Estrada Luna *et al.,* 2002). Whenever, the rootstock and scion components are sufficiently thick, wedge or cleft grafting procedures are used. This involves making a wedge on the scion material and inserting it into a corresponding cleft in the rootstock. Upon successful grafting, the scion and rootstock will coalesce to form a singular plant entity. To ensure the development of a healthy, cohesive plant, it is imperative to regularly examine newly grafted seedlings to eliminate any adventitious shoots that may emerge on or below the graft union.

3.4 Interactions of rootstock scion and signaling

A comprehensive understanding of the root system's influence on the scion necessitates advancements in our knowledge of communication mechanisms within the graft union and long-distance signaling pathways in plants (Else *et al.,* 2018) Numerous plant species, fruits, vegetables, and model species like *Arabidopsis thaliana*, have shown significant advancements in this regard. Research has shown that complex signaling pathways and communication processes occur throughout the graft union in vegetables, fruit crops and other horticulture crops. These processes help the rootstock and scion exchange different signaling chemicals, nutrients, and genetic information. Gaining an understanding of these processes is essential for improving agricultural output, creating hardy plant types and refining grafting methods. Understanding the physiological and molecular underpinnings of grafting responses has been made possible by model organisms like *A. thaliana*. The function of hormones, transcription factors and signaling pathways in long-distance communication between root and shoot systems has been clarified by research conducted on *Arabidopsis*. These observations offer insightful hints for comprehending related mechanisms in agronomically significant crops.

3.4.1 Hormonal and mineral nutrients

The effects of different hormones and mineral nutrients on different crops while micrografting has been tabulated in Table 1.

3.4.2 RNAs

The movement of messenger RNAs (mRNAs) and small RNAs across graft unions has been documented, indicating a form of genetic communication between different parts of the grafted plant. It has been noted that mRNA-protein complexes go through the graft union in the phloem (Hannapel, 2010). The migration of mRNA from a

rootstock can affect characteristics in the scion, including changing the form of the leaf, as pioneer investigations have shown. For example, Kudo and Harada's tests showed that mRNA from a tomato rootstock might change the morphology of a potato scion's leaves. Furthermore, in micrografts of apple and pear, it has been discovered that mRNA from certain genes, such as the gibberellic acid insensitive (gai) gene (Zhang *et al.,* 2012), travels from root to shoot and vice versa. Stress and signaling pathway-related mRNAs have been shown to be significantly abundant in grapevine. Studies, such as those conducted using DsRED transgenic walnut (Liu *et al*., 2017), have demonstrated the movement of mRNA from the rootstock to the wild-type scion. However, the efficiency and directionality of mRNA movement can be influenced by genetic factors inherent to the plant varieties involved in the grafting process. The ability to observe mRNA movement between different parts of grafted plants opens up new possibilities for both breeding programs and physiological research. By harnessing micrografting techniques, researchers can manipulate the movement of specific mRNAs to study gene expression patterns, investigate signaling pathways and potentially introduce genetic traits into new plant varieties.

Table 1: The effects of different hormones and mineral nutrients on different crops while micrografting

Hormones	Effects	Crop	Reference
Cytokinins	Vigour of scions	*Arabidopsis*	Werner *et al.,* 2001
Strigolactones (SLs)	Bud growth regulation	Grapevines	Gomez Roldan *et al.,* 2008
Gibberellins (GA12)	Shoot growth	Apple	Westwood and Batjer., 1960
Abscisic acid (ABA)	Signaling	Strawberries	Jia *et al.,* 2011
Jasmonic acid (JA)	Root to shoot signaling	Tomatoes and cucumbers	Müller *et al.,* 2015

3.4.3 Modifications to gene expression

Numerous grafting experiments, notably those involving vegetables and fruit crops, have reported alterations in gene activity within the scion triggered by signals originating from the rootstock. For example, in apples, grafting onto different rootstocks has been associated with changes in gene expression linked to other characteristics, such as disease resistance against fire blight, and tree size. Similarly, studies on grapes have reported significant alterations in leaf transcript profiles influenced by the rootstock. Furthermore, observations in grapevine (Yang *et al.,* 2015) and other crops have highlighted alterations in gene expression within the meristem of scion, demonstrating a specific emphasis on genes associated with chromatin regulation, cell organization, and hormone signalling (Aloni *et al.,* 2010). Epigenetic regulations, such as DNA methylation and histone modifications, play a crucial role in cell reprogramming. Although, there has been little specific study on woody plants, there is evidence that the scions of grafted vegetables have partially heritable changes in DNA methylation. The synergistic relationship between hormone action and epigenetic modifications is instrumental in governing plant growth and development. For instance, cytokinins (CKs) have been implicated in DNA methylation processes, while recent research has revealed connections between auxin biosynthesis, transport, signaling, and miRNAs, as well as epigenetic factors like histone modification. These findings underscore the complex regulatory mechanisms underlying plant grafting responses and highlight the importance of understanding both genetic and epigenetic factors in shaping plant phenotypes. The use of molecular markers, such as single nucleotide polymorphisms (SNPs), can help assess the genetic compatibility of rootstocks and scions. These markers can predict graft success and enhance the selection of suitable combinations for micrografting (Liu *et al.,* 2019).

Table 2: Micrografting studies in different fruit crops

S. No.	Name of the fruit crop	Scion cultivar	Rootstock	Success percentage of micrografts (%)	Reference
1	Pistachionut	*Pistacia vera* cv. Mateur	Elevated rootstock for seedlings	94-100	Abousalim and Mantell., 1992
2	Apple	*Malus domestica* var. Lal ambri	M.9 Rootstock	42.25	Huang *et al.,* 1982
3	Grape	*Vitis vinifera* cvs, Sahebi, Soltanin, Fakhri	41B	50.1-60.6	Aazami and Bagher. 2010
4	Pear	*Pyrus communis* cvs. Leconte	Shoots of *Pyrus betulaefolia* cultured *in vitro*	83.00	Hassanen., 2013
5	Walnut	*Juglans regia* cvs. Jinlong, No1, Xiangling	Elevated rootstock for seedlings	56.7-73.3	Payghamzadeh, and Kazemitabar, 2011
6	Citrus	I. Kinnow mandarin II. Satsuma mandarin	Germination of rough lemon seedlings *in vitro*	I 36 II 33.3	Jaskani and Abbas, 2007
7	Guava	Arka kiran	Germination through seedling and phyto-chemical properties	62	Lakshmi *et al*., 2022

4. Physiology of micrografting

4.1 Transverse slices of an area around graft components showing the primary results broken down

This includes active growth, differentiation, and integration processes occurring at the graft union, with cells undergoing various stages of development and differentiation to form functional xylem and phloem elements. The formation of callus tissue was observed, characterized by the proliferation of parenchymatous cells (Gebhardt and Goldbach, 1988). Some of these cells showed less dense cytoplasm, containing vacuoles and few organelles, indicating active growth and cell division. At the graft union, the observed enlargement of cells and occasional divisions of cambial initials suggested the incipient stages of tissue integration and fusion. Cambial cells were observed to be formed by a couple of cell layers. Some of these cells showed dense cytoplasm, indicating active metabolic activity. Phloem cells and xylem components were noted to be forming by a couple of cell layers, indicating the beginning of differentiation (Santarosa *et al.,* 2016). Tracheal elements, in the process of differentiation, had reached their normal size, and the cell lumen was dense with electrons. Phloem cells were described as round, isodiametric or hexagonal in shape with thin walls, and they were dispersed throughout the tissue.

5. Micrografting in different fruit crops

Micrografting in different fruit crops and their success percentage were tabulated as in Table 2.

5.1 Micrografting in different vegetable crops

Relationships between various rootstocks for solanaceae and cucurbitaceae crops and how to utilize rootstock to enhance characteristics were tabulated in Table 3.

Table 3: Micrografting in different vegetable crops

Name of the crop	Rootstock	Resistance	Reference
Melon	'TZ148'	*Fusarium* wilt	Cohen *et al.,* 2007
Tomato	'AR-9707' (*Solanum lycopersicum*)	Salinity	Fernandez Garcia *et al.,* 2004
Eggplant	*Solanum integrifolium*	-	Gisbert *et al.,* 2011
Sweet pepper	AR-96023 (*Capsicum annum*)	Root-knot nematode	Oka *et al.,* 2004

5.2 Micrografting in ornamentals

The chosen grafting technique is one of several variables that affect micrografting success. A healthy micro-scion-rootstock contact is essential for the micrograft union to occur because it promotes the cambial tissue's reconnection. Yýldýrým *et al.* (2019) discovered that in almond micrografts, the top-slit approach produced a superior connection, yielding fusions between scion and rootstock with 90-100% success rates. In comparison, a much higher number of misplaced micro-grafts and only 30-40% of effective grafts were created by top wedge micrografting (Onay *et al*., 2004; Suárez *et al.*, 2021). Opuntia spp. were micrografted using a comparison of wedge and horizontal grafts (Estrada-Luna *et al.,* 2002). They discovered that the horizontal grafts performed better because there was less scion displacement. Similarly, side grafting of small shoot apices outperformed apical grafting in cashews, yielding a considerably higher success rate of 66%.

5.3 Micrografting in medicinal plants

Micrografting plays a crucial role in the propagation of medicinally important horticultural plants. By allowing the rapid and reliable multiplication of selected plant varieties, micrografting ensures that high-quality, uniform plants are available for large-scale production of phytomedicinal products. For example, Citrus species, which are valued in phytomedicine for their antioxidant and anti-inflammatory properties, are commonly propagated using micrografting techniques to maintain desirable traits such as disease resistance and enhanced phytochemical content (Singh *et al.*, 2020). For instance, plants such as ginseng, *Panax ginseng* and *Taxus baccata*, known for their anticancer and anti-inflammatory properties, can be propagated and conserved through micrografting to ensure a sustainable supply for phytomedicine without depleting natural populations (Zhou *et al.*, 2018). For instance, *Aloe vera*, *Cinnamomum cassia*, *Asarum europeaum, Apium graveolens, Piper longum, Matricaria chamomilla, and Butea frondosa* are included and are commonly used in the phytomedicine industry (Bamne *et al.,* 2023). Further more, by using rootstocks with specific attributes, such as improved nutrient uptake or disease resistance, the yield and quality of phytochemicals in the scion can be optimized (Rout *et al.,* 2006). By synergizing the precision of micrografting with the advancements in plant biotechnology, incorporating advanced techniques such as genetic engineering, marker-assisted selection, transcriptomics, proteomics, metabolic engineering, and genome editing; we can achieve significant advancements in crop cultivation (Rai *et al.,* 2012) researchers can develop plants with enhanced resistance to pests and diseases, improved stress tolerance, and increased phytochemical production, making micrografting a powerful tool in modern phytomedicine (Gorecka *et al.,* 2010). Some of the azole derivatives showed potential reisistance to anticancer and antimicrobial activity in *in vitro*.

6. Field performance of *in vitro* micrografted plants

In vitro micrografted plants often show vigorous growth once acclimatized and transplanted to the field. This is due to the healthy, pathogen-free status of the plants and the enhanced rootstock-scion compatibility. For example, studies on micrografted grapevines show that they establish quickly in the field, leading to rapid growth and better vegetative development compared to traditionally grafted plants (Dolgov *et al.,* 2009). Acclimatized micrografted plants tend to exhibit higher survival rates when transplanted into the field. This is especially true for plants like citrus, where *in vitro* grafting under sterile conditions leads to improved scion-rootstock union and reduced mortality rates in the field (Navarro *et al.,* 2002). The survival rate of micrografted plants during acclimatization varies across species. For instance, apple grafted plants achieved a 100% survival rate (Dobránszky *et al.,* 2000), while almond plants had a survival rate between 85% and 100% (Yýldýrým *et al.,* 2010). Cacao showed an

82% survival rate (Miguelez-Sierra *et al.,* 2017), and passionfruit had a rate of 75% (Hieu *et al.,* 2022) In contrast, Tahitian lime and valencia orange had lower survival rates, ranging from 47% to 50% (Suárez *et al.,* 2021). Overall, micrografted plants showed a higher acclimatization success rate compared to ungrafted plants, particularly when the plants were challenging to handle in conventional tissue culture or had difficulty establishing roots (Channuntapipat *et al.,* 2003).

7. Applications of micrografting

7.1 Root promotion

Rooting is the main aspect of *in vitro* micrografting, in some species which have difficult to root character. This *in vitro* micrografting promotes rooting. Some of examples of *in vitro* micrografting are *Lens culinaris*, a significant pulse crop in the Mediterranean region, *Protea cynaroides*, An important ornamental plant indigenous to South Africa and some *Prunus* species had *in vitro* recalcitrance (Wu *et al.,* 2007). This was resolved by applying micro-shoots onto rootstock seedlings. Previous evaluations have emphasized the development of appropriate micrografting strategies to address plant species rooting challenges.

7.2 Promotion of shoot proliferation

When compared to traditionally *in vitro* cultivated segments, it was discovered that all micro-grafted plants had considerably better proliferation from shoot segments. The observation highlights the effectiveness of micrografting as a propagation technique. Factors to be considered are enhanced rooting as micrografting onto rootstocks can promote better root development compared to conventional *in vitro* culture methods. Genetic compatibility, which depicts matching scion and rootstock genotypes carefully in micrografting can ensure better compatibility, which in turn can positively influence shoot proliferation. Compatible graft unions facilitate efficient nutrient and resource exchange between the scion and rootstock. Micrografting can alter the hormonal balance within the plant, leading to enhanced shoot proliferation. The hormones that promote growth, like cytokinins and auxins can be produced and transported as a result of interactions between the tissues of the rootstock and scion (Li *et. al.,* 2022). Using disease-resistant rootstocks in micrografting can protect scion tissues from diseases that may inhibit proliferation in conventionally cultured segments. This protection allows for uninterrupted growth and proliferation of the micrografted plants.

7.3 Embryo rescue or encouraging the regrowth of organogenesis-derived shoots

In vitro, mutagenesis and the recovery of plants by *de novo* organogenesis and somatic embryogenesis (Cardoso *et. al*., 2017) may be crucial steps toward the production of genetically engineered plants. It may; however, provide issues with roots in certain horticultural species due to insufficient callus maturation and tissue culture. The pioneering documentation of shoot regeneration from somatic embryos (SEs) of cocoa plants was achieved in 1992 through the application of *in vitro* maturation (IVM) techniques.

7.4 Following cryopreservation, shoot regrowth

At the moment, cryopreservation is seen to be a useful tactic for enabling the long-term, economical protection of plant genetic resources. Many horticultural species shoot tips have been successfully cryopreserved in cryobanks; effective cryopreservation requires a high degree of post-thaw recovery. In certain species, like citrus, direct shoot tip recovery was not possible. To get around this, cryopreserved shoot tips were micrografted onto seedlings that had been grown *in vitro* technological developments in micrografting (Wang *et al.,* 2021). Due to the intricate nature of the procedures and the sometimes-poor success rate of the grafts, micrografting is a costly and time-consuming manufacturing process. Fruit plant *in vitro* grafts frequently fail as a result of incompatibility reactions, inadequate scion-stock contact, and phenolic browning of the cut surfaces. The development of alternative methods has overcome some of the challenges associated with micrografting, solidifying its position as a beneficial technology for technicians, researchers, nursery operators, and commercial tissue culture labs.

7.5 Enhancing stress tolerance and phytochemical production

In horticultural systems, rootstocks selected for their resistance to environmental stressors (*e.g*., drought, salinity, or soil-borne pathogens) can significantly improve the resilience of medicinal plants. Micrografting allows the combination of a stress-tolerant rootstock with a scion selected for its medicinal value. Studies have shown that rootstocks can influence the secondary metabolite content of the scion, enhancing the production of bioactive compounds such as flavonoids, alkaloids, and essential oils. This is particularly relevant for plants like *Lavandula* spp. and *Rosmarinus officinalis*, widely used in phytomedicine for their antioxidant and antimicrobial properties (Sorce *et al.*, 2002).

7.6 Enhancement of phytochemical profiles

The influence of rootstocks on the scion's secondary metabolite profile is well documented. Micrografting can be used to combine rootstocks and scions in a way that enhances the production of key phytochemicals. For example, certain rootstocks have been shown to increase the concentration of essential oils, flavonoids, or other bioactive compounds in medicinal plants. This has significant implications for the phytomedicine industry, where the potency of medicinal products is directly related to the concentration of these compounds. Studies on *Citrus* and *Mentha* spp. have demonstrated the effectiveness of micrografting in enhancing secondary metabolite production (Georgiou and Gregoriou, 1999).

8. Utilizations for micrografting

Many fruit crops have been improved and multiplied *via* the use of micrografting.

8.1 Virus and viroid elimination

The method of micrografting has been extensively employed in fruit crops to eradicate viruses, phytoplasma, and systemic diseases, resulting in the virus-free growth of several fruit plants. Spain employed *in vitro* grafting to create virus-free citrus trees. Since 1998, the method has been utilized in the native Arakapas mandarin of Cyprus to eradicate Citrus psorosis virus (CPsV), Citrus cachexia Virus (CCaVd), Citrus exocortis viroid (CEVd) and other related viroids. Citrus tristeza virus (CTV) was successfully eradicated from Satsuma mandarin in Croatia recently and using this method, it was possible to produce 91-95% kinnow mandarin (*Citrus reticulata*) and sweet orange (*Citrus sinensis*) cultivars that are free from Citrus tristeza virus (CTV). Since the development of the meristem outpaces the virus's systemic dissemination inside the plant, meristematic

tissues found in the axillary buds and shoot tips often stay virus-free (Cardoso *et al.,* 2017).

8.2 Evaluation of incompatibility with grafts

Graft incompatibility occurs when two separate plants are grafted together and are unable to successfully form a union and grow into a single composite plant. Fruit trees that exhibit graft incompatibility can be divided into two categories: Translocated and localized. The introduction of a mutually compatible inter-stock does not resolve translocated incompatibility, which is frequently linked to the migration of certain labile components between the grafting partners. The combination of "Non-pareli" almonds and "Mariana 2624" plums is an illustration of this category. Localized incompatibility is contingent upon the stock and scion coming into touch. This kind of incompatibility may be seen in bartlett pear scion directly grafted onto a quince rootstock. Poor vascular connection, phloem degradation and vascular discontinuity at the union location were found in anatomic examinations of incompatible grafts. Using the survival rate as an indicator, micrografting was utilized to investigate suitable and incompatible grape variety pairings. Among the various combinations tested, RizamatV/Baixiangjiao and Canepubu/Muscat Hamburg consistently exhibited the highest grafting survival rates, exceeding 85%. The survival rates under incompatible Canepubu/Baixiangjiao and Carignane/Baixiangjiao combinations were just 3.33 and 13.33%, respectively. Canepubu/Baixiangjiao has both translocated and localized incompatibilities, but Carignane/Baixiangjiao only has translocated incompatibility. Incompatible combinations can lead to vascular disconnection, a common cause of grafting failure. The persistence or partial dissipation of the necrotic layer can hinder successful graft union formation (Ji Ling, 2001).

8.3 Indexing viral diseases

This technique involves grafting a suspicious plant onto an indicator plant. Typical viral-induced symptoms appear on the indicator plant after the virus has spread if the suspect plant is infected with the target virus. In contrast to conventional propagation grafting, this diagnostic method does not need the establishment of a long-term, mutually compatible graft union. The development of micrografting techniques has greatly improved the effectiveness and speed of virus detection. Indicator plants may exhibit the characteristic symptoms of leaf roll virus within two to three weeks following the micrografting of infected scion material onto virus-free rootstocks, such as *Cabernet sauvignon* (Santarosa *et al.,* 2016). Cyprus uses indicator plants such as Madame Vinous, pineapple and sweet orange in addition to grapes to index the Citrus psorosis virus (CPsV) in native Arakapas Mandarin plants. With the use of this micro-indexing technique, post-entry quarantine protocols for imported plant materials can greatly aid in the timely and precise identification of viral infections before their further dissemination into nearby agricultural ecosystems.

9. Limitations of micrografting

Micrografting, though a highly effective propagation technique, comes with several limitations that affect its widespread adoption. These include graft incompatibility, acclimatization challenges, technical complexity, high costs, and sensitivity to environmental stressors. One of the significant limitations of micrografting is graft incompatibility between the scion and rootstock. Even in sterile, controlled conditions, there can be failures in graft union due to genetic differences or physiological incompatibilities. In Citrus, micrografting, incompatibility issues can still arise, especially with certain rootstock and scion combinations, leading to poor graft union and reduced survival rates in the field (Gambino *et al.,* 2005). Kobayashi *et al.* (2000) reported that the survival rate of micrografted sweet orange buds derived from organogenesis dropped significantly during the acclimatization phase, largely due to sensitivity to external environmental conditions. For small-scale and medium-scale fruit growers, the initial investment required for micrografting infrastructure is often not feasible, particularly in regions with limited access to such facilities (Peña *et al.,* 2008). Navarro *et al.* (2002) found that micro grafted citrus plants were more prone to environmental stress during field transplantation, particularly when acclimatization protocols were not strictly followed.

10. Recent studies on micrografting

López Cobollo *et al.* (2022) investigated that optimizing the grafting angle and the type of rootstock significantly improved the success rate of micro grafted plants. The combination of micrografting with specific growth conditions (such as humidity and temperature) led to higher graft survival rates. Ali *et al.* (2023) demonstrated that micrografting tomato scions onto resistant rootstocks significantly reduced the incidence of wilt disease and improved overall plant vigor compared to ungrafted controls. Chen *et al.* (2023) study revealed that micro grafted watermelon exhibited enhanced photosynthetic efficiency, improved root architecture, and better fruit yield compared to non-grafted plants, particularly under drought stress. Javed *et al.* (2023) examined the effects of various growth regulators on the success of micrografting in apple trees. Ochoa *et al.* (2022) demonstrated that micrografting can facilitate the incorporation of desirable traits from donor plants, leading to improved fruit quality and tree performance in avocado.

11. Future prospects

Fine-tuning hormonal regulation with deeper exploration into the intricate interplay of plant hormones in regulating graft compatibility, vigour, and stress response mechanisms, such as cytokinins, auxins, gibberellins, abscisic acid, and jasmonic acid,. Understanding how to manipulate these hormonal pathways through grafting techniques could lead to tailored approaches for optimizing plant growth and resilience. Investigating the genetic factors influencing graft compatibility and performance, including identifying key genes and regulatory elements involved in graft union formation, vascular reconnection, and signaling pathways. This knowledge can inform targeted breeding strategies aimed at developing rootstocks and scions with enhanced compatibility and desired traits. Examining the effects of epigenetic changes, including DNA methylation and histone modifications on grafting-induced changes in gene expression, phenotypic modulation and stress tolerance. Understanding how epigenetic regulation contributes to grafting success could pave the way for epigenetic engineering approaches to improve graft compatibility and plant performance. Further elucidating the mechanisms underlying long-distance signaling between rootstocks and scions, including the movement of mRNAs, proteins, and signaling molecules. Investigating how environmental factors influence these signaling pathways and their impact on plant growth and adaptation could provide insights into optimizing grafting techniques for diverse growing conditions. Integrating micrografting techniques with emerging technologies such as genome editing, transcriptomics, metabolomics, and bioinformatics to unravel complex molecular

networks underlying grafting responses and identify novel targets for genetic manipulation and crop improvement. Exploring the potential of micrografting for sustainable agriculture practices, including enhancing crop productivity, disease resistance, and abiotic stress tolerance while reducing chemical inputs and environmental impact. Creating micrografting procedures that work for a variety of crop species and environmental circumstances might help agriculture intensify sustainably.

12. Conclusion

Micrografting represents a versatile and powerful tool in plant science and agriculture, with the potential to drive innovation, enhance crop productivity, and contribute to sustainable agricultural practices. Continued research and development in micrografting techniques hold promise for addressing current and future challenges in plant production and breeding. It also plays a pivotal role in bridging the fields of horticulture and phytomedicine by ensuring the availability of high-quality, virus-free plants for phytomedicine. Micrografting supports the growing demand for herbal remedies and plant-based therapeutics in both traditional and modern medical systems. Also increasing crop output and decreasing waste, micrografting maximizes the use of resources including land, water, and inputs. This minimizes the impact on the environment and promotes resource efficiency, both of which support sustainable agriculture practices. In addition to supporting sustainable agricultural production, this responds to the increased demand for better cultivars. Increases climate change resistance and promotes sustainable agriculture.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

Abousalim, A. and Mantell, S.H. (1992). Micrografting of pistachio (*Pistacia vera* L. cv. Mateur). Plant Cell, Tissue and Organ Culture, **29**:231-234.

Agüero, C.B.; Uratsu, S.L.; Greve, C.; Powell, A.L.; Labavitch, J.M.; Meredith, C.P. and Dandekar, A.M. (2005). Evaluation of tolerance to Pierce's disease and Botrytis in transgenic plants of *Vitis vinifera* L. expressing the pear PGIP gene. Molecular Plant Pathology, **6**(1):43-51.

Albacete, A.; Martķnez Andśjar, C.; Pérez-Alfocea, F.; Lozano, J. and Asins, M.J. (2015). Rootstock-mediated variation in tomato vegetative growth under low potassium or phosphorous supplies. Acta Horticulturae, **(1086)**:147-152.

Ali, M.E.; Waliullah, S.; Kobayashi, K.; Yaeno, T.; Yamaoka, N. and Nishiguchi, M. (2016). Transmission of RNA silencing signal through grafting confers virus resistance from transgenically silenced tobacco rootstocks to non-transgenic tomato and tobacco scions. Journal of Plant Biochemistry and Biotechnology, **25**:245-252.

Aloni, B.; Cohen, R.; Karni, L.; Aktas, H.A.K.A.N. and Edelstein, M. (2010). Hormonal signaling in rootstock–scion interactions. Scientia Horticulturae, **127**(2):119-126.

Ļlvarez Hernţndez, J.C.; Castellanos-Ramos, J.Z.; Aguirre Mancilla, C.L.; Huitrón Ramķrez, M.V. and Camacho Ferre, F. (2015). Influence of rootstocks on fusarium wilt, nematode infestation, yield and fruit quality in watermelon production. Ciência e Agrotecnologia, **39**:323-330.

Amer, A.M.; Mohamed, G.M.; Hussein, M.H.; Sedik, M.Z. and Aly, U.I. (2017). Effect of some of the natural organic sources on rice tissue culture. Egyptian Pharmaceutical Journal, **16**(3):152-156.

Aazami, M.A. and Bagher, M. (2010). *In vitro* micro-grafting of some Iranian grapevine cultivars. Romanian Biotechnology, **15**:5576-5580.

Badalamenti, O.; Carra, A.; Oddo, E.; Carimi, F. and Sajeva, M. (2016). Is *in vitro* micrografting a possible valid alternative to traditional micropropagation in Cactaceae *Pelecyphora aselliformis* as a case study. Springer Plus, **5**:1-4.

Bamne, F.; Shaikh, N.; Momin, M.; Khan, T. and Ali, A. (2023). Phytochemical analysis, antioxidant and DNA nicking protection assay of some selected medicinal plants. Ann. Phytomed., **12**(2):406-413.

Bettoni, J.C.; Bonnart, R. and Volk, G.M. (2021). Challenges in implementing plant shoot tip cryopreservation technologies. Plant Cell, Tissue and Organ Culture (PCTOC), **144**(1):21-34.

Bhowal, R.; Kumari, S.; Sarma, C.; Suprasanna, P. and Roy, P. (2022). Phytochemical constituents and bioactivity profiles of citrus genus from India. Analytical Chemistry Letters, **12**(6):770-787.

Black, L.L.; Wu, D.L.; Wang, J.F.; Kalb, T.; Abbass, D. and Chen, J.H. (2003). Grafting tomatoes for production in the hot-wet season. Asian Vegetable Research and Development Center. AVRDC Publication, **3**(2):551-555.

Calatayud, Ļ.; San Bautista, A.; Pascual, B.; Maroto, J.V. and López Galarza, S. (2013). Use of chlorophyll fluorescence imaging as diagnostic technique to predict compatibility in melon graft. Scientia Horticulturae, **149**:13-18.

Cardoso, J.C.; Curtolo, M.; Latado, R.R. and Martinelli, A.P. (2017). Somatic embryogenesis of a seedless sweet orange (*Citrus sinensis* L.) Osbeck). *In vitro* Cellular and Developmental Biology-Plant, **53**:619-623.

Chandran, S.; Sarangapani, S.; Prabakaran, V.; Subramanian, S.; Chokkalingam, S. and Jayakumar, S. (2023). Micrografting: A *in vitro* technique for sustainable horticulture production and its applications. The Journal of Phytopharmacology, **12**(2):111-118.

Channuntapipat, C.; Sedgley, M. and Collins, G. (2003). Micropropagation of almond cultivars Nonpareil and Ne Plus Ultra and the hybrid rootstock Titan× Nemaguard. Scientia Horticulturae, **98**(4):473-484.

Chuck, G. and O'Connor, D. (2010). Small RNAs going the distance during plant development. Current Opinion in Plant Biology, **13**(1):40-45.

Cohen, R.; Burger, Y.; Horev, C. and Koren, A. (2007). Introducing grafted cucurbits to modern agriculture: The Israeli experience. Plant Disease, **91**(8):916-923.

Conejero, A.; Romero, C.; Cunill, M.; Mestre, M.A.; Martķnez Calvo, J.; Badenes, M.L. and Llįcer, G. (2013). *In vitro* shoot-tip grafting for safe Prunus budwood exchange. Scientia Horticulturae, **150**:365-370.

Cotado, A.; Müller, M.; Morales, M. and Munné Bosch, S. (2018). Linking jasmonates with pigment accumulation and photo protection in a high-mountain endemic plant, *Saxifraga longifolia*. Environmental and Experimental Botany, **154**:56-65.

De Pasquale, F.; Giuffrida, S. and Carimi, F. (1999). Minigrafting of shoots, roots, inverted roots, and somatic embryos for rescue of *in vitro* Citrus regenerants. Journal of the American Society for Horticultural Science, **124**(2):152-157.

Desai, S.P.; Momin, Y.H.; Taralekar, S.T.; Dange, Y.D.; Jagtap, S.R. and Khade, H.P. (2021). Evaluation of potential *in vitro* anticancer and antimicrobial activities of synthesized 5-mercapto-4-substituted 1, 2, 4 triazole derivatives. Ann. Phytomed., **10**(2):273-279.

Dobrjnszky, J.; Magyar Tjbori, K.; Jjmbor Benczsr, E. and Lazjnyi, J. (2000). New *in vitro* micrografting method for apple by sticking. International Journal of Horticultural Science, **6**(4):79-83.

Else, M.A.; Taylor, J.M.; Young, S. and Atkinson, C.J. (2018). The effect of the graft union on hormonal and ionic signalling between rootstocks and scions of grafted apple (*Malus pumila* L. Mill.). Environmental and Experimental Botany, **156**:325-336.

Estrada-Luna, A.A.; López Peralta, C. and Cjrdenas-Soriano, E. (2002). *In vitro* micrografting and the histology of graft union formation of selected species of prickly pear cactus (Opuntia spp.). Scientia Horticulturae, **92**(3-4):317-327.

Fallik, E.; Alkalai Tuvia, S.; Chalupowicz, D.; Zutahy, Y.; Zaaroor, M.; Beniches, M. and Gamliel, A. (2016). Effects of rootstock and soil disinfection on quality of grafted watermelon fruit (*Citrullus lanatus* L.): A two-year study. Israel Journal of Plant Sciences, **63**(1):38-44.

Fan, J.; Yang, R.; Li, X.; Zhao, W.; Zhao, F. and Wang, S. (2015). The processes of graft union formation in tomato. Horticulture, Environment, and Biotechnology, **56**:569-574.

Fernjndez Garcka, N.; Martknez, V. and Carvajal, M. (2004). Effect of salinity on growth, mineral composition, and water relations of grafted tomato plants. Journal of Plant Nutrition and Soil Science, **167**(5):616-622.

Gabarro, P. (1943). A new method of grafting. British Medical Journal, **1**(4301):723.

Galatti, F.D.S.; Franco, A.J.; Ito, L.A.; Charlo, H.D.O.; Gaion, L.A. and Braz, L.T. (2013). Rootstocks resistant to *Meloidogyne incognita* and compatibility of grafting in net melon. Revista Ceres, **60**:432-436.

Gambino, G.; Ruffa, P. and Schubert, A. (2005). Acclimatization and field performance of *in vitro* micrografted plants of grapevine (*Vitis vinifera*). Scientia Horticulturae, **103**(1):1-9.

Gao, P.; Xing, W.W.; Li, S.H.; Shu, S.; Li, H.; Li, N.; Shao, Q.S. and Guo, S.R. (2014). Effect of pumpkin rootstock on antioxidant enzyme activities and photosynthetic fluorescence characteristics of cucumber under Ca (NO3) 2 stress. In: International Symposium on Vegetable Grafting, **1086**:177-187.

Gautier, A.T.; Cochetel, N.; Merlin, I.; Hevin, C.; Lauvergeat, V.; Vivin, P.; Mollier, A.; Ollat, N. and Cookson, S.J. (2020). Scion genotypes exert long distance control over rootstock transcriptome responses to low phosphate in grafted grapevine. BMC Plant Biology, **20**:1-15.

Gebhardt, K. and Goldbach, H. (1988). Establishment, graft union characteristics and growth of Prunus micrografts. Physiologia Plantarum, **72**(1):153-159.

Gentile, A.; Frattarelli, A.; Nota, P., Condello, E. and Caboni, E. (2017). The aromatic cytokinin meta-topolin promotes *in vitro* propagation, shoot quality and micrografting in *Corylus colurna* L. Plant Cell, Tissue and Organ Culture (PCTOC), **128**:693-703.

Ghate, T.H.; Sharma, P.; Kondhare, K.R.; Hannapel, D.J. and Banerjee, A.K. (2017). The mobile RNAs, StBEL11 and StBEL29, suppress growth of tubers in potato. Plant Molecular Biology, **93**:563-578.

Gisbert, C.; Prohens, J.; Raigón, M.D.; Stommel, J.R. and Nuez, F. (2011). Eggplant relatives as sources of variation for developing new rootstocks: Effects of grafting on eggplant yield and fruit apparent quality and composition. Scientia Horticulturae, **128**(1):14-22.

Giuffrida, F.; Cassaniti, C.; Agnello, M. and Leonardi, C. (2014). Growth and ionic concentration of eggplant as influenced by rootstocks under saline conditions. In: International Symposium on Vegetable Grafting, **1086**:161-166.

Gomez-Roldan, V.; Fermas, S.; Brewer, P.B.; Puech Pagčs, V.; Dun, E.A.; Pillot, J.P.; Letisse, F.; Matusova, R.; Danoun, S.; Portais, J.C. and Bouwmeester, H. (2008). Strigolactone inhibition of shoot branching. Nature, **455**(7210):189-194.

Gregory, P.J.; Atkinson, C.J.; Bengough, A.G.; Else, M.A.; Fernjndez Fernjndez, F.; Harrison, R.J. and Schmidt, S. (2013). Contributions of roots and rootstocks to sustainable, intensified crop production. Journal of Experimental Botany, **64**(5):1209-1222.

Hannapel, D.J. (2010). A model system of development regulated by the long distance transport of mRNA. Journal of Integrative Plant Biology, **52**(1):40-52.

Hassanen, S.A. (2013). *In vitro* grafting of pear (Pyrus spp.). World Appl. Sci. J; **21**(5):705-709.

He, J.; Zhou, J.; Wan, H.; Zhuang, X.; Li, H.; Qin, S. and Lyu, D. (2020). Rootstock–scion interaction affects cadmium accumulation and tolerance of *Malus*. Frontiers in Plant Science, **11**:1264.

Hieu, T.; Phong, T.H.; Khai, H.D.; Mai, N.T.N.; Cuong, D.M.; Luan, V.Q.; Tung, H.T.; Nam, N.B. and Tan Nhut, D. (2022). Efficient production of vigorous passion fruit rootstock for *in vitro* grafting. Plant Cell, Tissue and Organ Culture (PCTOC), **148**(3):635-648.

Huang, S.C. and Millikan, D.F. (1981). *In vitro* micrografting of apple shoot tips. HortScience, **15**(6):741-743.

Huetteman, C.A. and Preece, J.E. (1993). Thidiazuron: A potent cytokinin for woody plant tissue culture. Plant cell, Tissue and Organ Culture, **33**:105-119.

Hussain, G.; Wani, M.S.; Mir, M.A.; Rather, Z.A. and Bhat, K.M. (2014). Micrografting for fruit crop improvement. African Journal of Biotechnology, **13**(25).

Jaskani, M. and Abbas, H. (2007). *In vitro* studies on micrografting technique in two cultivars of citrus to produce virus free plants. Pak. J. Bot., **39**(5):1773-1778.

Jonard, R. (1986). Micrografting and its applications to tree improvement. Berlin, Heidelberg: Springer Berlin Heidelberg. In Trees, pp:31-48.

Jia, H.F.; Chai, Y.M.; Li, C.L.; Lu, D.; Luo, J.J.; Qin, L. and Shen, Y.Y. (2011). Abscisic acid plays an important role in the regulation of strawberry fruit ripening. Plant Physiology, **157**(1):188-199.

Kanehira, A.; Yamada, K.; Iwaya, T.; Tsuwamoto, R.; Kasai, A.; Nakazono, M. and Harada, T. (2010). Apple phloem cells contain some mRNAs transported over long distances. Tree Genetics and Genomes, **6**:635-642.

Kehr, J. and Kragler, F. (2018). Long distance RNA movement. New Phytologist, **218**(1):29-40.

Kobayashi, A.K.; Bespalhok, J.C.; Pereira, L.F.P. and Vieira, L.G.E. (2003). Plant regeneration of sweet orange (*Citrus sinensis*) from thin sections of mature stem segments. Plant Cell, Tissue and Organ Culture, **74**:99-102.

Kudo, H. and Harada, T. (2007). A graft-transmissible RNA from tomato rootstock changes leaf morphology of potato scion. Hort. Science, **42**(2):225-226.

Lakshmi, E.N.V.S.; Kumar, S.; Sudhir, D.A.; Jitendrabhai, P.S.; Singh, S. and Jangir, S. (2022). A review on nutritional and medicinal properties of guava (*Psidium guajava* L.). Ann. Phytomed., **11**(2):240-244.

Li, G.; Tan, M.; Liu, X.; Mao, J.; Song, C.; Li, K.; Ma, J.; Xing, L.; Zhang, D.; Shao, J. and Ge, H. (2022). The nutrient, hormone, and antioxidant status of scion affects the rootstock activity in apple. Scientia Horticulturae, **302**:111157.

Liu, X.; Walawage, S.L.; Leslie, C.A.; Dandekar, A.M.; Tricoli, D.M.; Hu, H.; Huang, Y.; Zhang, J.; Xv, C.; Huang, J. and Zhang, Q. (2017). *In vitro* gene expression and mRNA translocation from transformed walnut (*Juglans regia*) rootstocks expressing DsRED fluorescent protein to wild-type scions. Plant Cell Reports, **36**:877-885.

Liu, S.; Yang, Z.; Li, G.; Li, C.; Luo, Y.; Gong, Q.; Wu, X.; Li, T.; Zhang, Z.; Xing, B. and Xu, X. (2019). Multi-omics analysis of primary cell culture models reveals genetic and epigenetic basis of intratumoral phenotypic diversity. Genomics, Proteomics and Bioinformatics, **17**(6):576-589.

Mandache, M.B. and Cosmulescu, S.N. (2023). Exploration of the bioactive compounds of *Malus domestica* and possibilities of using by-products of fruit processing in the juice industry: Review. Annals of the University of Craiova, Biology, Horticulture, Food Products Processing Technology, Environmental Engineering, **28**(64).

Mangoldt, F.V. (1895). Die Ueberhäutung von Wundflächen und Wundhöhlen durch Epithelaussaat, eine neue Methode der Transplantation1. DMW-Deutsche Medizinische Wochenschrift, **21**(48):798-799.

Miguelez Sierra, Y.; Hernȷndez Rodrķguez, A.; Acebo Guerrero, Y.; Baucher, M. and El Jaziri, M. (2017). *In vitro* micrografting of apical and axillary buds of cacao. The Journal of Horticultural Science and Biotechnology, **92**(1):25-30.

Navarro, L.; Roistacher, C.N. and Murashige, T. (1975). Improvement of shoot-tip grafting *in vitro* for virus-free Citrus 1. Journal of the American Society for Horticultural Science, **100**(5):471-479.

Ochoa, R.; Carrillo, M. and Martķnez, M. (2022). Micrografting as a tool for genetic improvement in avocado (*Persea americana* Mill.). Journal of Horticultural Science and Biotechnology, **97**(4):451-458.

Onay, A.; Pirinē, V.; Yżldżrżm, H. and Basaran, D. (2004). *In vitro* micrografting of mature pistachio (*Pistacia vera* var. Siirt). Plant Cell, Tissue and Organ Culture, **77**:215-219.

Oztekin, G.B.; Giuffrida, F.; Tuzel, Y. and Leonardi, C. (2009). Is the vigour of grafted tomato plants related to root characteristics J. Food Agric. Environ., **7**:364-368.

Padilla, I.M.G. and Encina, C.L. (2011). The use of consecutive micrografting improves micropropagation of cherimoya (*Annona cherimola* Mill.) cultivars. Scientia Horticulturae, **129**(1):167-169.

Panis, B. (2018). Sixty years of plant cryopreservation: From freezing hardy mulberry twigs to establishing reference crop collections for future generations. In: International Symposium on Plant Cryopreservation, **1234**:1-8.

Payghamzadeh, K. and Kazemitabar, S.K. (2011). *In vitro* propagation of walnut : A review. African Journal of Biotechnology, **10**(3):290-311.

Raharjo, S.H.; Witjaksono, Gomez-Lim, M.A.; Padilla, G. and Litz, R.E. (2008). Recovery of avocado (*Persea americana* Mill.) plants transformed with the antifungal plant defensin gene PDF1. 2. *In vitro* Cellular and Developmental Biology-Plant, **44**:254-262.

Rai, S.K.; Neha, A.; Neha, P.; Meena, R.P.; Kavita S. and Rai, S.P. (2012). Nutraceutical enriched vegetables: Molecular approaches for crop improvement. International Journal of Pharma and Bio Sciences, **3**(2):363-379.

Ribeiro, L.M.; Nery, L.A.; Vieira, L.M. and Mercadante-Simões, M.O. (2015). Histological study of micrografting in passionfruit. Plant Cell, Tissue and Organ Culture (PCTOC), **123**:173-181.

Rout, G.R. and Jain, S.M. (2020). Advances in tissue culture techniques for ornamental plant propagation. Achieving Sustainable Cultivation of Ornamental Plants, pp:149-188.

San Bautista, A.; Calatayud, A.; Nebauer, S.G.; Pascual, B.; Maroto, J.V. and López Galarza, S. (2011). Effects of simple and double grafting melon plants on mineral absorption, photosynthesis, biomass and yield. Scientia Horticulturae, **130**(3):575-580.

Santarosa, E.; de Souza, P.V.D.; de Araujo Mariath, J.E. and Lourosa, G.V. (2016). Physiological interaction between rootstock-scion: Effects on xylem vessels in *Cabernet sauvignon* and *Merlot grapevines*. American Journal of Enology and Viticulture, **67**(1):65-76.

Sarvade, S.; Singh, R.; Vikas, G.; Kachawaya, D.S. and Khachi, B. (2014). Agroforestry: An approach for food security. Indian J. Ecol., **41**(1)95-98.

Savvas, D.; Colla, G.; Rouphael, Y. and Schwarz, D. (2010). Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. Scientia Horticulturae, **127**(2):156-161.

Selvaraj, K.V.; Sivakumar, P.; Shalom, S.V.; Bharathi, A.; Velayutham, A. and Prabha, T. (2022). A review on the *in vitro* regeneration of the timeless panacean medicinal and aromatic herb: *Rosmarinus officinalis* (L.). Ann. Phytomed., **11**(1):247-252.

Singh, S.K. and Khawale, R.K. (2008). *In vitro* propagation of tropical and sub-tropical fruit crops. Recent advances in plant biotechnology. IK International, New Delhi, pp:157-184.

Smith, M.A.L. and Obeidy, A.A. (1991). Micropropagation of a mature, male Kentucky coffee tree. Hort. Science. **26**(11):1426.

Suįrez, I.E.; Correa, C.Į. and Dķaz, C.L. (2021). Microinjertación de naranja Valencia y lima Tahití. Temas Agrarios, **26**(1):26-35.

Teixeira da Silva, J.A.; Gulyįs, A.; Magyar-Tįbori, K.; Wang, M.R.; Wang, Q.C. and Dobrįnszki, J. (2019). *In vitro* tissue culture of apple and other Malus species: Recent advances and applications. Planta, **249**:975-1006.

Temgire, S.; Mirza, A.A.; Wani, A.W.; Ravi, K.; Tanpure, P. and Kadlag, S. (2023). Unveiling the potential: Colour enhancement and pharmaceutical properties of Crimson seedless grapes. Ann. Phytomed., **12**(1):105-114.

Torres-Viñals, M.; Sabaté-Casaseca, S.; Aktouche, N.; Grenan, S.; Lopez, G.I.L.B.E.R.T.; Porta-Falguera, M. and Torregrosa, L. (2004). Large-scale production of somatic embryos as a source of hypocotyl explants for *Vitis vinifera* micrografting. Vitis, **43**(4):163-168.

Tsaballa, A.; Xanthopoulou, A.; Madesis, P.; Tsaftaris, A. and Nianiou Obeidat, I. (2021). Vegetable grafting from a molecular point of view: The involvement of epigenetics in rootstock-scion interactions. Frontiers in Plant Science, **11**:621999.

Turnbull, C.G.; Booker, J.P. and Leyser, H.O. (2002). Micrografting techniques for testing long distance signalling in *Arabidopsis*. The Plant Journal, **32**(2):255-262.

Urias Garcia, C.; Perez Alvarez, S.; Escobedo Bonilla, C.M.; Gonzalez-Vega, M.E.; Flores Cordoba, M.A.; Guerrero Morales, S. and Palacios-Monarrez, A. (2023). Micropropagation of Vachellia spp. as an alternative for the reforestation of desert areas in Mexico/Micropropagacion de Vachellia spp. como una alternativa para la reforestacion de zonas deserticas en Mexico. Cultivos Tropicales, **44**(2):1i-1i.

Volk, G.M.; Bonnart, R.; Shepherd, A.; Yin, Z.; Lee, R.; Polek, M. and Krueger, R. (2017). Citrus cryopreservation: viability of diverse taxa and histological observations. Plant Cell, Tissue and Organ Culture (PCTOC), **128**:327-334.

Wang, M.R.; Lambardi, M.; Engelmann, F.; Pathirana, R.; Panis, B.; Volk, G.M. and Wang, Q.C. (2021). Advances in cryopreservation of *in vitro*-derived propagules: Technologies and explant sources. Plant Cell, Tissue and Organ Culture (PCTOC), **144**:7-20.

Werner, T.; Motyka, V.; Strnad, M. and Schmülling, T. (2001). Regulation of plant growth by cytokinin. Proceedings of the National Academy of Sciences, **98**(18):10487-10492.

Westwood, M.N. and Bjornstad, H.O. (1974). Fruit Set as Related to Girdling, Early Cluster Thinning and Pruning of 'Anjou'and 'Comice' Pear1. Hort. Science, **9**(4):342-344.

Wu, H.C.; Du Toit, E.S. and Reinhardt, C.F. (2007). Micrografting of *Protea cynaroides*. Plant Cell, Tissue and Organ Culture, **89**:23-28.

Xu, H.; Zhang, W.; Li, M.; Harada, T.; Han, Z. and Li, T. (2010). Gibberellic acid insensitive mRNA transport in both directions between stock and scion in *Malus*. Tree Genetics and Genomes, **6**:1013-1019.

Yao, W.; Kong, L.; Lei, D.; Zhao, B.; Tang, H.; Zhou, X.; Lin, Y.; Zhang, Y.; Wang, Y.; He, W. and Li, M. (2023). Establishment of an efficient method for kiwifruit *in vitro* micrografting. Plant Cell, Tissue and Organ Culture (PCTOC), **152**(2):427-436.

Yang, Y.; Mao, L.; Jittayasothorn, Y.; Kang, Y.; Jiao, C.; Fei, Z. and Zhong, G.Y. (2015). Messenger RNA exchange between scions and rootstocks in grafted grapevines. BMC Plant Biology, **15**:1-14.

Yang, N.; Wang, D.; Gao, Y.; Hu, E.; Yu, X.; Peng, S.; Ji, J. and Zhang, M.S. (2022). An efficient micropropagation protocol, chemical components, and hypoglycemic activity for Cremastra appendiculata (D. Don) Makino pseudobulbs. *In vitro* Cellular and Developmental Biology-Plant, **58**(2):213-224.

Yżldżrżm, H.; Onay, A.; Süzerer, V.; Tilkat, E.; Ozden Tokatli, Y. and Akdemir, H. (2010). Micrografting of almond (*Prunus dulcis* Mill.) cultivars "Ferragnes" and "Ferraduel". Scientia Horticulturae, **125**(3):361-367.

Yildirim, M.U. (2013). Micropropagation of *Origanum acutidens* (Hand Mazz) IETSWAART Using Stem Node Explants. The Scientific World Journal, **2013**(1):276464.

Zhang, W.N.; Gong, L.; Ma, C.; Xu, H.Y.; Hu, J.F.; Harada, T. and Li, T.Z. (2012). Gibberellic acid insensitive mRNA transport in Pyrus. Plant Molecular Biology Reporter, **30**:614-623.

Zilka, S.; Faingersh, E.; Rotbaum, A.; Tam, Y.; Spiegel, S. and Malca, N. (2002). *In vitro* production of virus-free pear plants. Acta Hortic. **596**:477-479.

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