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## ORIGINAL ARTICLE

# Using putrescine to increase the rooting ability of hardwood cuttings of the peach × almond hybrid GF677

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**Abstract**
GF677 is an interspecific hybrid which has important value in terms of economic and horticultural characteristics. The use of this rootstock for some stone fruit trees including almond and peach has been increased dramatically in recent years. It has some useful characteristics i.e. high tolerance to calcareous soil, a strong root system and suitability for poor soils, but its propagation is difficult. This study aimed to evaluate the effect of putrescine (PUT) on the rooting of GF677 cuttings under mist conditions. Treatments used include PUT concentrations (2 and 4 mM for 5 minutes), indole-3-butyric acid (IBA) using the quick-dip method (1500 and 3000 mg $l^{-1}$ for five seconds), and a commercial rooting powder and control (without any rooting substances). Treatments were applied at the end of the dormant season. Growth data were obtained 120 days after the treatments. The results showed that application of 1500 mg $l^{-1}$ of IBA and commercial rooting powder improved GF677 rooting, but 3000 mg $l^{-1}$ of IBA resulted in toxic effects on rooting and foliage of the cuttings. Cuttings treated with PUT solutions showed the best roots and shoots in terms of the number and quality. In conclusion, IBA may be replaced by PUT in the rooting process of GF677 cuttings.

**Key words:** almond rootstock; callus; GF677; indole-3-butyric acid; propagation; putrescine

## INTRODUCTION

Almond (*Prunus dulcis* Mill.) is one of the oldest nuts in the world and can be consumed as edible fruit and also exploited in the cosmetic industry. It is native to large areas of Iran, Tajikistan, Afghanistan and west of Pakistan. Over history, the almond has been transferred from above-mentioned areas to regions such as the United States of America, Europe, Australia and Africa. Recently, California has been the biggest producer of almonds in the word. In some areas like Iran, the almond is propagated mainly by seed and this is one of the main problems for almond production because of variations in the quality and the standardization of crops resulting from it. Such a problem is very important for exports and can be removed by using appropriate varieties

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and then grafting them on suitable rootstocks. Today across the word, peach rootstocks and interspecific peach × almond rootstocks are being used in good fertile soils and Mariana rootstock in heavy and wet soils.

The use of interspecific hybrid GF667 has been recently increased in the almond industry. GF667 is a rootstock which was identified by chance in France in 1940 (Fernandez et al. 1994) and was used as the first hybrid between peach and almond at a commercial level (Felipe et al. 1998). Following improvements in methods of vegetative propagation GF667 rootstock has, since 1975, become the predominant rootstock used for peach and almond in France. This rootstock is mainly noted for its vigour and excellent compatibility with scion cultivars, which caused trees grafted on it to grow well under drought or wet conditions (Fernandez et al. 1994). Almond trees grafted on GF677 show good results (De Salvador 2004) and their growth and yield are better than almond seedlings (Massai et al. 2002). GF677 is less injured during transplantation. High tolerance to calcareous soil, high quality of fruit and adaptability to dry climates (Fontanet et al. 1998) have also helped almond trees grafted on GF677 to have high growth and yield (Fernadez et al. 1994).

In general, all the above-mentioned characteristics have led to the introduction of GF677 as a suitable rootstock for almond. Although it has some very good characteristics, some of them are susceptible to *Agrobacterium* and nematodes and all of them are susceptible to root rot, and it does not have the easy propagation required of a rootstock (Felipe 1998). The problem of difficult propagation resulted from the introduction of the *P. dulcis* genome to its genetic structure. The auxin-type growth regulators are traditionally used for the enhancement of the rooting of difficult-to-root woody plants (Hartmann et al. 1997). For GF677 propagation, indole-3-butyric acid (IBA) has been used more than other auxin-type substances. Although IBA increases the rooting percentage of GF677, the results of its use are not desirable. Therefore, much research has been conducted to increase the rooting of this rootstock by using other growth regulators and various methods (Touqeer et al. 2004) such as: the use of *Agrobacterium* (Damiano et al. 1995) or ethylene (Marino 1997); modification of cultivation methods and pretreatments before cutting (Tsipouridis and Thomidis 2004); and change in the mineral ratio in the tissue culture (Kalinina and Brown 2007).

Polyamines are a new group of growth regulators, which have some important roles in physiological processes such as embryogenesis, cell division, development and root formation (Liu et al. 2006). It has been recently shown that these substances are able to promote root formation and root development in hard-to-root plants, and putrescine (PUT) has shown a better response compared to other polyamine substances (Wu et al. 2010). The effects of polyamines on rooting were shown for the first time in the rooting of olive branches, which did not show sufficient endogenous polyamine (Rugini et al. 1997). It has now been made clear that trees that have enough endogenous polyamine, do not respond to exogenous polyamine. The effects of polyamines on rooting enhancement of cuttings have been linked with an increase of peroxidase activity at the basal end of cuttings (Rugini et al. 1997).

The objective of the current study was to evaluate the effects of PUT application on the rooting of hardwood cutting of almond GF677 rootstock and to compare the results with the effects of IBA application.

## MATERIAL AND METHODS

Uniform cuttings of GF677 used for this study were prepared at the end of the dormant season during late winter from wood 10±4 mm in diameter of maternal plants kept in a greenhouse. The cuttings were made 20 cm long. After preliminary washing, cuttings were dipped into a 3000 mg $l^{-1}$ solution of the fungicide Captan (Ariashimi, Iran) for 2 hours and then the cuttings were allowed to dry for 20 minutes (Tsipouridis and Thomidis 2004). Before insertion of the cuttings into the rooting media, the bottom 2cm part of the cuttings was treated with *i)* PUT (2 or 4 mM for 5 min), *ii)* IBA (1500 or 3000 mg $l^{-1}$ for 5 seconds), *iii)* a commercial rooting powder ('Richgro Root Strike', containing 3 g $kg^{-1}$ IBA, Richgro, Australia), and iv) distilled water as the control treatment. For the preparation of the IBA solution, the required amount of IBA was dissolved in 20 ml ethanol and then filled to the desired volume (Hartmann et al. 1997). With regard to the treatment with commercial rooting powder, the bottom 2 cm part of the cuttings was wetted with water shortly before being dipped into the powder in order to help the powder adhere better. Finally, after insertion of cuttings into the washed sand medium they were kept under mist conditions for 120 days. The cuttings were placed under environmental conditions favourable for rooting in which temperature was adjusted to 25 °C during the day and 22 °C during the night at 12/12 photoperiod (Tsipouridis and Thomidis 2004, Tajbakhsh et al. 2009). During the first month of the experiment, the mist system operated for 40 seconds in 20-minute intervals (40 seconds ON and 20 minutes OFF) and for the rest of the experiment, it operated for 40 seconds in 50-minute intervals (40 seconds ON and 50 minutes OFF). To reduce the risk of fungal infections, a 3000 mg $l^{-1}$ solution of fungicide Zineb (Ariashimi, Iran) was applied during the second stage of the experiment (on day 60).

At the end of the experiment, data including the number of rooted cuttings, the number of adventitious roots, root length, the fresh and dry weight of roots, the number of lateral shoots, shoot length, the fresh and dry weight of shoots, internode length and callus formation were measured and recorded. The experiment was carried out based on a completely randomised design (CRD) with 4 replications and 25 cuttings per each of the replications. A simple regression analysis was used to evaluate the correlation between basal callus formation, the percentage of rooted GF677 and the root number of the cuttings. Statistical analysis of the data was carried out using SPSS 16 software and differences among the treatment means were compared using Duncan's multiple range test.

## RESULTS AND DISCUSSION

The results of the effects of the experimental treatments on root formation and root growth indices are shown in Fig. 1. In general,

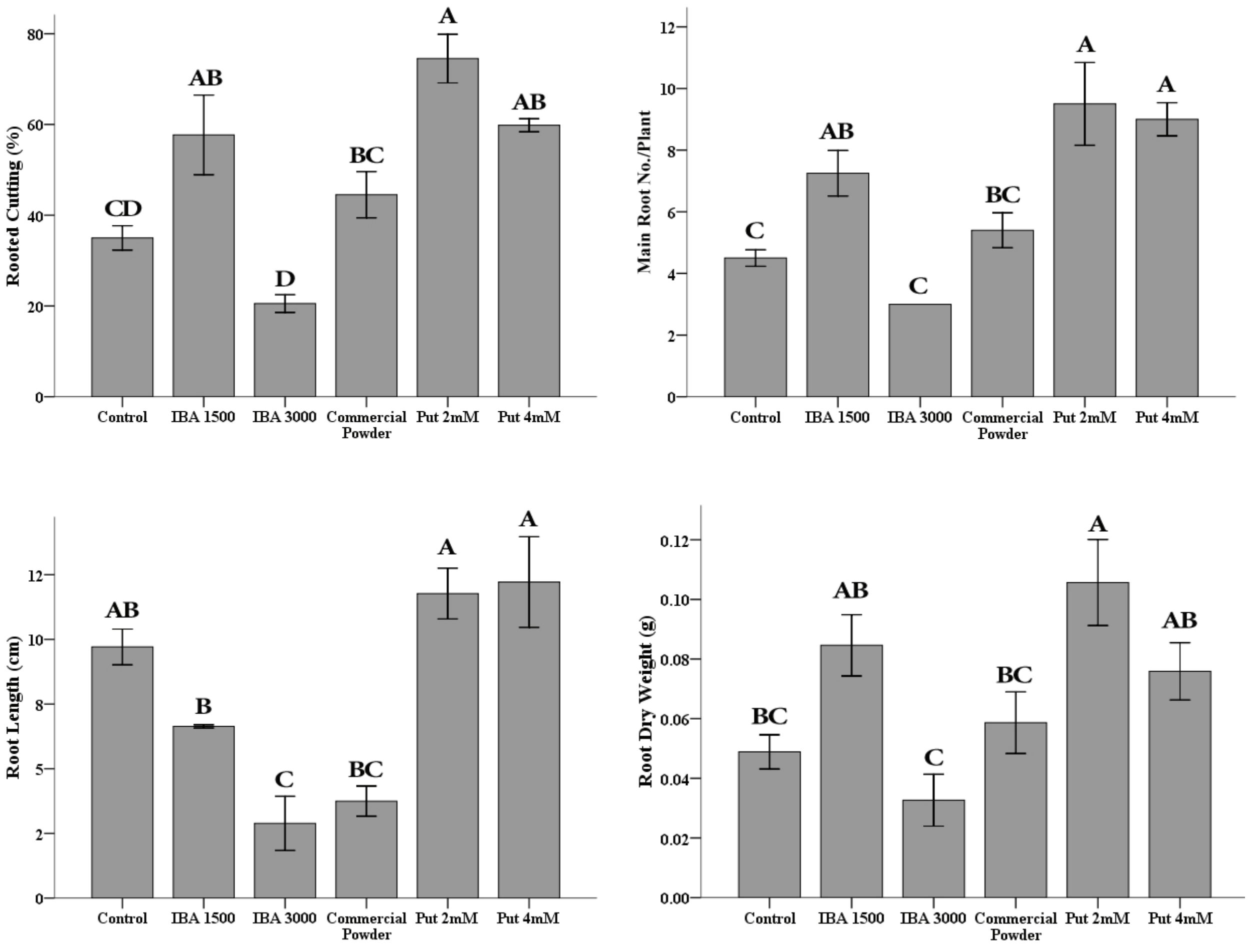

**Fig. 1.** Root formation and root growth indices of GF677 cuttings treated with different concentrations of IBA and PUT. Means with the same letter do not differ at $P<0.05$ by Duncan's Multiple Range Test.

stimulation of root cuttings with the help of auxin or without auxin differs based on the genotype, environmental conditions and plant growth stage. According to various studies, IBA have been introduced as the most effective plant growth regulator for the rooting stage under tissue culture conditions and greenhouse conditions (Brhadda et al. 2003, Naghmouchi et al. 2008).

The results obtained from this study showed that the application of IBA at a concentration of 1500 mg l$^{-1}$ by the quick-dip method is effective in the rooting of GF677 cuttings and may increase the proportion of rooted GF677 cuttings up to 57%. It is also clear that the application of 3000 mg l$^{-1}$ of IBA in the quick-dip method resulted in a toxic effect and reduced the number of rooted cuttings of GF677. As shown in the data on the length and dry weight, excessive concentration of IBA brought about weak and stunted roots. Rooting powders, produced commercially and used by farmers, contain some auxin and increase rooting. Our results show that application of such powders sometimes does not lead to desirable results.

The use of polyamines as the new group of plant hormones exogenously has promoting effects on rooting. In the current study, the application of PUT has significantly increased the number of rooted GF677 cuttings. The results showed that the application of PUT at a concentration of 2 mM for 5 minutes has led to the highest proportion of rooting (74.6%) of GF677 cuttings. The application of a higher concentration of PUT did not increase the percentage of the rooted cuttings.

The comparison of PUT with IBA shows that PUT not only has less toxicity for GF677 cuttings, but it can also stimulate rooting of the cuttings more than IBA. It may be concluded that the concentration of endogenous auxins of GF677 cuttings at the end of the dormant season is sufficient for rooting, and that the lack of auxins probably is not a limiting factor for rooting of GF677 cuttings.

Root growth data show that plants treated with 2 mM or 4mM PUT not only had a higher number of roots (9.5 or 9, respectively), but that they also had the longest roots (11.7 or 12.2 cm, respectively). On the other hand, the lowest number of roots were observed in the control (4.5) and after 3000 mg l$^{-1}$ IBA (3.0) treatments; and the shortest roots were obtained in the cuttings treated with 3000 mg l$^{-1}$ IBA (2.8 cm).

The highest dry weight of roots was obtained in 2 mM PUT treatment (0.10 g), and the lowest dry weight of roots was observed in 3000 mg l$^{-1}$ IBA treatment (0.03 g). Data on length and dry weight of roots show that application of PUT in the rooting of GF677 resulted in roots with better quality compared to the IBA treatments. Having a higher number of, and more vigorous roots led to a lower loss after transplanting the plants to the field. The trees will experience a lesser shock and finally the loss of transplantation will be reduced. It is also possible that, because of their stronger root system, almond trees grafted on such rootstock have a greater tolerance to hot and dry summers, and to drought conditions existing in rain-fed lands. The percentage of cuttings with callus was significantly higher in 2 and 4 mM PUT and 1500 mg l$^{-1}$ IBA treatments, and the lowest number of the cuttings with callus was observed in 3000 mg l$^{-1}$ IBA treatment (Fig. 2).

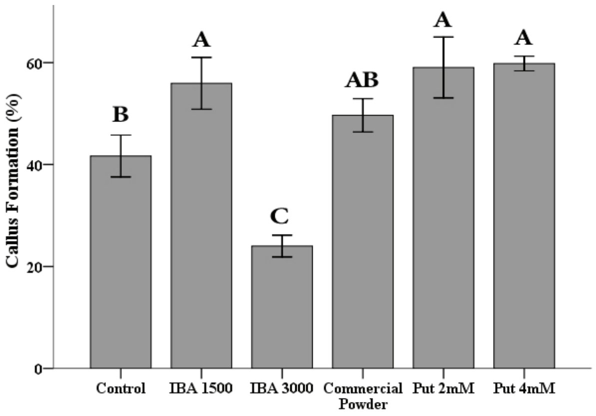

**Fig. 2.** Basal callus formation of GF677 cuttings treated with different concentrations of IBA and PUT. Means with the same letter do not differ at $P<0.05$ by Duncan's Multiple Range Test.

Auxins have a wide variety of effects on plant growth and morphogenesis. They are involved in regulating cell elongation, cell division and differentiation (Dietz et al. 1990). They are also known to promote callus formation (Skoog and Armstrong 1970, Letham 1974, Akiyoshi et al. 1983); however, our results have shown that a high concentration of IBA may adversely affect the basal callus formation of GF677 cuttings. The callus formation induced by PUT treatment is probably due to the stimulatory effect of PUT on cell division (Palavan and Galston 1982, Smith et al. 1985, Galston et al. 1997, Iqbal et al. 2006).

Callus is an irregular mass of parenchyma cells in various stages of lignification. Adventitious roots may emerge through the callus (Hartmann et al. 1997) or may originate directly from different tissues such as vascular cambium and secondary phloem cells in cuttings (Koyuncu and Balta 2004). Hartmann and Kester (1975) stated that in most cases the formation of callus and the formation of roots were independent of each other. In some species, however, the basal callus and callus formation have been found to be a precursor of adventitious root formation (Girouard 1967).

Callus formation of GF677 was in coincidence with the data on rooting percentage and number of roots (Fig. 3). Our results suggest that basal callus formation may follow rooting of GF677 hardwood cuttings. Bhella and Roberts (1975), Davies et al. (1982), Koyuncu and Balta (2004), and Tajbakhsh et al. (2009) also showed that callus formation may help the rooting of some hard to root woody plant species.

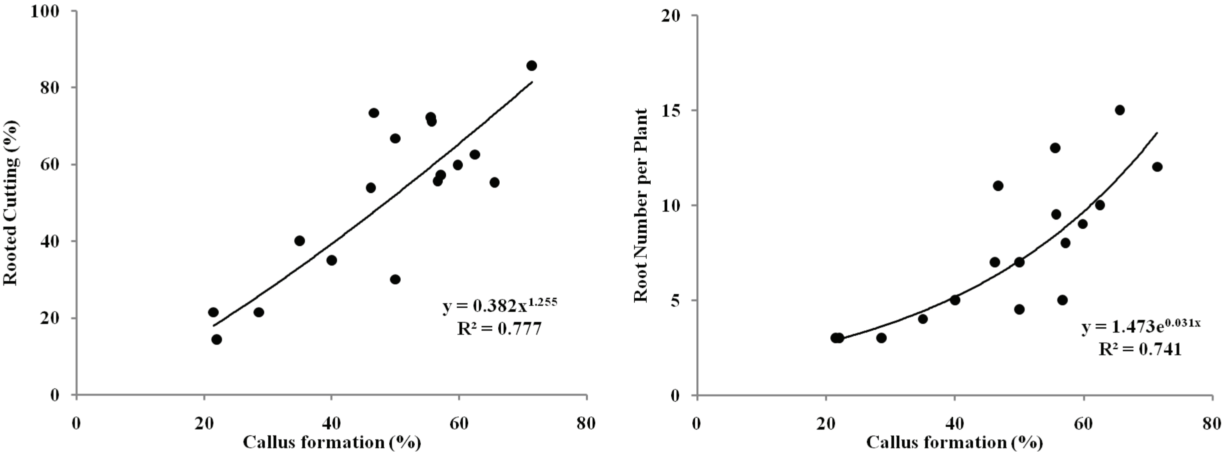

**Fig. 3.** Correlation between basal callus formation and percent rooted GF677 and root number of the cuttings

The effects of treatments on shoot growth indices are shown in Table 1. The result of shoot growth indices indicate that shoot growth under PUT application is stable and that no toxic effect was observed (Table 1). The number of shoots was significantly higher in the control, IBA 1500 mg $l^{-1}$, and PUT 4 mM (2.1 shoots per plant); however, plants had the lowest number of shoots in the IBA 3000 mg $l^{-1}$ (1 shoot per plant). Internode length of plants treated with 4 mM PUT was significantly higher (1.2 cm) than the shortest internodes found in auxin treated plants (0.3–0.5 cm). Shoot length and shoot dry weight of PUT treated plants were significantly higher than the other treatments.

**Table 1.** Effects of the treatment with IBA and PUT on shoot growth indices of GF677 cuttings

| Treatment | Shoot No./Plant | | Internode length (cm) | | Shoot length (cm) | | Shoot dry weight (g) | |
|---|---|---|---|---|---|---|---|---|
| Control | 2.1 | A* | 0.9 | AB | 4.2 | B | 0.3 | AB |
| IBA 1500 mg $l^{-1}$ | 2.1 | A | 0.5 | B | 6.1 | AB | 0.3 | AB |
| IBA 3000 mg $l^{-1}$ | 1.0 | B | 0.3 | B | 4.0 | B | 0.1 | B |
| Commercial powder | 1.2 | AB | 0.5 | B | 4.1 | B | 0.3 | AB |
| PUT 2 mM | 1.7 | AB | 0.8 | AB | 6.8 | A | 0.4 | A |
| PUT 4 mM | 2.1 | A | 1.2 | A | 6.9 | A | 0.5 | A |

* Means within columns followed by the same letter do not differ at $P<0.05$ by Duncan's Multiple Range Test.

In relation to shoot growth, IBA at high concentrations may not only prevent root formation of GF677, but also adversely affect the growth and development of shoots. Data on internode length also show that the application of PUT causes a significant increase in internode length. Such results may be caused by the effect of PUT as a plant growth regulator, which can stimulate plant growth and development, by its effects on stimulatory plant hormones (Galston et al. 1997, Iqbal et al. 2006, Karimi and Rahemi 2012), or may be indirectly caused by a higher root formation, which actively provides water, nutrition and phytohormones to support shoot growth. This is very important in commercial rootstock propagation due to its providing the possibility of early grafting as a result of an increase in suitable plant growth vigour.

Based on the results of the current study it can be concluded that the basal callus and callus formation play a key role in the rooting of GF677 hardwood cuttings. The results show that the optimum concentration of IBA for rooting of GF677 is 1500 mg $l^{-1}$ for 5 seconds. Application of the commercial powders for rooting should be used with caution due to its limited proper effect on rooting. Using a low concentration of putrescine for the rooting of GF677 cuttings can be a more appropriate option compared to IBA or commercial rooting powders. Putrescine not only has less toxicity and easier application, but it also accelerates shoot and root growth.

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