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Micrografting: A *in-vitro* technique for sustainable horticulture production and its applications

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ABSTRACT

A variety of plant species, especially woody plant species, can be micropropagated using the crucial technique of *in vitro* micrografting. *In vitro* micrografting has developed over the past few decades into a method to speed up shoot recovery and adaptation of horticultural species cultivated *in vitro*. This review analyses studies on horticultural crops that address the development of *in vitro* micrografting, factors influencing its performance, and the contribution of micrografting applications to the field of micropropagation. The potential application of this technique is based on the significant contributions that micrografting makes to the recovery of vigour and rooting competence, the promotion of shoot recovery following somatic embryogenesis and organogenesis, and the facilitation of shoot regrowth following cryopreservation. This technique facilitates the genetic engineering and preservation of horticultural crops are highlighted.

Keywords: Micrografting, Horticultural Crops, *In-vitro* technique.

INTRODUCTION

Grafting refers to the accidental or intentional joining of two distinct plant segments and is a typical technique for the vegetative multiplication of crops [1]. In addition to conferring significant agronomic features to scions, such as uniformity of plant architecture and tolerance to biotic and abiotic stressors, grafting can be utilised to prevent juvenility in perennial woody species [2]. Along with influencing tree vigour, productivity, and fruit quality, the scion-rootstock combination can also lengthen the harvest season [3]. After the development of *in vitro* plant tissue culturing in the early 1900s, demonstration was carried using a grafting method by tissue culture (micrografting) in ivy and chrysanthemum, respectively, in the 1950s [4, 5]. This method was later developed and standardised for virus eradication from citrus species [6]. *In vitro* micrografting (IVM) has been used extensively to date in the following ways: (1) in pathogen management to facilitate the eradication, indexing, and transmission of pathogens; (2) to facilitate *in vitro* rooting; (3) to stimulate regenerating plant tissue cultures during micropropagation; (4) to assess graft incompatibility caused by pathogen infection; and (5) in studies focusing on the molecular basis of disease. Because of the following qualities, IVM is a crucial technology that aids in the micropropagation of horticulture plants and forest species. IVM can reduce species-specific responses of scions to the culture medium because the rootstock mediates the delivery of the hormonal and nutritional requirements necessary for the scion regrowth from the medium [7,8]. Additionally, IVM can be performed throughout the year using scions and rootstocks at the same physiological stage [9]. IVM is carried out in a humid, aseptic environment under strict monitoring. This stable *in vitro* environment and the micro-scion/likely rootstock's pathogen-free condition may favour callus formation and the speedy construction of the vascular reconnection between scions and rootstocks that are necessary for successful grafting [10,11]. There are numerous fruit crops for which micrografting protocols have been developed, viz., almond [12], apple [13], apricot [14], avocado [15], cacao [16], cashew [17], cherimoya [18], cherry [9], citrus [19], guava [2], grape [10], jujube [20], mulberry [21], hazelnut [22], kiwifruit [23], passion fruit [24], olive [25], peach [26], pear [27], pistachio [28], plum [29], walnut [30], and watermelon [31]. This chapter includes results from recent IVM-based experiments to demonstrate how IVM can be utilised to enhance micropropagation in horticulture.

Preparation of Scions

Successful micrografting depends on a number of factors, including the origin and type of scion material [32]. The scions utilised in micrografting, which are often shoot or shoot tips, can be obtained from *in vitro* or *ex-vitro*-produced plants. Scion material has traditionally been obtained from *in vitro* plants, which has the advantages of being free from microbial and fungal contaminations, the required size, and being readily available year-round [33]. However, using *ex-vitro* material could add a seasonality element to the process because the excised plant material might still be in a dormant stage [34].

Preparation of Scions

However, fresh shoot apices from trees that are actively growing in the field or in a greenhouse can be used in micrografting techniques [10]. Before preparation of the shoot/shoot tip and micrografting, in vivo plant-derived shoot apices are promptly surface sterilised. For surface sterilisation, a brief treatment with 70% ethyl alcohol is typically paired with a lengthier treatment with sodium hypochlorite or mercuric chloride [35]. During the development of in vitro cultures, tissue browning is a frequent concern [36]. Similar to this, in IVM, wounding during scion/rootstock preparation may also result in the browning and oxidation of plant tissues, which would negatively impact the effectiveness of the graft [37]. By presoaking scions in antioxidant solutions, the damaging effects of tissue browning may be reduced [38]. By presoaking the cut edge of the scion with 0.01% ascorbic acid and 0.015% citric acid (1:1) before in vitro grafting respectively, reduced phenolic exudation in cashew (*Anacardium occidentale* L.) and apple (*Malus domestica*) was noticed [39, 40]. Before preparing the scion, in vitro stock shoots can be pre-conditioned in culture conditions enriched with 0.1% polyvinylpyrrolidone to reduce tissue browning in cashew. In creating a procedure for micrografting of native and commercial roses, demonstration was conducted with silver nitrate, as an antioxidant, was essential in limiting the development of phenolic chemicals that could result in micrografting failure [41]. Prior to micrografting, they discovered that a brief dip treatment (5–10 min) with silver nitrate (50 mg L⁻¹) might stop tissue browning and hence increase the survival of micrografts on injured explants (scions and rootstocks). As opposed to this, researchers reported that *Protea cynaroides* micrografts showed decreased viability when scions were presoaked in ascorbic acid and citric acid solution; treatment with antioxidant solution caused greater browning in scions than in untreated scions [33]. These findings supported Navarro's hypothesis [42] that pre-treatment was less effective at preventing tissue browning than the quick micrografting procedure. Another reason would be that the graft site was more moist than usual, which led to an insufficient concentration of antioxidants, which inadvertently encouraged the propagation of phenolic oxidation [33]. Therefore, the doses of antioxidants and/or their combinations are other crucial issues that need to be addressed. Antioxidant's responses to the reduction or inhibition of phenolic browning may also depend on the species.

Preparation of Rootstocks

The two main sources of rootstocks used in micrografting are segments of in vitro cultivated shoots and in vitro germinated seedlings. In vitro germination of seeds is the initial stage in preparing rootstock seedlings for micrografting, and several methods are required to induce germination. According to earlier researchers, cacao seeds were extracted from mature pods, surface sterilised, inoculated in culture media, and three-week-old seedlings were utilised as rootstocks. They discovered that cacao seeds did not need any pretreatment for effective in vitro seed germination [16]. In many instances of in vitro germination, it is important to remove the seed coat. For instance, to prepare almond in vitro rootstocks, the endocarps (hard seed coats) were first removed from the seeds, which were then surface sterilised. Similarly to this, after removing the outer pericarp and shells from pistachio seeds, the mature kernels were surface sterilised [28]. To encourage seed germination, mature cashew [39] and jujube [20] seeds were scarified in strong hydrochloric acid and sulfuric acid, respectively, before being surface sterilised. Some surface-sterilized seeds have had their embryos extracted and cultured

in a germination medium to promote good germination. A successful branch tip micrografting method for *Protea cynaroides* was created utilising rootstock made of in vitro germinated embryos that were 30 days old [33]. Following seed germination, which takes different amounts of time depending on the species, the seedlings are either beheaded above the cotyledons to use the epicotyls as a grafting site [15] or cut below the cotyledons to use the hypocotyls [42]. In vitro shoots can also be employed as rootstocks in species with significant adventitious rooting rates [40, 43]. The grafted shoots are next cultivated on a medium used for root induction of the rootstock genotype, which calls for the optimization of the rooting medium beforehand. When branches (rootstock) were stimulated to root before grafting, [9] Bourrain and Charlot, 2014 found that cherry had a 79% success rate for grafting. [44] Obeidy and Smith, 1991 reported that using rooted in vitro shoots as rootstocks and apical 2-cm shoots as scions resulted in a graft success rate of up to 45% in apples. [45] Nkanaunena *et al.*, 2001 reported that the three-month-old rooted rootstocks produced the highest graft success rate (at least 60%), with better development of grafted shoots, when comparing the performance of *Uapaca kirkiana* (Muell. Arg) micrografts derived from in vitro rooted and unrooted rootstocks. The species under study may have an impact on the good reaction to the success of micrografting from the usage of rooted rootstocks. [46] Sammona *et al.*, 2018 examined the grapevine cultivar (cv.) "Superior" micrografted onto several rootstocks and discovered no distinctions in grafting performance between rooted and unrooted rootstocks.

Grafting techniques

The effective fusion of the rootstock and scion is crucial to the outcome of a micrografting technique. A significant factor in the effectiveness of in vitro grafts is the grafter's ability. The type and size of the scion propagule as well as the goal of the micrografting may influence the choice of grafting technique to be used. The most popular in vitro grafting methods, top-slit or top-wedge, have been tried out on a wide variety of genera. On the rootstock, a slit or cleft is formed, and wedge-shaped scions are put into the cleft [47]. Apical micrografting is the term used to describe the placing of small shoot tips into the slit produced at the top or immediately over the rootstocks when they are employed as scions [48]. Side grafting (also known as a side insertion) is the process of inserting tiny shoot tips into a slit on one side of the rootstock [49]. It has been successfully employed in citrus to side graft shoot apices into inverted T-cuts of rootstocks [50]. When longer shoots or nodal portions were employed as scions, side insertion was also used [31]. Due to the great susceptibility of the in vitro propagules utilised in micrografting processes to moisture, drying of the cut scion or rootstock surface may have a detrimental effect on the graft's success. Therefore, IVM should be carried out right away following the preparation of rootstock and scions to prevent dehydration [9]. To create a strong graft union, it is also crucial to guarantee solid contact between the rootstock and scion [33]. The elastic electric-wire tube [51], aluminum foil [44], Parafilm strip [20], silicon tube [52], paper bridge [41], silicone chip [53], plastic clamps [30], or alginate gel beads [54] are just a few of the tools that have been used to enable quick and efficient union between the rootstock and scion. In particular, for the top-slit and top-wedge methods, these grafting devices are used to support the graft and keep the scion and rootstock together during graft healing. Agar solution applied to the grafting zone as an adhesive material [13] or the practice of dipping the lower end of the scion in the culture medium before fitting it into the rootstock are other methods to establish and fix the graft union, particularly when the scion does not fit into the

rootstock properly. The method of dipping the lower end of the scion in the culture medium before grafting, according to [55] Pathirana and McKenzie, 2005, not only delivers nutrients directly to the graft site but also maintains moisture on the cut surfaces until the high relative humidity inside the vessel is restored after closure. With this method, micrografting has a high success rate of 75–85% in grapevine. According to earlier studies an efficient method for apple IVM involves using an agar-agar solution to affix the scion to the rootstock's vertical slit [13]. To prevent oxidative browning, they treated the V-shaped cut scion base with an antioxidant solution (0.15 mg L⁻¹, 0.1 mg L⁻¹, ascorbic acid, and 0.1 mg L⁻¹), then treated it with 1% agar-agar solution. Finally, two drops of agar solution were applied to the graft zone before the scion was attached to the rootstock. All acclimatized plants survived this procedure, and the transplant success rate was 95%. In contrast, *Protea cynaroides* micrograft survival rates were lower after medium-supported grafting than after unsupported grafting [33]. Similar to this, when antioxidant supplements or culture medium solution was administered to the micrograft union, and reported either a reduction in graft union success or no reaction, respectively. As a result, we advise applying medium-supported grafting as a fallback method for IVM [56, 57].

Culture conditions

The success of testing different culture conditions to optimize micrograft regrowth depends on the plant species and the source of the plant material used. To successfully grow rootstock seedlings, seeds are often continuously dark-lit for 1 to 6 weeks [58, 42, 59]. However, effective methods utilizing seedlings that were light-grown have also been described [60, 31, 61]. Previous studies demonstrated that the success of the grafting was correlated with the light conditions during seedling development using grapefruit micrografted onto seedling sour orange [58]. When rootstock seedlings were produced from seeds that had been continuously in the dark for two weeks as opposed to seeds that had been continuously in the light, the frequency of graft success increased from 5 to 50%. Similarly, work has been discovered that rootstock seedlings from seeds germination and growth in darkness had a higher graft success rate than seedlings germination and growth in light (2.7%) in Citrus cultivars viz., Cadenera Fina and Pera (sweet oranges) [6]. Contrarily, earlier record showed moderate success rates on rootstocks from seeds sprouted under light circumstances, ranging from 14 to 28%, for Tahitian lime and Valencia orange micrografted to seedlings from the mandarin (Cleopatra) [62]. After micrografting, plant growth circumstances can have an impact on graft success. Jujube tree (*Ziziphus mauritiana* 'Gola') micrografted plants were first grown in darkness for 10 days before being moved to light conditions [20]. They discovered that spending time in the dark before and after grafting was crucial to prevent photooxidation at the grafting site and to reduce the loss of auxins produced in the scion. Before in vivo acclimatization, micrografted almond plants were cultivated on a rooting medium and incubated in the dark for seven days, then moved to the light of 35–40 mol m⁻² s⁻¹ for two weeks, and lastly to 60 mol m⁻² s⁻¹ for one week [43]. Various supporting structures and growth mediums have been employed in micrografting methods. Micrografted plants have been supported using paper bridges, perlite, and vermiculite in liquid culture medium, as well as solid and semi-solid culture media. For instance, a paper bridge or liquid medium with perlite was used as the supporting system in the micrografting of lime [63], Cashew [39] and cut rose [41] micrografting have both been done using a liquid medium. To provide greater support for the micrografts cultivated in liquid media, a movable paper bridge can be created. The benefits of employing

liquid media include increased nutrient availability and absorption as well as less root system injury when transplanting plants. An agar-solidified medium with vermiculite was successfully used in cherry micro grafting to produce high-quality grafted plants [9]. When apple micrografted plants were grown on agar-solidified media, maximum graft success was attained [64].

Acclimatization

After a progressive change in light intensity and ventilation, micrografted plants can be transplanted into potting soil once they have developed strong roots and visible scion regrowth [28, 12]. Acclimatization is a crucial stage in micrografting protocols since transplanting micrografted plants into ex-vitro settings might result in severe losses. To remove any residual media from the roots, micrografts are taken out of their in vitro environments and rinsed with tap water [14, 8, 66]. Grafted plants are then put into pots with the substrate. Micrografted plants are kept in high relative humidity for the first few days before being gradually moved to ex-vitro conditions [67, 62]. It was possible to successfully acclimatize (83–87%) micrografted jujube plants that had been propagated for a month on a growth medium with the scion having grown to 5–10 cm in length (scions were initially 5–10 mm) [20]. Cacao plants that had been micrografted and cultivated in vitro for two weeks could be transplanted into ex-vitro environments, according to [16]. Only plants with two enlarged leaves and a scion that was at least 1 cm long survived the acclimatization process. For micrografted plants to survive acclimatization, there must be roots on the rootstock. According to the recent research, passion fruit micrografted plants without roots did not survive acclimation; plants with roots created in vitro had the highest survival rate [24]. Species-to-species variation exists in the survival rate of micrografted plants after acclimation. For instance, the survival rate of grafted plants in the apple reached 100% in the almond [67] it varied from 85 to 100%, in the cacao [13] and in the passionfruit [24] it was 82% and 75%, respectively, while in the Tahitian lime and Valencia orange it ranged from 47 to 50% [62]. Conflicting results were reported when they micrografted sweet orange buds produced through organogenesis [68]. In that instance, the fully developed in vitro micrografts grew slowly in the greenhouse, so the micrografted plants were re-grafted onto three-month-old seedlings of Rangpur lime for rapid acclimatization and normal development of the plants. However, when plants were challenging to manage in traditional tissue culture or to establish roots on, micrografted plants generally had a higher success rate of acclimatization than ungrafted plants [43].

Applications of micrografting

Root establishment

A crucial step in micropropagation procedures is in vitro rooting. The fundamental barrier to micropropagation for several species has been the inability to induce adventitious root development. To produce roots and get around rooting issues in the vegetative multiplication of these species, IVM is an alternate method. For instance, the in vitro recalcitrance of *Protea cynaroides*, a significant ornamental species endemic to South Africa, *Lens culinaris*, a significant pulse crop of the Mediterranean region, and some Prunus species was overcome using IVM of micro shoots onto rootstock seedlings. Previous reviews have emphasized the need for the development of appropriate micrografting techniques to address plant species' rooting challenges [69, 70]. The limiting phase in *Garcinia indica*'s in vitro propagation is root induction [71]. Also, devised a micrograft methodology to address

this issue by periodically grafting shoot tips onto in vitro juvenile seedlings to reestablish rooting competency. They used micro grafting to revitalize in vitro shoots generated from 20-year-old trees by employing the 2-month-old in vitro seedlings as rootstocks. After being severed at the lowest node, the elongated shoots (scions), which were about 0.5–1.0 cm in length, were cut into a V-shape at the bottom and put into a vertical incision in the rootstock. The apical part of the scion was clipped (1–1.5 cm in length) after the graft union had formed (6–8 weeks), and it was regrafted onto fresh in vitro rootstock. *Annona cherimola* shoot in vitro rooting was challenging, but it was possible after 1–2 cycles of micrografting onto rootstock seedlings [18]. Similarly to this, three cycles of in vitro grafting enhanced the jujube's root capacity [20]. Even after two successive rounds of micrografting onto rootstock seedlings, *Juglans regia* (walnut) in vitro adult clones failed to establish adventitious roots, even though several research had used micro grafting to encourage rooting. After 30 cycles of in vitro subcultures, nevertheless, a satisfactory root induction rate could be achieved [72]. Therefore, more research is still required to enhance the stimulation of roots in walnut. In vitro germinated seedlings are frequently used as rootstocks in micrografting for better rooting.

Shoot establishment

In vitro plants that have been grown for a long time frequently show diminished regeneration capacity [73]. After consecutive grafting onto robust rootstocks, the lowered proliferation might be restored in vitro [74]. Earlier researchers have micrografted the nodal segments of three cherimoya cultivars onto in vitro germinated seedlings to enhance the micropropagation procedure [18]. When compared to normally in vitro cultivated segments, proliferation from shoot segments was observed to be significantly improved in all micrografted plants. The Ferragnes and Ferraduel almond cultivars micrografted onto in-vitro grown wild almond seedlings also successfully restored shoot proliferation [65]. For better shoot proliferation of the cultivar Zard throughout several culture cycles. After the third successive micrografting, the success of the micrograft, shoot elongation, and bud sprouting all improved [75]. The growth of the scions and the proportion of rooted micrografts were both enhanced in *Ziziphus mauritiana* by repeated micrografting (2 times) of in vitro shoots onto in vitro germinated seedlings. Following micrografting, numerous plant species, including cherimoya, mandarin and sweet orange, improved in vitro roots and shoot proliferation.

Embryo rescue

In vitro mutagenesis and the production of genetically modified plants may benefit from the recovery of plants through de novo organogenesis and somatic embryogenesis [76]. However, it can be problematic in some horticultural species because of issues with roots or because of insufficient callus maturation and tissue culture. Many regenerants formed from organogenesis are unable to easily produce roots, therefore micrografting has been used to solve this problem [77]. Sunflower shoots grown from leaves were micrografted onto in vitro-germinated seedlings using a side insertion technique to address the poor rooting capacity shown in these shoots [31]. With this technique, the 0.5–1.0 cm-long shoots with a wedge-shaped base were inserted into the longitudinal cut at the hypocotyl to achieve the best survival (75%) rate. The acclimatized micrografted sunflowers flowered and produced seeds satisfactorily. IVM was used to enhance the growth of sweet orange (*Citrus sinensis*) stem segments that were regenerated from thin sections [68]. [78] Almeida *et al.*, 2003 evaluated the use of micrografting with plantlets of *Carrizo citrange* as rootstocks to assist

shoot recovery following genetic transformation using the same regeneration strategy in four sweet orange cultivars. IVM was also used on the pepper plant (*Capsicum annuum*) to produce rooted transgenic plants that had undergone organogenesis from cotyledons [79]. Notably, field pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L) ex-vitro micrografting were applied in legumes employing in vitro-regenerated shoots as scions and ex- vitro germinated seedlings as rootstocks to permit grafting and acclimation simultaneously [80]. Similar procedures were also applied to produce rooted plants from adventitious pear branches generated from cotyledons [81]. The use of IVM to enhance the recovery of shoots regenerated from somatic embryos (SEs) of cocoa plants was first documented [82]. Raharjo and Litz, 2005 suggested an efficient micrografting method for SE shot rescue in avocados. They grafted (V-shaped cut) SE-derived shoots that were 5 to 10 mm in length onto in vitro rootstock seedlings for their investigation, and the grafted plants were subsequently cultivated on a phytohormone-free medium. This procedure allowed for the establishment of micrografted plants after 3–4 weeks and the recovery of 70.5% of the SE-derived shoots, compared to just 30.4% of non micrografted SE shoots and no recovery of normal plantlets. Additionally, ex-vitro grafting was done in accordance with the micrografting procedure, and this served as a protocol for saving modified avocado materials. In the case of avocado, effective IVM recovery of transgenic plants was reported [83]. In this study, *Agrobacterium* was used to convert globular somatic embryos developed from immature zygotic embryos. To improve recovery after selection on kanamycin, the germinated somatic embryos were then lengthened to 3–5 mm before being micrografted onto in vitro-germinated seedlings. To induce complete recovery or somatic organogenesis in seedless sweet orange, micrografting of ovary-derived somatic embryos onto in vitro seedlings proved effective [84].

Cryopreservation

The long-term, cost-effective management of plant genetic resources is now thought to be facilitated by cryopreservation [85, 86]. Many horticultural species' shoot tips have been preserved in cryobanks; successful cryopreservation calls for a high level of post-thaw recovery [87]. In some species, such as citrus, direct shoot tip recovery was not possible; to address this, cryopreserved shoot tips were micrografted onto in- vitro produced seedlings. Method of vitrification were used to successfully recover citrus shoot tips that had been cryopreserved [88]. They prepared six-week-old in vitro 'Carrizo' citrange seedlings as rootstocks to support the shoot tips that had been cryopreserved. In their study, rootstock seedlings with a height of at least 3 cm were decapitated 1 cm above the cotyledonary node with a 2-mm deep incision made into the cut surface, then a horizontal cut across the seedling was done to create a "ledge" or "step" at the cut surface. The basal section of cryopreserved branch tips was removed and put on this rootstock ledge. For eight Citrus and Fortunella species, this post-thaw procedure led to an average of 53% of regrowth. To enable the post-thaw recovery of 150 pathogen-free citrus accessions representing 32 taxa following droplet-vitrification cryopreservation, used the same IVM technique [88]. 24 taxa that underwent this technique exhibited mean rates of regeneration after cryopreservation of above 40%. There are ongoing efforts to utilize this effective method to restore plants after cryopreserving a variety of citrus species [89]. The recovery of cryopreserved Chinese jujube (*Ziziphus jujuba*) shoot tips required micrografting [90]. Without micrografting, cryopreserved shoot tips cultivated on recovery medium only produced leaves. There was no shoot regeneration. In

contrast, when shoot tips were micrografted onto sour jujube (*Ziziphus spinosa*) rootstock seedlings, a high shoot recovery rate of 75% was achieved. This process worked well to create plants free of phytoplasmas associated with jujube witches' broom. To produce plants devoid of phytoplasma and enable cryopreservation, and micrografting was used. While micrografting has been thoroughly investigated in Citrus to assist cryopreservation methods, it has not been thoroughly investigated in other species^[88, 89]. Micrografting may help woody plants that are still resistant to cryopreservation grow back their tried processed shoot tips. Pistacia species, where shoot tip cryopreservation led to poor recovery levels ranging from 5.0 to 17.6%, are among the plants that might profit from this strategy^[91]. Though studies concentrating on shoot tip cryopreservation are still required for the safe conservation of elite avocado cultivars, SEs have been proven to be responsive to cryopreservation in the case of avocados^[92]. Therefore, in the case of pistachio, avocado, and other resistant plant species, micrografting could be seen as a method to enhance shoot tip regrowth following cryopreservation.

CONCLUSION AND FUTURE PROSPECTS

For numerous plant species, IVM procedures have been created with varying degrees of success. The origin and preparation of rootstocks and scions, grafting techniques, graft growth conditions, and acclimatization are just a few of the variables that affect how successfully plants recover after micrografting. These variables are genotype- and species-specific, just like in vitro tissue culture. Therefore, to increase the utilization of micrografting in micropropagation, additional development and optimization of micrografting procedures are required, especially for refractory species. Although the majority of horticultural species have successful micrografting protocols in place, these protocols are technically challenging and must be carried out by specialists who have the right tools, skills, and training. Therefore, advancement is still required to streamline micrografting processes. The programs using IVM for micropropagation also always involve moving the micrografted plants into ex-vitro environments, so it is important to assess how acclimation affects micrograft plantlet survival as well as long-term survival in the event of partial graft incompatibility. IVM is still used in some plant species as an essential step to give roots for in-vitro grown propagules, permitting further acclimatization, despite advancements in vitro plant tissue cultures over the years. Seedlings grown from in vitro germinated seeds are frequently employed as rootstocks in micrografting techniques in species with problems in vitro rooting. In some cases, successive micrografting of shoots onto in vitro germinated seedlings has been successful in restoring vigor and rooting competence for species that demonstrated decreased rooting and shoot proliferation after lengthy in vitro cultures. Additionally, IVM has aided in the recovery of shoots produced by somatic embryogenesis and de novo organogenesis, which are frequently used as sources of explants for genetic modification. To facilitate the genetic modification of horticulture plants like citrus and avocado, IVM has been implemented. IVM is also necessary for citrus shoot tips to survive cryopreservation to support post-thaw healing and shoot regrowth. With the aid of micrografting, large-scale cryopreservation of citrus has been achieved, guaranteeing the secure and long-term preservation of its priceless genetic resources. To restore cryopreserved species that are still resistant to cryopreservation techniques, micrografting may be used in light of the accomplishments seen in citrus cryopreservation.

Conflict of interest

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