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Rooting of peach [*Prunus persica* (L.) Batsch] hardwood cuttings as affected by IBA concentration and substrate pH

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Abstract

In order to study the effects of four IBA concentrations (0, 1000, 2000 and 3000 mg L^{-1}) and three substrate pH levels [pH 5, 7 and 9] on rooting of hardwood cuttings of peach *cv.* Red May, experiment were conducted at the Department of Horticulture, College of Agricultural Sciences, University of Sulaimani/ Kurdistan,Iraq, . The experiment was laid down in a factorial RCBD with three replications. Comparison among means was done using Duncan's multiple range test ($P \leq 0.05$). Rooting and callusing percentage, root number, root length, root fresh weight, root dry weight, shoot length, shoot diameter, leaf number, leaf area, shoot fresh weight and shoot dry weight were examined. The highest rooting percentage (40 %) was achieved from 2000 mg L^{-1} IBA. However, effects of IBA levels were not significant on callusing. Control gave no rooting and other root traits. Effects of the IBA concentration on vegetative traits were not different with each other except shoot diameter and leaf number. Shoot diameter and leaf number were the highest (1.12 mm and 4.51, respectively) at 2000 mg L^{-1} IBA. Rooting and other parameters were the best in pH 5 and 7 substrates. Substrate pH 9 gave minimum rooting and other parameters. Effects of interactions between the two factors showed that cuttings treated with 2000 mg L^{-1} IBA and planted in pH 7 substrate gave the highest (60 %) rooting percentage, and cuttings dipped in 1000 mg L^{-1} IBA and planted in pH 7 substrate gave the best other root traits. Interaction effects of the two factors on shoot traits showed that the maximum shoot traits were observed at interaction between 2000 mg L^{-1} IBA and pH 5 substrate.

Key words: *Prunus persica*, hardwood cuttings, IBA, substrate pH, rooting, vegetative traits

Introduction

The botanical name of peach [*Prunus persica* (L.) Batsch] is based on the country of origin *i.e.* Persia (actual Iran), and peach firstly was named by Linne (1758) as *Amygdalus persica*. Finally in 19^{th} century, it was acknowledged that western China is the geographical origin of peach (De Candolle, 1883). Peach is a deciduous tree, and attributed to Rosaceae family, included under the genus *Prunus* (Bailey, 1927). According to Foreign Agricultural Service/USDA Office of Global Analysis 2015, production of peach in the world was about 20.5 million tons.

Peach propagation is sometimes conducted through seed, but seed has low germination due to its hard covers and immature embryo, and the seedlings may not be true to the type (Parvez *et al.*, 2007). Also, peach is propagated by grafting scion at top of the rootstocks with exclusive cultivars, but the drawbacks of this propagation are sometimes death of the seedlings because of incompatibility, and it is expensive, especially in high density patterns. For this reason, growers have considered peach cuttings as a mean of multiplication (Overcash *et al.*, 1983).

In propagation of peach of peach plants by stem cutting, softwood (Liu *et al.*, 1989), semi-hardwood (Avery and Beyl, 1991) and hardwood (Reighard *et al.*, 1990) cuttings were used. Hardwood cuttings is better, because they are easy to prepare, are not readily perishable, may be shipped safely over long distances if necessary, and require little or no special equipment during rooting. Because of some reasons, rooting of peach cuttings are difficult-to-root (Hartmann *et al.*, 2002). However, the reasons of rooting failure are not clearly understood. Prevention of rooting by sclerification was reported in stone fruit rootstocks by Beakbane (1961), biochemical factors may influence rooting of peach cutting as well (Caboni *et al.*, 1997 and Naija *et al.*, 2008). Also, factors such as cultivar, age of tree, collection date, cuttings length, degree of hardening of the cuttings, wounding, heat treatment and concentration of auxin-like compounds could influence rooting of the cuttings (Oliveira *et al.*, 2003; Dick and Leakey, 2006 and Husen *et al.*, 2015).

Researchers found that rooting of hardwood cutting of peach is influenced by applying synthetic auxin, and the most frequently used auxin is IBA (Desmond and Daniele, 2008). Moreover, substrate pH has its role in rooting of cuttings (Brian, 2000). The objective of this study was to investigate the effect of different IBA concentrations and substrate pH on rooting of peach hardwood cuttings of cultivar, Red May, which is one of the old cultivars, freestone, yellow-white flesh, sweet and juicy, and is one of the best early peach cultivars. Also, it is widely cultivated in Kurdistan region and Iraq, particularly in warm climate zones.

Materials and methods

This research was conducted to investigate the effect of different IBA concentration and substrate pH on rooting of peach hardwood cuttings in a greenhouse at the College of Agricultural Sciences, University of Sulaimani, Kurdistan region, Iraq. The experiment was laid down in a factorial RCBD with 3 replications. The cuttings were taken from an orchard at Sirwan sub-district which belongs to Halabja Governorate.

Preparation of cuttings: The cuttings were prepared from 8-year old trees of peach cultivar (Red May). The weekly average temperatures of the orchard position are shown in (Table 1). 180 hardwood cuttings were taken on February 19, 2015. One year old branches were cut, wrapped with plastic sheets, and brought to the laboratory, where the cuttings taken from middle part of branches with 20 cm length (Tsipouridis *et al.*, 2006), with 0.5-1 cm diameter (Zencirkiran and Erken, 2012). The base of cuttings were cut 0.5 cm below the lowest node (Sándor *et al.*, 2008), and the cuttings were soaked in water for 12 hours (Ling and Zhong, 2012) and then exposed to air until surface was dry. Then two tangential cuts of 0.5 cm depth were made on opposite sides of base of the cuttings (Tworkoski and Takeda, 2007).

Table 1. Weekly average temperature in of the orchard from December 1, 2014 to March 10, 2015

Date			Temperature (°C)		
			Maximum	Minimum	Mean
2014	December	Week 1	15.4	6.5	10.8
		Week 3	11.8	5.6	8.9
		Week 4	13.1	3.7	8.3
2015	January	Week 1	11.9	3.7	8.3
		Week 2	8.4	0.6	4.1
		Week 3	13.9	1.8	7.7
		Week 4	14.4	5.1	9.7
	Feruary.	Week 1	15.6	3.9	9.8
		Week 2	15.5	8.3	11.9
		Week 3	11.6	3.8	7.7
		Week 4	15.2	3.4	9.3
	March	1-10	16.8	5.5	11.3

Preparation of chemical solutions: IBA solution was prepared by dissolving IBA in 50 % ethanol (with a purity of 96 %) (Evert and Smittle, 1990) with different concentrations (0, 1000, 2000 and 3000 mg L^{-1}). The cuttings were randomly divided in four lots, each lot included 45 cuttings, then every lot separately dipped in control (ethanol and distilled water without IBA) and different IBA concentrations for 10 seconds (Sebastiani and Tognetti, 2004). The cuttings were left until surface dry, after that the base of cuttings were treated with Captan 75, 1:9 in talc powder (Tsipouridis *et al.*, 2003). The treated cuttings were kept in plastic bags then sealed and stored at 3±1°C (Xu and Chen, 1989) for 20 days.

Preparation of rooting substrate: Rooting substrate was prepared from perlite (Tsipouridis *et al.,* 2005), 7.5 L perlite was put in polyethylene bags and 1.5 L of H_2O_2 3 % (in tap water) was added instead of water with different pH (5, 7 and 9), to reach 20 % moisture (Tsipouridis and Thomidis, 2004). The pH was adjusted by HCl and NaOH. The polyethylene bags were sealed to prevent moisture loss and inserted in a plastic pot with 24 cm diameter and 27 cm depth. All pots were placed in a greenhouse and arranged in 3 blocks. The temperature of the greenhouse was partially controlled by split air conditioner. Temperature and humidity inside the greenhouse are shown in Table 2.

Table 2. Average weekly temperature and humidity inside the greenhouse during the study period

Date		Avgerage Temperature (°C)	Humidity (%)
March 10-16	Week 1	19.5	39
March 17-23	Week 2	16.2	59
March 24-30	Week 3	17.7	59
March 31-April 6	Week 4	22.6	35
April 7-13	Week 5	20.9	38
April 14-20	Week 6	22.2	36
April 21-27	Week 7	22.3	29
April 28-May 4	Week 8	26.9	23
May 6-12	Week 9	22	41

Planting of cuttings: The cuttings were removed from the cold storage on March 10 and directly stuck in the pots. Cuttings treated with 4 IBA concentrations were planted in 3 substrate pH each pot containing 5 cuttings and replicated 3 times in the 3 blocks. After six weeks, 120 mL of tap water was added to each pot. Finally, after 9 weeks, the experiment was terminated and the cuttings were lift to study the effect of studied treatments.

Results and discussion

As shown in Table 3, rooting percentage was significantly affected by IBA concentrations. The highest rooting percentage (40 %) was achieved from 2000 mg L^{-1} IBA, besides rooting was reduced at 3000 mg L^{-1} IBA. However, effects of IBA levels were not significant on callusing and root number, length, fresh weight and dry weight. No rooting was observd in control. Loach (1988) observed that inducing adventitious root formation in cuttings was affected by concentration of the applied auxin. Tsipourdis *et al.* (2003) found that 2000 mg L^{-1} IBA was the best for rooting of peach hardwood and semi-hardwood cuttings depending on cultivars. In addition, the adverse influence of IBA was found when higher concentrations were applied (Erez and Yablowitz, 1981). Another research found that application of 3000 mg L^{-1} of IBA by quick dipping method caused toxic effect on rooting of hardwood cuttings in GF766 peach (Karimi and Yadollahi, 2012). Also, da Costa *et al.* (2013) reported that the comparatively higher auxin concentration is required for adventitious root induction, but its formation was adversely supraoptimal auxin concentration.

Table 4 showed that the effect of IBA concentrations on vegetative traits were not different with each other significantly except in shoot diameter and leaf number. Shoot diameter and leaf number were the highest (1.12 mm and 4.51, respectively) at 2000 mg L^{-1} IBA, but, control and 3000 mg L^{-1} IBA gave no or the lowest results. This may be due to 2000 mg L^{-1} exhibited early rooting,

Table 3. Effect of IBA concentrations on rooting percentage, callusing percentage and other root traits of peach *cv.* Red May

IBA concentrations (mg L^{-1})	Rooting (%)	Callusing (%)	Root number	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
0	0 c	22.22 a	0 b	0 b	0 b	0 b
1000	22.22 b	15.55 a	6.33 a	9.74 a	0.39 a	0.059 a
2000	40 a	8.88 a	6.38 a	7.77 a	0.317 a	0.041 a
3000	17.77 b	17.77 a	6.5 a	5.35 a	0.314 a	0.04 a

* The treatment means in each column with the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test.

Table 4. Effect of IBA concentrations on vegetative traits of peach *cv.* Red May hardwood cuttings

IBA concentrations (mg L^{-1})	Shoot length (cm)	Shoot diameter (mm)	Leaf number	Leaf area (cm^2)	Shoot fresh weight (g)	Shoot dry weight (g)
0	0 b	0 c	0 b	0 b	0 b	0 b
1000	0.5 ab	0.66 ab	1.55 b	22.77 a	0.170 a	0.054 ab
2000	0.67 a	1.12 a	4.51 a	28.2 a	0.240 a	0.070 a
3000	0.37 ab	0.507 bc	1.88 b	15.95 ab	0.119 ab	0.050 ab

* The values in each column with the same letter do not differ significantly ($P\leq0.05$) according to Duncan's Multiple Range Test.

hence absorption is increased (Parvez *et al.*, 2007), hence shoot diameter and leaf number increased.

Substrate pH 5 and 7 were not significantly different in rooting percentage, callusing percentage and other root traits except root number but they were different with pH 9 (Table 5). Rooting and other parameters were the best in pH 5 and 7 substrates. Substrate of pH 9 gave minimum rooting percentage (5 %), callusing percentage (1.66 %) and other root traits. Holt *et al.* (1998) reported that rooting of stem cutting of rhododendron was enhanced by low substrate pH, additionally peach GF677 hardwood cuttings demonstrated the best rooting at substrate pH 3 to 5 (Tsipourdis and Thomidis, 2004).

Effect of substrate pH on vegetative traits was similar to root traits (Table 6). Vegetative traits were the best in pH 5 and 7 substrates, and they were not significantly different, but they were different with pH 9. These results indicate that, at high substrate pH, nutrients become less available to be absorbed by the roots, and this lead to inferior shoot growth (Cavins *et al.*, 2000). Medium pH affected the uptake of rooting medium ingredients, and also affected on chemical reactions, particularly those catalyzed by enzymes (Thorpe *et al.*, 2008).

Table 7 show the interaction effects between IBA treatments and substrate pH on rooting and callusing percentage and other root traits. Cuttings treated with 2000 mg L^{-1} IBA and planted in pH 7 substrate gave the highest (60 %) rooting percentage. Generally, cuttings treated with IBA and planted in low substrate pH (pH 5 and 7) exhibited the best rooting percentage. Interaction effects between the two factors on callusing percentage were not significant. Moreover, cuttings dipped in 1000 mg L^{-1} IBA and planted in pH 7 substrate gave the highest root number (15.33), root length (15.43 cm), root fresh weight (0.78 g) and root dry weight (0.114 g).

Rooting could not take place in IBA-untreated cuttings planted in the three substrate pH levels. Also, cuttings dipped in 3000 mg L^{-1} IBA and planted in pH 9 substrate gave no rooting. At low pH level, the highest IBA uptake, and rooting percentage were observed in apple (Harbage *et al.*, 1998) and smoke tree (Ilczuk and Jacygrad, 2016). Also the effect of IBA and substrate pH on adventitious root formation in cuttings may be due to their effects on expansin protein in the cell wall. During adventitious root induction, an increase in expansin mRNA levels were observed in conifers hypocotyls after application of exogenous auxin (Hutchison *et al.*, 1999), and expansin considered to be

Table 5. Effect of substrate pH on rooting percentage, callusing percentage and other root traits of peach *cv.* Red May hardwood cuttings

Substrate pH	Rooting (%)	Callusing (%)	Root number	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
pH 5	28.33 a	26.66 a	5.6 b	7.85 a	0.42 a	0.056 a
pH 7	26.66 a	20 a	8.55 a	8.59 a	0.33 a	0.048 a
pH 9	5 b	1.66 b	0.25 c	0.71 b	0.009 b	0.001 b

* The values in each column with the same letter do not differ significantly ($P\leq0.05$) according to Duncan's Multiple Range Test.

Table 6. Effect of substrate pH on vegetative traits of peach *cv.* Red May hardwood cuttings

Substrate pH	Shoot length (cm)	Shoot diameter (mm)	Leaf number	Leaf area (cm^2)	Shoot fresh weight (g)	Shoot dry weight (g)
pH 5	0.76 a	0.71 a	2.88 a	24.92 a	0.210 a	0.073 a
pH 7	0.35 ab	0.9 a	2.58 a	23.28 a	0.167 a	0.051 ab
pH 9	0.05 b	0.108 b	0.5 b	1.99 b	0.020 b	0.007 b

Table 7. Interaction effect between IBA concentrations and substrate pH on rooting percentage, callusing percentage and other root traits of peach *cv.* Red May hardwood cuttings

IBA concentrations (mg L^{-1})	Substrate pH	Rooting (%)	Callusing (%)	Root number	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
0	5	0 e	26.66 a	0 d	0 c	0 d	0 c
0	7	0 e	40 a	0 d	0 c	0 d	0 c
0	9	0 e	0 a	0 d	0 c	0 d	0 c
1000	5	40 abc	26.66 a	3.33 cd	11.2 a	0.36 bcd	0.061 abc
1000	7	20 cde	13.33 a	15.33 a	15.43 a	0.78 a	0.114 a
1000	9	6.66 de	6.66 a	0.33 d	2.6 bc	0.034 d	0.003 c
2000	5	46.66 ab	26.66 a	7.1 bc	13.4 a	0.7 ab	0.093 ab
2000	7	60 a	0 a	11.4 ab	9.66 abc	0.24 cd	0.03 bc
2000	9	13.33 de	0 a	0.66 d	0.26 c	0.003 d	0.0004 c
3000	5	26.66 bcd	26.66 a	12 a	6.8 abc	0.62 abc	0.072 abc
3000	7	26.66 bcd	26.66 a	7.5 bc	9.26 abc	0.32 bcd	0.047 abc
3000	9	0 e	0 a	0 d	0 c	0 d	0 c

* The values in each column with the same letter do not differ significantly ($P\leq0.05$) according to Duncan's Multiple Range Test.

Table 8. Interaction effect between IBA concentrations and substrate pH on vegetative traits of peach *cv.* Red May hardwood cuttings.

IBA concentrations (mg L^{-1})	Substrate pH	Shoot length (cm)	Shoot diameter (mm)	Leaf number	Leaf area (cm^2)	Shoot fresh weight (g)	Shoot dry weight (g)
0	5	0 b	0 c	0 b	0 c	0 b	0 b
	7	0 b	0 c	0 b	0 c	0 b	0 b
	9	0 b	0 c	0 b	0 c	0 b	0 b
1000	5	0.71 ab	0.91 ab	1.33 b	33.59 ab	0.222 ab	0.065 ab
	7	0.8 ab	1.09 ab	3.33 b	34.72 ab	0.289 ab	0.096 ab
	9	0 b	0 c	0 b	0 c	0 b	0 b
2000	5	1.52 a	1.55 a	7.55 a	47.24 a	0.447 a	0.126 a
	7	0.3 b	1.38 ab	4 ab	29.39 abc	0.195 ab	0.057 ab
	9	0.2 b	0.43 bc	2 b	7.96 bc	0.079 b	0.029 ab
3000	5	0.81 ab	0.39 bc	2.66 b	18.84 abc	0.172 ab	0.1 ab
	7	0.3 b	1.13 ab	3 b	29.02 abc	0.185 ab	0.051 ab
	9	0 b	0 c	0 b	0 c	0 b	0 b

* The values in each column with the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's Multiple Range Test.

responsible for acid-induced cell wall loosening (Cosgrove and Li, 1993; McQueen-Mason, 1995 and Ludwig-Müller *et al.*, 2005).

Table 8 shows the interaction effects between the two factors on vegetative traits. The maximum shoot traits were observed at interaction between 2000 mg L^{-1} IBA and pH 5 substrate. Cuttings planted in pH 9 substrate gave shoot traits only with 2000 mg L^{-1} IBA. Besides, shoot could not develp in control cuttings planted in the three pH substrate.

IBA concentrations and substrate pH were effective on rooting of hardwood cuttings of peach. 2000 mg L^{-1} IBA concentration and low substrate pH were the best for propagation of peach *cv.* Red May. Additionally, influence of the two factors became more considerable when they interacted together, however, the molecular mechanism of their effects require more investigation in the future.

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