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RESEARCH ARTICLE

Micrografting of *Pistacia vera* L.: A review

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Abstract

Pistacia vera is widely propagated by budding or via grafting onto a convenient rootstock to obtain determined sex type trees at the early stage of growth or deal with the environmental issues that may face *P. vera* during production. Conventional budding and grafting will have certain problems such as season-dependent and waiting for the seedling for a long time to reach budding and grafting size. Therefore, micrografting has been adopted in the last decades to overcome grafting problems effectively. Micrografting of *P. vera* essentially depends on many factors, for instance, the type of media along with supporting materials applied in the media, whether the micrografting is conducted *in vitro* or *in vivo*, the species of the rootstock used, age of the donor plant of the explant, scion size, the technique employed to the micrografting process. According to the results that have been achieved, pistachio micrografting could be conducted when the above-mentioned factors were controlled. However, the effect of growth regulators and the age of scion need to be further investigated in the future. In this review, the results of *P. vera* micrografting are highlighted, which have been obtained in the last decades, aiming to update the expertise in this area with the latest information about *P. vera* micrografting and provide clarity about the gaps that still exist in the micrografting of this species.

Keywords: Media; Pistachio; Rootstock; Size; Technique.

1. Introduction

Pistachio (*Pistacia vera* L.) is a member of the Anacardiaceae family which has a dioecious nature (Torun *et al*., 2021). The ancient Mesopotamia region, encompassing Syria, Iraq, Iran, and certain areas of Turkey, is the origin place of *P. vera* (Nezami and Gallego, 2023; Ibrahim and Mayi, 2023). The genus *Pistacia* embraces 13 species, among them; *P. vera* is the only one that is commercially cultivated for its valuable nuts (Onay *et al.*, 2005). The nuts contain magnesium, potassium, calcium, protein, carbohydrates, dietary fibres, fat, folic acid, vitamin K, gamma-tocopherols, phytochemicals, and polyphenols (Dreher, 2012). Collectively, nuts significantly improve heart-related conditions, blood pressure, cholesterol level, vascular stiffness, endothelium, and gastrointestinal functions, controlling weight, metabolism of glucose, kidney function, and allergies (Nadimi *et al*., 2019). The thriving areas of pistachio have sufficient chilling requirements to break the dormancy of the buds and long warm, summers. A warm and long summer provides the necessary heat for the trees to accumulate enough growing degree days, which helps maturation of the nuts with good flavor and splitting shell, and guarantees pollen is transferred from male to female trees by wind or pollinators (Ferguson *et al*., 2005). Therefore, it is tolerable for high summer temperatures, but excessive dampness

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and high humidity are unfavorable for the growth of this species (Esmaeilpour *et al*., 2015). The trees of *P. vera* are propagated from seedlings grown from seeds, budded and grafted seedlings, or micro-propagated seedlings. The most common one is obtained from budding or grafting on suitable rootstocks. The reasons why *P. vera* is commonly propagated from budding or grafting increasing disease resistance, drought and salinity tolerance, enhanced tree performance to absorb water and minerals, also obtaining seedlings with certain sex types at the early stages of growth and reducing juvenile phase (Rahneshan *et al*., 2018; Moriana *et al*., 2018; Mohammed, 2022).

The rootstocks that have been used for budding or grafting *P. vera* are *P. atlantica*, *P. chinensis*, *P. integerrima, P. khinjuk, P. lentiscus, P. vera, P. mutica, P. palaestina,* and *P. terebinthus (*Sheikhi *et al*., 2019). However, budding and grafting of *P. vera* are challenging owing to dilatory growth and inadequate success percentage (Acar *et al*., 2017). Furthermore, Marín *et al*. (2016) referred that grafting on suitable rootstocks is needed for cultivars of *P. vera,* but the uncertainty of grafting success prevents farmers from meeting the demanding plants. Several grafting failures have been exhibited in pistachio. According to Guerrero *et al*. (2004), many variables, including temperature, humidity, oxygenation, rootstock activity, and rootstock diameter, affect the success of pistachio grafting. Given that environmental conditions are out of our control, all of these contribute to graft success rates that are typically poor. In contrast to other fruit plants where graft compatibility is the main concern, pistachio grafting appears to be impacted by the scion's and rootstock's physiological conditions (Lorente *et al*., 2011). Thus, a better grafting method that contributes to elevated success percentage is inevitable.

In the last few decades, plant tissue culture techniques have been widely used in various domains such as micropropagation, callus induction, cell and protoplast culture, and plant breeding. Micrografting, one of the applications of micropropagation, is an alternative technique to obtain grafted plants. In this technique, a shoot tip or meristem explant is placed onto a rootstock which is aseptically raised from micropropagation or seed (Hussain *et al*., 2014). Despite micropropagation, micrografting is used for many other purposes, such as studying plant signaling, developing virus-free plants, virus indexing, validating graft incompatibility via grafting *in vitro*, shortening the hardening time of micro-propagated plants, and rejuvenation and/or reinvigoration of tree species (Mondal *et al*., 2005; Chilukamarri *et al*., 2021). Micrografting also eliminates the essential season-dependent aspect of conventional grafting (Pant and Husen, 2022). For the micrografting of *P. vera*, the *in vitro* plantlets that micro-grafted were successfully acclimatized, and the establishment of micro-grafted plants *in vivo* was achieved without any problem encountering (Onay, 2003a). The findings of numerous investigations on *P. vera* micrografting will be examined in depth in this review to update the expertise in this area with the latest information about *P. vera* micrografting and provide clarity about the gaps that still exist in the micrografting of this species.

2. Explant selection and preparation along with sterilization

The main difficulty in aseptic cultures of *P. vera* explants grown in a field is slow growth as a result of browning and blackening. A large quantity of polyphenols is released from the explants of mature *P. vera* into the media during a few hours after culturing, which causes the death of cultures. To get rid of this problem, Onay *et al*. (2007) reported that this issue was resolved very well by a series of bleach treatments and washes with sterile distilled water. They further indicated that the explant position did not affect the survival percentage, but apical tip explants taken from the pruned shoot trunks showed the

longest shoot compared to those from the orthotropic trunk's end or the plagiotropic trunk's end. Marín *et al*. (2016) collected explants of *P. vera* cultivars ('Kerman', 'Larnaka', 'Peters', 'C-Especial', and AD15), then they were immediately submerged for 15 min into 1 mM citric acid + 1 mM ascorbic acid antioxidant solution. Explants early death has occurred to all cultivars except for AD15. Therefore, the explants of 'Peters', 'Kerman', and 'Larnaka' were submerged again in the same antioxidant for 7 h and 'C-Especial' for 24 h. Following, antioxidant treatment the explants were washed for 1 h under tap water, then disinfected with 0.05 % (w/v) $HgCl_2$ or bleach-detergent solution (5 g active Cl L^{-1}) for 15 min. The results confirmed that short treatment with the antioxidants was not effective, in contrast, lengthening the treatment with the same antioxidant for 7 h permitted initiating cultures from 'Larnaka', 'Kerman', and 'Peters' cultivars, but not from 'C-Especial'. Contamination was reduced by the application of diluted bleach and $HgCl_2$ disinfectant solutions. Moreover, Benmahioul *et al*. (2016) found that *P. vera* explants from young seedlings were easily disinfected and $HgCl_2$ or NaOCl disinfectants decreased contamination percentage.

3. Media for micrografting of *P. vera*

Culture medium type is among the important factors that impact the growth rate of plantlets micro-grafted *in vitro*. Rehman and Gill (2014) reported that the phase and composition of the medium have a major impact on graft success, necrosis, vigor, and verification. Liquid, semi-solid, and solid are the three types of media prevalently used in micrografting. The three media have been used with micrografting of *P. vera*. Abousalim (1990) and Abousalim and Mantell (1992) employed MS liquid medium supplemented with 30 g L^{-1}. Similarly, a liquid MS medium enhanced with 0.5 mg L^{-1} BAP, 250 mg L^{-1} casein hydrolysate, 250 mg L^{-1} malt extract, and 1% activated charcoal was utilized for micrografting of *P. vera*. Semi-solid MS medium supplemented with 30 g L^{-1} sucrose was used by Onay *et al*. (2004); whereas, Onay *et al*. (2003b) utilized a solid medium containing Gamborg vitamins, 20 g L^{-1} sucrose, and 7 g L^{-1} agar. Liquid medium works well for *P. vera* and other fruits micrografting most of the time (Rehman and Gill, 2015). Liquid media has many advantages over semi-solid and solid. It provides micro-grafted plants in terms of better growth, higher nutrient absorption, and lesser root system damage (Wang *et al*., 2022).

4. Role of plant growth regulators in *P. vera* micrografting

Growth regulators play inevitable role because scions for micrografting are obtained *in vitro* through micropropagation. Also, in the matter of using micro-cuttings, the rootstocks are produced via micropropagation. In spite of these, auxin and cytokinin growth regulators increased the successful rate of micrografting when micro-scions were quickly dipped in these growth regulators and inserted into the rootstock (Hussain *et al*., 2014), but there are no reports of *P. vera* micrografting. In the case of *P. vera* explant initiation, Gabr and Hassanen (2012) achieved the best shoots number and length at 1 mg L^{-1} 6-benzyl adenine (BA), and root initiation and growth at 2 mg L^{-1} indol-3-butyric acid (IBA). Besides, Benmahioul *et al*. (2016) excised nodes of *P. vera* and cultured in an MS medium supplemented with different cytokinins at various concentrations. BA at 1 mg L^{-1} was the best for shoot induction and growth, and 2 mg L^{-1} meta-topoline was excellent for axillary shoot proliferation. The shoots that were longer than 2 cm could be rooted *ex vitro* by treatment with Rhizopon® (2% IBA), a commercial rooting powder.

5. Using media support materials in micrografting of *P. vera*

In the cases of using liquid media for micrografting of pistachio, supporting materials have been used (Rehman and Gill, 2015). The advantages of using liquid media are the dispersion of the nutrients in a homogenous way which allows the explant to absorb the medium components effectively and reduces the explant's oxidative stress (Shukla *et al*., 2020). On the contrary, the drawback of this medium is hyperhydricity, which consequently leads to failure of shoot development and rooting as a result of anatomical deformities originating from a physiological abnormality of the tissues (Prasad and Gupta 2006). Various supporting materials like polypropylene fibre (milcap), filter paper bridge, vermiculite, cotton fibres, cellulose fibres, or glass seeds can be applied aiming to overcome hyperhydricity (Grzegorczyk-Karolak *et al*., 2017). polypropylene fibre (milcap) and filter paper bridge were the two supporting materials studied in micrografting of pistachio (Chilukamarri *et al*., 2021). Polypropylene fibre was first applied to Micrografting by Deogratias *et al*. (1986) to avoid asphyxiation and increase ventilation in the rooting media of the rootstocks. They observed that this substrate, moistened by the nutrient medium, has the effect, on the one hand, of simplifying handling initially (time-saving, ease of transfer into tube); on the other hand, to allow harmonious development of the root system of the rootstock whose vigor affects the graft recovery. Besides, Davoudi Pahnekolayi *et al*. (2019) indicated some benefits of filter paper bridges in micrografting media. First, it is inexpensive and can be easily disinfected for *in vitro* purposes. Second, it is modifiable by using two movable arms regarding the diameter of the rootstock and scion. Third, more growth agents can be absorbed from the culture medium, and more contact with the graft area is ensured because it has a paper texture, which may enhance callus production. Finally, detaching from the grafting region will be easy without damaging, after successful micrografting. However, the results of micrografting of *P. vera* revealed that milcap (polypropylene fibre) was the best when the seeds were directly germinated on the top of the 'Milcap' support as an alternative for using filter paper bridges (Abousalim, 1990). Moreover, Abousalim and Mantell (1992) found that 'Milcap' support system is the most reliable technique for *P. vera* micrografting, which provided the root system with good growth and branching, and pistachio micrografts reached 100% as a result of this system. Also, a more rapid and extensive scion elongation was detected on top of this system. Further, this system facilitated grafting manipulations, and root damage was prevented during the micrografting.

6. *In vivo* and *in vitro* micrografting of *P. vera*

The two practiced method applied in micrografting are *in vivo* and *in vitro*. Considering *in vitro* micrografting, the rootstocks are produced from either the seeds after germination on an aseptic medium or from micro-propagated micro-cutting. The scion for *in vitro* micrografting is achieved from *in vitro* propagated shoots (Sanjaya and Rai, 2003). While *in vivo* micrografting involves the grafting of a rootstock obtained from a seedling or cutting propagated under greenhouse or nursery conditions. In this method, the scions are prepared from a shoot produced *in vitro* or from newly formed plants grown in greenhouses (Sanjaya *et al*., 2006). An aseptic, humid environment with strict monitoring is employed for *in vitro* micrografting. Construction of the vascular reconnection between micro-scion and rootstock will occur under this pathogen-free, *in vitro* condition (Ashrafzadeh, 2020). Furthermore, in some species physiological and anatomical problems will be encountered as conventional grafting is used, but *in vitro* micrografting gives the opportunity to overcome these problems (Aazami and Bagher, 2010).

Regarding *in vivo* method, is a modified method of *in vitro* micrografting in which disinfected tips from the flushes of terminal buds are excised and inarched onto healthy rootstocks (Takahara *et al*., 1986). Certain advantages can be guaranteed with *in vivo* micrografting in comparison to the *in vitro* method; for instance, it is a simpler procedure that requires no aseptic handling and transplant shock will not happen to micro-grafted plants compared to the ones transferred to *in vivo* condition after *in vitro* micrografting (Ogata and Yamanaka, 2021). The investigation with *P. vera* micrografting proved that *in vivo* and *in vitro* methods could be successfully conducted. Abousalim (1990) studied *in vivo* and *in vitro* micrografting of *P. vera*. He observed (83 - 92%) success for *in vitro*, and *in vivo* micrografting resulted in (83%) success when seven-week-old *P. vera* seedling rootstocks were grafted with scion originating from micro-propagated of four-year-old *P. vera*. Another study showed that *P. vera* can be grafted *in vitro* or *in vivo* and the best results were observed as the seedling rootstocks of *P. vera* micro-grafted with *in vitro* scions of *P. vera* initiated from 1-year-old plants (Onay *et al*., 2003b). Marín *et al*. (2016) germinated seeds of *P. terebinthus* L. in Pindstrup substrate inside a greenhouse and after 4 weeks they *in vivo* micro-grafted with the acclimatized *P. vera* scion of AD15 and different non-acclimatized 'Kerman', 'Larnaka', and 'Peters' scions. The results confirmed that (61 %) of acclimatized AD15 and (75.7%) of *in vitro* 'Larnaka' succeeded, but scion taken from acclimatized AD15 developed new shoots in a shorter time. Despite the production of rootstocks from seeds for micrografting *in vitro* or *in vivo*, *P. terebinthus* rootstocks were micro-propagated *in vitro*, and the produced micro-cuttings with and without roots were micro-grafted *in vitro* with *in vitro* scions of *P. vera*. Some of the successful grafted plants could be acclimatized *in vivo* (Lorente *et al*., 2011).

7. Micrografting of *P. vera* onto different rootstocks

Grafting of *P. vera* onto certain rootstock is conducted as a means to propagate or to control some edaphic and climatic issues will face pistachio production worldwide. Nowadays, the most prominent rootstocks used for *P. vera* are *Pistacia vera* L., *Pistacia atlantica* Desf, *Pistacia khinjuk*, *Pistacia terebinthus* L., *Pistacia integerrima* L., and *P. integerrima* × *P. atlantica* hybrid (UCB1) (Parfitt *et al*., 2012; Surucu *et al*., 2020). Whereas, the region of the production has a decisive role in determining which rootstock is the best. *P. terebinthus* rootstock in the Mediterranean basin, *P. integerrima* and UCB1 in the USA, and *P. vera* in Iran are widely used (del Carmen Gijón *et al*., 2010). Despite this, the strength of the rootstocks of *P. vera* is different to reach grafting size, for example, *P. vera* itself reaches grafting size earlier more than other rootstocks (Mohammed, 2022). *P. terebinthus* highly resists drought and cold, inversely it is easily infected with verticillium and more difficult to graft than *P. atlantica* and *P. integerrima* (Ferguson *et al*., 2016). *P. integerrima* is tolerable for verticillium, but susceptible to forest. While *P. atlantica* is the most susceptible to verticillium, but tolerable to cold more than *P. integerrima* and lower than *P. terebinthus* (Ferguson *et al*., 2005). The rootstocks of these species are obtained by either seeds or cloning via *in vitro* techniques (Onay, 2003a), then they are grafted by conventional methods or *in vitro* and *in vivo* micrografting. For the first time, *P. vera* microscion could be *in vitro* and *in vivo* micro-grafted onto *P. vera* rootstocks by Abousalim (1990). In another attempt, Abousalim and Mantell (1992) developed a successful micrografting technique for *P. vera*, in which they micro-grafted *in vitro*-derived seedling rootstocks of *P. vera* with micro-scions of the same species. *P. vera* rootstocks were achieved from germinated seeds *in vitro*, and achieved *in vivo* via growing seedlings in pots

inside a greenhouse. When the *in vitro* and *in vivo* seedlings respectively reached 12-day-old and 3- to 5-month-old, they micro-grafted with *P. vera* cv. 'Siirt' scion. The results indicated that micrografting was effective both *in vivo* and *in vitro*, but the *in vivo* micrografting method allowed the new axillary branches to grow and mature well (Onay *et al*., 2003b). Shoot tips of *P. ver* cv. 'Siirt' were collected and multiplied *in vitro*, then scions prepared from regenerated shoots of these shoot tips and grafted onto *P. vera* rootstocks raised *in vitro* from nuts. The micrografting was successful and the *in vivo* establishment of micro-grafted plants encountered no problems (Onay *et al*., 2004). Additionally, *in vitro* germination of mature dry nuts of *P. atlantica, P. terebinthus, P. khinjuk,* and *P. mutica* was carried out and used as rootstock for micrografting of *P. vera* cv. 'Siirt' scion. The scions of 'Siirt' pistachio were taken *in vivo* from shoot tips of grown trees, after a series of sterilization they directly micro-grafted onto the different rootstocks *in vitro*. No growth between the scion and the rootstocks was achieved (Can *et al*., 2006). Onay *et al*. (2007) assessed *P. vera, P. terebinthus,* and *P. khinjuk in vitro* seedling rootstocks for micrografting with *in vitro* obtained scions of *P. vera* cv. 'Siirt'. The results showed that *P. vera* gave rise to the best stem diameter in comparison to *P. terebinthus* and *P. khinjuk*, which is crucial for holding the micro-scions. In the continuation of the *P. vera* micrografting, Lorente *et al*. (2011) obtained *P. terebinthus* rootstocks *in vitro* as micro-cuttings, and rooted and unrooted ones were micro-grafted with *in vitro* scions of *P. vera*. Following 3-5 weeks, 44% of the grafted plants survived and growth continuing was noted in some of them in the greenhouse. Moreover, taking into account *P. terebinthus* as rootstock, Marín *et al*. (2016) employed different *in vitro* scions of *P. vera* belonging to acclimatized AD15 and non-acclimatized 'Kerman', 'Larnaka', and 'Peters', to graft onto *in vivo* seedling rootstocks of *P. terebinthus* in a greenhouse. Micrografting of both types of scions (acclimatized and non-acclimatized) was successful onto *P. terebinthus* rootstock. Rootstocks of *P. vera* cv. 'Akbari' and 'Badami-Riz-Zarand' were derived via *in vitro* seed germination and grafted with *in vitro* micro-scions of 'Ahmad Aghaei', 'Akbari', 'Badami Sefid', and 'Kalle Ghochi'. The best outcome was in grafting of 'Badami-Riz-Zarand' rootstock with 'Badami Sefid' scion and the worst was in the grafting of 'Akbari' rootstock with 'Kalle Ghochi' scion (Tabeei *et al*., 2020). Moreover, *P. vera* can be used as rootstock to micrograft the scion of other *Pistacia* species, Süzerer *et al*. (2014) successfully micro-grafted *in vitro* scions of *Pistacia lentiscus* L. onto seedling rootstock of *P.vera*. In comparison to *P. terebinthus, P. atlantica, and P. khinjuk* rootstocks, *P. vera* rootstock reached grafting size sooner and had the largest stem diameter.

8. Donor plant age of explant of the scion

The final success of micrografting largely relies on several different factors that make micrografting a quite complex procedure. The physiological state and the age of the donor plant of the explant from which the scion is multiplied are one of them (Selby *et al*., 2005). Generally, the explants are collected from aged plants not favorable in micropropagation because callus initiation is difficult from these explants, and exudation of toxic phenols in aged explants is more than those explants from plants at the juvenile stage (Ahmad *et al*., 2013). Besides, rejuvenation of the plants through *in vitro* culture may be difficult if the explant is prepared from aged plants (Basto *et al*., 2012). Furthermore, the age of the donor plant correlates with the ability of the explant to disinfection. Aged explants with poor physiological state are sensitive to disinfection (da Silva *et al*., 2016). Regarding micrografting of *P. vera*, the studies have explained that the scions raised from young tree explants gave rise to better results. In his pioneer work, Abousalim (1990) selected *in vitro* scions

of *P. vera* originating from 4-year-old trees grown in the greenhouse over the scions raised from a 30-year-old tree grown in the field to micrograft onto seedling rootstocks of *P. vera* produced *in vitro* and *in vivo*. The purpose of this selection was to get rid of the adverse consequences of browning and take benefits of vigor and faster growth of the young scions. Additionally, scions of *P. vera* trees at different ages (1-, 5-, 10- and 30-year-old) were developed *in vitro* and micro-grafted onto *P. vera* seedling rootstocks *in vitro* and *in vivo*. In both methods, the scions developed from the 1-year-old plant were the best and grew into new shoots rather than the scions that belonged to the tree with the 30-year-old. Moreover, axillary shoot development on the scions derived from 10- or 30-year-old trees was few (Onay *et al*., 2003b).

9. Effect of scion size

One of the effective characteristics of the scion directly related to a successful micrografting process is the size of the scion. It is hard to graft a small scion and requires additional components in the culture media. Inversely, the application of a large enough scion may guarantee higher plant growth regulators together with nutrient reserves (Smith, 2012). On the other hand, large scions may contain more leaf primordia which in turn will grow into leaves earlier and help an effective micrografting (Naddaf *et al*., 2023). Necrosis and micrograft vigor are significantly influenced by the scion size as well (Sridhar and Venugopal, 2019). Similarly, in the micrografting of *P. vera*, the size of the scion has an apparent role in obtaining an effective micrografting. Grafting success was 100% with 10 mm *P. vera* scions, 83-92% was gained with 1-3 mm scion, only 33% of the grafted plants survived at 0.5-0.7 mm scion, and scion length lower than 0.3 mm was irresponsive to micrografting (Abousalim, 1990). Various *P. vera* scion length was estimated at <0.5, 0.5–1, 2–4, 4–6 and >10 mm. The analyzed results proved that a parallel increase in micrografting success was noted with an increase in scion length to 6 mm, but at >10 mm decreased. Whereas, shoot tips completely become necrotic at <0.5 mm scion length (Onay *et al*., 2004). In addition, Tabeei *et al*. (2020) studied two *P. vera* scion sizes (<5 and between 5-10 mm) from four cultivars ('Ahmad Aghaei', 'Akbari', 'Badami Sefid', and 'Kalle Ghochi') for micrografting on two *P. vera* rootstocks ('Akbari' and 'Badami-Riz-Zarand'). The best result was recorded at Badami Sefid scion at 5-10 mm size onto the 'Badami-Riz-Zarand' rootstock.

10. Micrografting techniques of *P. vera*

The studies confirm that close contact between the cambial tissues of the scion and rootstock ascertains an efficient micrografting (Channuntapipat *et al*., 2003). To make this firm contact between the scion and rootstock happen, the skillful of the grafter and the technique by which the scion is put onto the rootstock are the key determinant (Wang *et al*., 2022). Many different techniques have been applied in the micrografting process, and the type and size of the scion together with the aim of the micrografting decide which one should be selected (Yıldırım *et al*., 2010). For micrografting of *P. vera*, slit micrografting, wedge micrografting on the stump, and wedge micrografting in the leaf axil have been investigated (Onay *et al*., 2007). In slit micrografting, the scion is cut in a V-shape and inserted into a vertical slit produced in the decapitated rootstock. Wedge micrografting on the stump comprises a wedge that is made into the stump of decapitated rootstock to match with a V-shape cut scion. While wedge micrografting in the leaf axil consists of leaving a single leaf on the rootstock after decapitation, then the leaf blade is removed and the leaf axil is cut in a wedge shape to insert the scion into it. (Onay *et al*., 2004) found that slit micrografting was the best technique for *P. vera* related to wedge

micrografting on the stump and wedge micrografting in the leaf axil. They further demonstrated that the plants micro-grafted with the slit technique formed a good union (80%), and 75% of them developed axillary shoots. In the context of employing various techniques to micrograft of *Pistacia* spp., slit micrografting and wedge micrografting on the stump were used for micrografting *P. lentiscus* onto *P. vera, P. terebinthus, P. atlantica, and P. khinjuk* rootstocks. Higher percentages of mature shoot tips were successfully micro-grafted as a result of slit micrografting (Süzerer *et al*., 2014).

11. Conclusion

The current review could shed light on the findings that have been obtained in various studies on the micrografting of *P. vera*. According to the initial studies on *P. vera* micrografting, supporting materials used in media of the micrografting process were effective and polypropylene fibre (milcap) was outstanding. Furthermore, micrografting can be conducted in two ways *in vitro* and *in vivo*, both of them are effective for *P. vera*, but acclimatization is not needed for *in vivo* micrografting. In the case of *in vitro* micrografting, liquid media was better than solid and semi-solid media. Several rootstocks have successfully been applied for micrografting of *P. ver*a including *P. vera*, *P. terebinthus, P. atlantica, and P. khinjuk*, however, *P. vera* reached grafting size earlier and had a larger diameter. Besides, *P. vera* can be used as a rootstock for other species of *Pistacia* such as *P. lentiscus.* The age of the donor plant of the explant from which the scion is multiplied decisively played a vital role in *P. vera* micrografting. The scions derived from younger trees, 1- and 4-year-olds, are much better than those from aged trees, particularly 10- and 30-year-olds. The size of the scion should be at a proper length (5-10 mm), but <0.5 mm and >10 mm are inconvenient. Among the three techniques (slit micrografting, wedge micrografting on the stump, wedge micrografting in the leaf axil have been used with *P. vera* micrografting, slit micrografting was the best and provided a good union of the micro-scion and rootstock. Despite these, there is no data about the effect of the age of the scion or using growth regulators before insertion of the scion into the rootstock on micrografting of pistachio.

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