



ORIGINAL ARTICLE

The Effects of Various Concentrations of IBA Hormone on Rooting of ‘TF92’; New Peach-Almond Hybrid under *Ex- vivo* and *In - vitro* Conditions

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KEY WORDS

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ABSTRACT

One of the main problems of fruit cultivation, especially peaches and almonds, is the lack of the application of the existing clone rootstocks such as the promising hybrid of peaches and almonds and its unavailability due to its propagation in Iran. So the seedling rootstocks are inevitably used. However these rootstocks cause many problems for the growers due to the lack of uniformity. In order to solve this problem, the effect of different concentrations of indole butyric acid on the rooting of hardwood cuttings ‘TF92’ (promising hybrid of peach and almond) was tested in a private sector greenhouse in Karaj equipped with a heating pad during 2019. In this paper, the effect of 4 different levels of indole butyric acid hormone (IBA) 0, 1500, 2500 and 3500 parts per million in solution on hardwood cuttings was studied in a completely randomized design in three replications. The results showed that there was a significant difference between various concentrations of indole butyric acid in terms of the effect on the characteristics of the mode of investigation such as the percentage of rooting, the number of roots formed, the dry weight of the roots, the length of the roots and the length of the resulting branches in the cuttings. Thus the highest rooting percentage (78.45) was found in the indole butyric acid (IBA) hormone treatment with a concentration of 2500 mg L⁻¹ compared to other treatments. Therefore, this method can be recommended as a suitable treatment for the rooting of wood cuttings of this promising emerging rootstock. Also, the highest percentage of rooted plantlets was obtained on Murashige and Skoog medium (MS) supplemented with 1 mg L⁻¹ IBA. According to this study, the concentrations of 1 mg L⁻¹ IBA results in the maximum rooting, and it has been suggested as a suitable hormone for in vitro rooting of ‘TF92’.

Introduction

One of the most important problems of fruit orchards, especially in peaches and almonds, is their propagation through seedling rootstocks, which causes non-uniformity of growth and, as a result, it decreases the productivity in orchards (Kester and Grasselly,

1987; Ak *et al.*, 2021). Therefore, the best way to preserve the genetic properties of the fruit trees is to use a vegetative propagation method, in which the plants are identical in terms of appearance and genetics (Socias i Company and Gradziel, 2017).

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Because of the mentioned problems, for more than 20 years, vegetative rootstocks have been used instead of seedling ones, and these new rootstocks are mainly the result of improvement programs (Socias i Company and Gradziel, 2017). In these programs, the ability of asexual propagation, resistance to soil diseases and nematodes, and resistance to high soil moisture are considered (Loreti and Morini, 2008).

The goal of any country is to achieve and introduce superior rootstocks, so all the researchers are interested in it (Vahdati *et al.*, 2021). Therefore, research should be done in parallel to make the path to realize this goal. In addition, it should be noted that most countries planting the peach and almond have almost similar environmental and soil conditions. Therefore, their problems are also similar in many aspects regarding rootstocks (Loreti and Morini, 2008; Socias i Company and Gradziel, 2017). Different rootstocks can be used for peaches and almonds due to the large number of species or interspecies hybrids that can be used for this purpose (Kester and Sartori, 1966; Kester *et al.*, 1991; Socias i Company *et al.*, 1995) and among these, the GF677 rootstock is one of the most important, most common and most used rootstocks. Peach and almond hybrids are widely used to resist iron deficiency caused by lime in many countries, especially in Mediterranean countries and Iran (Day, 1953; Kester and Grasselly, 1987; Coli *et al.*, 2018; Bielsa *et al.*, 2019). Another characteristic of these rootstocks is their compatibility with peaches and almonds (Moreno and Cabra 1994; Moreno *et al.*, 1994; Felipe *et al.*, 1997). These rootstocks are often strong and are suitable for dry and poor soils and can be used for the replacement of orchards (Socias i Company *et al.*, 1995; Duncan *et al.*, 2020). In our country, in recent years, actions have been taken to use vegetative rootstocks in the production of fruit tree nursery plants, and there is an increasing desire to use transplanted plants on vegetative rootstocks among growers and fruit producers (Vahdati *et al.*, 2021).

One obstacle to cultivate fruit trees using vegetative rootstocks in Iran is the restriction of the access to this type of rootstocks due to the obstacles and problems in the propagation of these rootstocks (Sarikhani and Sarikhani-khorami, 2020). One of the main problems of fruit cultivation, especially in peaches and almonds, is the lack of using the existing clone rootstocks such as GF667 and its unavailability due to the problem of its propagation in Iran, so seedling rootstocks are inevitably used, which due to the lack of uniformity cause many problems for orchards. Therefore, the availability of clone rootstocks such as 'TF92' (the new promising hybrid rootstock of peach and almond) in bulk and with the simplest propagation method is quite necessary. For this reason, in this research, the effect of different concentrations of IBA hormone on rooting of cuttings and explants 'TF92' are investigated. There have been many reports about the effect of auxin on rooting improvement, the speed of the root formation, the number, quality, and uniformity of roots in cuttings, and indole butyric acid is probably the best substance for rooting (Loreti and Morini, 2008). This is because its high concentrations are non-toxic for the plant and are effective in rooting a large number of species. According to Kasim *et al.* (2009) report auxin treatment causes an increase, and cytokinin and gibberellin treatment causes a decrease in the number of adventitious roots. One of the best and most common substances used in the rooting is indole butyric acid, which is slowly destroyed by the enzyme system that destroys auxin. Consequently, a stable chemical has a greater effect on root development than a non-stable chemical (Loreti and Morini, 2008). Therefore, auxin plays an essential role in the formation of the rooting process and produces the root initiator and affects the growth of new root forms and its development (Stefanic and Vodnik, 2007). The application of auxins leads to an increase in the percentage of the rooting of cuttings, the formation of primary roots, as well as the number and quality of

roots (Kasim *et al.*, 2009). Short-term chilling increases the amount of auxin in the cuttings, compared to no chilling. Also, during the dormancy period the amount of hormones inside the bud's changes and the concentration of auxin increases (Pallardy, 2008; Wojtania *et al.*, 2022). The highest percentage of rooting was obtained by the treatment of 4000 ppm indole butyric acid after 30 days in almond hardwood cuttings. Rooting of hard and semi-hard wood cuttings of peach indicated that one-year-old wood cuttings had the highest percentage of rooting after being stored at 2-4°C for 2-3 months, and indole butyric acid with a concentration of 2000 mg. per liter caused the most root formation on GF 677 rootstock (Kasim *et al.*, 2009). According to a report, the highest percentage of rooting was obtained in nemaguard rootstock hardwood cuttings at a concentration of 4000 ppm of indole butyric acid, so that the cuttings at the beginning of the branch rooted better than the middle and end cuttings of the branch (Tewfik, 2002). Also, GF 667 rootstock has the highest percentage of root formation at a concentration of 3000 ppm of indole butyric acid and the lowest percentage of root formation was obtained at a concentration of 4000 ppm for indole butyric acid, as well as the maximum number of roots at a concentration of 2500 ppm of indole butyric acid and the longest root length at a concentration of 2500 ppm (Shamshad and Abbasi, 2003). On the other hand, in vitro propagation is an alternative to overcome this problem. Since the tissue culture technology has been widely used for a large-scale micropropagation of uniform commercial plants, most research on this interspecies hybrid (peach-almond hybrid) was focused on the optimization of reproduction steps by using explants mainly axillary buds or branch tips (Kodad *et al.*, 2021). Nevertheless, organogenesis seems to be highly dependent on genotype and an efficient protocol for shoot formation depends mainly on the type of media, plants growth regulators combinations, medium composition and

culture conditions (Tewfic, 2002; Magyar-Tabori *et al.*, 2010). Indole-3-butyric acid (IBA) is the most extensively used auxin for peach-almond hybrids rootstock propagation by cuttings because of its role in enhancing root initiation and the number, quality, and uniformity of roots (Pinto *et al.*, 2020). The peach-almond hybrids present successful rooting rates when treated with indole-3-butyric acid (IBA) in adequate concentration, which is the optimal dosage (Kaur, 2017). Therefore, the use of plant growth regulators (PGRs) is one of the most effective ways to increase root initiation, rooting percentage, and root quality and uniformity. The most widely used PGR for this purpose is indole butyric acid (Kaur, 2017; Pinto *et al.*, 2020; Kordzadeh and Sarikhani, 2021). Therefore, this research is carried out to achieve this goal. For this reason, in this research, the effect of different concentrations of IBA hormone on rooting of cuttings and explants 'TF92' (new peach-almond hybrid) has been evaluated.

Materials and Methods

Rooting of cuttings *ex vivo*

This research was carried out in mist greenhouse located in Karaj during 2019 and the plant materials were hard cuttings of 'TF92' rootstock (Fig. 1). 'TF92' rootstock (*P. persica* x *P. dulcis*), is a hybrid rootstock developed from a cross between 'Tuono' almond (*P. Amygdalus Batsch*, syn. *P. dulcis* (Mill.)) and 'Filamina' peach (*P. persica*) in Iran. Although it was developed for almond, it shows good compatibility (data not published) with peach and nectarine cultivars. 'TF92' is characterized by strong growth, easy propagation and adaptation to calcareous soils. Hard cuttings of 'TF92' rootstock are obtained from one-year branches with optimal distance between nodes and branch diameter from mother trees located in Karaj region (Horticultural Station of Mishkinabad) and then they were transferred to the greenhouse as

soon as possible. Among the facilities and materials required for the experiment, we can mention IBA hormone, greenhouse, perlite-sand cultivation bed, mist system, heating system and fan for humidity control. This experiment was carried out in order to stimulate rooting and to achieve optimal use of the hormone indole butyric acid (IBA) on the rooting of new cuttings of 'TF92' rootstock. For this purpose, indole butyric acid hormone produced by the German company SIGMA (Purity 99.8%) was used at concentrations of 0, 3500, 2500, 1500 mg liter⁻¹. To prepare the cuttings, 20 cm long cuttings were prepared from the selected branches of the mother plants and were grouped into 15 packs, and at the time of transfer to the bed, 2 cm long cuttings were cut using a sterile blade. Both sides of the end of the cutting was scratched in such a way that part of the wood texture was injured, but the bud at the bottom of the cutting was not injured. After wounding, the cuttings were placed in 3% benomyl solution for 60 seconds for disinfection, and they were placed in the open air for 15 minutes, then hormonal treatment was applied. 'TF92' cuttings were cultivated in perlite substrate in three replicates in a randomized complete design. Perlite has been giving better response to the cutting roots due to its substrate (Kreen *et al.*, 2002).

After planting the cuttings in the bed, the optimal temperature of the culture bed was 20-23°C and the ambient air temperature of the greenhouse was 15-20°C. The decrease in substrate temperature from the planting cuttings stage to the harvesting of rooted cuttings was adjusted to 23°C in the planting stage, 20°C in the callus formation stage, and 18 °c in the stage of primary root emergence. The relative humidity was 90% in the greenhouse during the first week, 80% humidity in the second week and 60% in the third week. The humidity decreased accordingly until rooting, and this was controlled using a hygrometer every day. At the end of the experiment, traits like the percentage of rooted cuttings, average

number of roots, average root length, average dry weight of roots, average length of new shoots and amount of carbohydrates were studied by Yang *et al* (2015) method.

Rooting of cuttings in vitro

'TF92' was used in this research. 'TF92' is an almond and peach hybrid and is one of the promising genotypes obtained from the improvement programs of the National Horticulture Research Institute in Iran, which has favorable characteristics such as resistance to the adverse climatic conditions of Iran. Explants were obtained from the horticultural research station Mishkinabad (Horticultural Station of Mishkinabad). In-vitro experiments were performed in the tissue culture laboratory of National Horticulture Research Institute of Iran. The Explants were prepared from healthy five –years –old mature trees with appropriate vegetative growth. Sodium hypochlorite was used to disinfect the samples from surface and internal contamination. After transferring to the laboratory, the leaves of the branches were removed and the stem buds and explants containing a single bud with a piece of stem node were isolated. After that, the explants were washed for 60 minutes in running water and placed in 70% alcohol solution under the aluminum hood for 30 seconds, and then they were washed with sterile distilled water, and then disinfected with potassium hypochlorite with 1.5 active ingredients in 15 minutes. Then the contamination-free samples were cultured in jars containing the culture medium, one explant per jar. Finishing the proliferation test, suitable propagated branches with a size of three to four centimeters were selected and used for this purpose. The experiment was conducted as a completely randomized design with plant materials included 'TF92' explants and IAA hormone at four levels (0, 0.5, 1, 1.5 mg liter⁻¹). After four weeks of cultivation, the characteristics of rooting percentage, root number and root length in each explant were evaluated and recorded. It should be noted that in this

stage of the experiment, EDDHA - Fe iron was used by 80 mg liter⁻¹ and sucrose by 30 grams liter⁻¹, and for each of the treatment, four jars were considered as four replications and 15 explants were cultured in each jar. Then the samples were transferred to the growth room. In the growth room, the samples were kept in the mean temperatures of 24°C and 18°C at night, 16

hours in light and 8 hours in darkness. They were kept with active photosynthetic radiation (PAR) equal to 25 Jmol m⁻² per second. Then the characteristics of rooting were recorded. The data were analyzed using SAS software version 9.1 and the comparison of means was done using Duncan's multiple range tests at the level One and five percent.



Fig. 1. The interspecific hybrid of Flamina nectarine and Tuono variety almond (the first rootstock resulting from interspecies hybridization of peach and almond in the cultivar and almond rootstock breeding program in Iran)

Results

Rooting ex vivo

The results of the Analysis of variances of the data obtained from the wooden cuttings in the SAS software were checked and the means were compared

using Duncan's multi-range test by the Mini tab software (Tables 1), which are as follows:

Table 1. Effect of indole butyric acid hormone on traits related to rooting of 'TF92' root cuttings

Indole butyric acid hormone treatment (ppm)	Percent rooting	Root length of wooden cuttings	Root volume (mm ³)	Root dry weight (gram)
0	9.31d	5.34c	1.67abc	0.06c
500	33.17c	8.98b	2.10ab	0.09b
2500	78.45a	17.33a	2.04ab	0.50a
3500	46.43b	16.97a	2.06ab	0.41a
Significant	**	**	**	*

*,*P*≤0.05,and**,*P*≤0.01

The results of the analysis of variance in Table 1

showed that different hormone treatments had a

significant effect on the percentage of rooting of ‘TF92’ cuttings and there was a significant difference between the different concentrations of indole butyric acid in terms of the effect on the characteristics of the mode of investigation such as the percentage of rooting, the number of roots formed, the dry weight of the roots, the length of the roots and the length of the resulting branches in the cuttings at the level of 1%. Also, by comparing the means in Table 1, it is clear that all characteristics in the indole butyric acid (IBA) hormone treatment with a concentration of 2500 mg were improved compared to other treatments. For

instance, the highest rooting percentage (78.45) was found in the indole butyric acid (IBA) hormone treatment with a concentration of 2500 mg liter⁻¹.

Rooting in vitro

The optimal in vitro conditions to induce explant rooting of the ‘TF92’ peach-almond hybrid was determined by comparing the different concentrations of auxinic. In this experiment three IBA concentrations were studied: 0, 0.5 and 1 mg l⁻¹ using the in vitro culture medium created specifically for this hybrid

Table 2.effect of IBA concentration on in vitro rooting of ‘TF92’ (peach-almond hybrid)

IBA(mg liter ⁻¹)	rooting	Root number
0.00	16.43c	0.8c
0.5	43.35b	2.03b
1	87.56a	5.6a
Significant	*	**

*, P≤0.05, and **, P≤0.01

The results of the analysis of variance showed that IBA concentrations had significant effects on in vitro rooting parameters (Table 2). The highest percentage of rooted shoots was obtained on MS supplemented with 1 mg L⁻¹ IBA (Table 2).

Discussion

The results showed that the effect of different hormone treatments on the characteristics of the rootings of ‘TF92’ cuttings was significant. On the other hand, it is clear that the rooting by the hormone treatment of indole butyric acid 2500 mg liter⁻¹ in wooden cuttings was better than other treatments. The reason for this may be the fact that auxin plays an essential role in the induction of rooting and leads to the formation of root initiator. Also, auxin has an effect on the speed of rooting of cuttings. Plants produce natural auxin in young branches and leaves, but it is better to use artificial auxin for rooting. One of the most important and useful material is indole butyric acid. Natural indole butyric acid plays a more

important role in the stimulation phase of the formation of root initiators, but it is more sensitive to auxin degrading enzymes than naphthalene acid (Stefanic and Vodnik 2007). However, if the higher concentration of the hormone is used during rooting, or if it is used more than necessary, it will disturb the balance of the rooting hormone for the propagation of fruit tree species (Hartman and Kester 1983). On the other hand, Mirsolimani and Rahimi (2015) also found similar results regarding the number of main roots of 677GF rootstock in treatment with indole butyric acid. According to their experimental findings, 2000 ppm of IBA was used in Myrobolan GF-31 and Myrobolan GF8-1 varieties for which the rooting level of 80% was obtained. Differences may be due to the high rate of hormone application which affects the root formation in some plant varieties. Indeed, though many researchers have supported the positive effects and usefulness of IBA to support rooting. The the results by Ahmed *et al.* (2003), showed that the effect of IBA was varied for different characteristics studied

with respect to its different concentrations. In general, as for the effects of different treatments on plant adventive root formation, it has been observed that there are significant differences between applications regardless of the growing media.

In terms of rooting percentage, the results of the present study showed that different concentrations of indole butyric acid had different effects on the rooting of cuttings, so that the highest one was related to the concentration of 2500 mg/liter and the lowest one was related to zero concentration (control) (Table 1). The effects of the applied concentrations of indole butyric acid on the percentage of rooting of the cuttings show a significant increase in the percentage of root formation in all three applied levels compared to the control. This result is consistent with the results of the researches by Mirsolimani and Rahimi (2006). The reason of this is the effect of auxin on the rooting of cuttings, which is considered as a stimulating and developing hormone for the initiation of adventitious roots (Swedan *et al.*, 1993). Swedan *et al.* (1993) reported that the hardwood cuttings of plum and peach and 677GF collected in November had the highest rooting at the concentrations of 1000 and 3000 of indole butyric acid solution. The best rooting in almond hardwood cuttings was obtained in 85% at a concentration of 4000 ppm of indole butyric acid (Felipe 1984). Also, bitter almond hardwood cuttings at a concentration of 8000 ppm had a significantly higher rooting percentage than other concentration treatments (Kasim *et al.* 2009). It has been reported that the percentage of rooting in Mit Gomer peach variety was proportional to the low amount of phenolic content and the amount of rooting of this variety increased with the decrease in the amount of phenols (Howard *et al.*, 1983). According to these findings, the concentration of hormones used to stimulate rooting depends on the time of cutting, the type of substrate and the type of genotype.

According to the analysis of variance table (Table

2), there is a significant difference in the effect of indole butyric acid hormone on the root length of 'TF92' cuttings. Comparing the means in Table 2, the highest number of roots is related to the hormonal treatment of 2500 mg liter⁻¹ indole butyric acid in wooden cuttings. Similar results have reported that the production of ethylene increases with the increase in the auxin concentration; ethylene prevents the growth of root length and causes cell division (Loreti and Morini 2008). An increase in the number of lateral roots in high concentrations of indole butyric acid has also been previously reported by Gudarowska and Malaczukm (2006). Examining the effect of the indole butyric acid hormone on the average root length of 'TF92' rootstock cuttings, in which root length is considered as an important parameter in the evaluation of rooted cuttings, it was found that the higher its amount per unit of soil volume, the strength of the plant will increase. This is relevant in the absorption of water and minerals (Loreti and Morini 2008).

Investigating the effect of indole butyric acid hormone on the root volume of 'TF92' rootstock cuttings showed that the root volume increased in cuttings treated with different hormone concentrations (Table 1). The increase in the root volume due to the hormone application up to a certain concentration has been reported by many researchers (Shamshad and Abbasi 2003, Loreti and Morini 2008).

Also, the effect of indole butyric acid hormone on root dry weight is significant at level 1%. So the comparison of the means in Table 2 showed that the highest root dry weight is related to the hormonal treatment of 2500 mg/liter indole butyric acid in wooden cuttings. Similar results show that after hormone treatment, the cuttings are planted in a bed with good drainage; it makes the cuttings benefit from sufficient oxygen and moisture and grow in higher rate (Antonopoulou *et al.*, 2005, Loreti and Morini 2008). Most of the new research on rootstock propagation in recent years has focused on improving

the success achieved by conventional cutting techniques and developing the allied technique of micropropagation. Usually, cutting techniques have been developed to aid the propagation of recalcitrant rootstock clones or to circumvent disease, soil, or site problems experienced with more conventional division techniques (Webster 1995).

Rooting in vitro

Results of the effects of IBA on the in vitro rooting of the ‘TF92’ peach-almond hybrid showed that IBA concentrations had significant effects on the in vitro rooting feature. The highest percentage of the rooted shoots was obtained on MS supplemented with 1 mg L⁻¹ IBA. (Table 2). In a previous study on micropropagation of *Prunus* sp., rooting is considered as a critical stage, since it determines the plant survival during the acclimatization (Rogalski *et al.*, 2003). According to this study, the concentrations of 1 mg L⁻¹ IBA lead to the maximum rooting (Table 2), and it has been suggested as a suitable hormone for in vitro rooting of ‘TF92’ (peach-almond hybrid). This is because IBA is more stable and less sensitive to auxin degrading enzymes, and would slowly be metabolized by the peroxidase enzyme (Miri, 2017). In a previous study on peach rootstock Guardian, IBA plays a cardinal role in rooting, where its utilization in culture media presents the highest percentage of roots, and also contributes to a better induction of lateral roots (Bertoni, 2011; McLeod *et al.*, 2020). Of course, there are significant differences in the rate of rooting depending on genotype, concentration, type of auxin, and culture conditions (Tewfic, 2002; Johnson *et al.*, 2020). According to the reports, the role of IBA has been more effective in the root induction stage than other auxin hormones. As an instance, IBA plays an essential role in the induction of rooting, followed by the highest percentage of rooting and better induction of lateral roots compared to indole acetic acid (IAA) in almond cultivars and peach and almond

interspecific hybrids (Dobr´anszki and Teixeira da Silva, 2010; Silva *et al.*, 2016; Kodad *et al.*, 2021).

When plants with developed roots were transferred to the pot containing peat: perlite in the ratio of 1:1, the survival rate of the plantlets was found to be 58.3%. It should be noted that plants with long roots are easily damaged during the transplantation process or being contaminated by pathogens, which can affect the survival of the plants. If these cases are considered in the transplantation process, the plants planted in the soil will continue to grow naturally without any morphological abnormalities.

Conclusions

The results of the present study about the propagation of ‘TF92’ rootstock (first promising peach and almond hybrid rootstock), in terms of the rooting traits under *ex vivo* and *in vitro* condition treated with different concentrations of indole butyric acid (IBA) hormone, indicated a good rooting traits variety among the treatments. The general conclusion from the results of this research was that there is a significant difference between various concentrations of indole butyric acid in terms of the effect on rooting characteristics such as the percentage of rooting and the number of roots, the length of the resulting branches in the cuttings. For instance, the highest percentage of root formation (78.45) was found in the treatment of indole butyric acid (IBA) hormone with a concentration of 2500 mg liter⁻¹ compared to other treatments. Therefore, it can be recommended as a suitable treatment for the rooting of wood cuttings of this emerging promising rootstock (‘TF92’). Also, the highest percentage of the rooted shoots was obtained on MS supplemented with 1 mg L⁻¹ IBA. According to this study, the concentrations of 1 mg L⁻¹ IBA caused the maximum rooting, and it has been suggested as a suitable hormone for in vitro rooting of ‘TF92’ (peach-almond hybrid).

Conflict of interests

The authors declare that there are no conflicts of interest.

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