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Water soaking and benzyladenine treatment for higher grafting success in grapevine

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Abstract

The grafting of grapevines has become essential to overcome biotic and abiotic stresses. Also, stimulating the rapid formation of the grafting union is the key to grafting success. This investigation was an attempt to study the effect of removing growth inhibitors through soaking in water for 24 hours versus adding growth stimulation through fast dipping in 250 ppm benzyladenine (BA) for 30 seconds of graft wood before grafting, on the grafting success of Flame Seedless and Early Sweet scions (*Vitis vinifera*) grafted onto Freedom rootstock (*Vitis champinii x 1613C*) in addition to the control treatment. Water soaking came first, followed by the treatment of 250 ppm BA, which significantly improved the grafting success of both cultivars. Grafting success was positively associated with increasing callus formation at the grafting zone, accompanied by the highest total indols content, the lowest total phenols content and peroxidase activity above and below the grafting zone. However, water soaking significantly increased total indols and decreased the total phenols content of the ungrafted cuttings. This study suggests that applying water soaking to grape cuttings before grafting is an environmentally friendly, sustainable and alternative practice for synthetic growth regulators to improve grafted cuttings' success.

Key words: Callus degree, indols, phenols, peroxidase, *Vitis vinifera*

Introduction

The grapevine (*Vitis vinifera*) is one of the crops of great economic importance to many countries, Egypt included. But it is susceptible to many biotic stresses, such as infection with phylloxera and nematodes, which cause reduced vine productivity (Rubio *et al.*, 2020). So grafting vines, on American rootstocks, resistant to biotic and abiotic stresses is required for viticulture success (Opazo *et al.*, 2020; Tedesco *et al.*, 2020). Also, other characteristics are required to select grapevine rootstocks, such as grafting compatibility (Köse *et al.*, 2015; Fayek *et al.*, 2017). In Egypt, Freedom (*V. champinii x 1613C*) is one of the most commonly used commercial rootstocks for grafting in viticulture due to its high resistance to nematode infection (El-Nabi *et al.*, 2013; Wallis, 2020), but its grafting success is lower with some grapevine cultivars such as Flame Seedless and Early Sweet (Fayek *et al.*, 2017).

Callus formation at the grafting zone is essential for vascular connection formation between scion and rootstock, which is related to the grafting success of plants (Vrsic *et al.,* 2015; Rashedy, 2016; Tedesco *et al.*, 2020). Previous studies indicated that hormonal application increased callus formation and new vascular tissue by promoting cell division and development (Yin *et al.*, 2012; Aghaei *et al.*, 2013), which is related to improving grafting success in almond (Isıkalan *et al.*, 2011), mulberry (Kako, 2012; Zenginbal and Eşitken, 2016) and walnut trees **(**Farsi *et al.*, 2018). Also, in grapevine, grafting success was improved by the application of hormones such as auxins and cytokinins (Bidabadi *et al.*, 2018) or the application of plant growth-promoting rhizobacteria (PGPR) (Sabir, 2013). On the other side, many studies indicate that soaking grapevine cuttings in water before propagation increases callus formation at the cutting base (Mohamed, 2017). Recently, in jackfruit, Basalo and Lina (2020) reported that soaking water treatment of scions of the Eviarc Sweet cultivar increased grafting success. However, there is scarce information concerning the influence of water soaking and hormonal application on grape grafting success. Therefore, this study was conducted to determine the effects of soaking grafting wood in water and dipping in benzyladenine (BA) treatments on improving the grafting success of two cultivars of grapevine (Flame Seedless and Early Sweet) on Freedom rootstock.

Materials and methods

This experiment was carried out during winter 2019 and 2020 to evaluate the effect of water soaking and benzyladenine treatments on the bench grafting success of Flame Seedless and Early Sweet grapevines (*vitis vinifera*) on Freedom rootstock (*V. champinii x* 1613C) at the nursery and laboratory of the Pomology Department, Faculty of Agriculture, Cairo University at Giza, Egypt (30°01'04"N31°12'30"E).

Plant materials: Woody cuttings of Flame Seedless and Early Sweet scions and Freedom rootstock were obtained in the first week of January and cold stored for one month at 4° C and 70-80 RH before grafting (Korkutal *et al.*, 2011).

Grafting and soaking treatments: The cuttings were cut into 5-7 cm lengths with a single bud for scions (Flame Seedless and Early Sweet) and 25cm lengths with 3-4 nodes for rootstock (Freedom). Buds of rootstock cuttings were then removed with a knife. Grafting was done using the tongue graft technique on 1st February. The following treatments were as follows: the

cuttings were grafted without any application as control (T_1), the cuttings of both the scion and rootstock were soaked in water for 24 hours before grafting (T_2), and the cut grafting surfaces of both the scions and rootstock were dipped in 250 ppm BA for 30 seconds immediately before grafting (T_3). After grafting, the grafted areas were covered (rolled and tied) with special plastic parafilm and dipped for one second into hot grafting-wax paraffin at 40°C. The cutting bases of the rootstocks were fast dipped in 2000ppm indole-3-butyric acid for 5 seconds, then stored in a wetted peat and sawdust mixture (1:4 V/V) at 28°C and 95 % RH for 30 days before being planted in the nursery conditions (Paunović *et al.*, 2012).

Planting and growing conditions: Grafted cuttings were planted in a black plastic bag 30 cm x 30 cm filled with washed sand under a plastic tunnel in a shade-net greenhouse (shade rating of 40 %) for one month before being removed on 1st April and kept under greenhouse conditions up to the end of the experiment. The grafts were irrigated with tap water every second day. During the experiment period, the application of fertilization was added at a weekly rate of 0.25 strength Hoagland nutrients concentration (Fozouni *et al.*, 2012) and the pests were treated when needed by usual agricultural practices.

Morphological parameters: Callus degree was assessed at the grafting union after 30 days of grafting (1st March) based on visible observations: 0 = no callus, 1 = 25 % callus, 2 = 50 % callus, 3 = 75 % callus, and 4 = 100 % callus (Çelik, 2000). The following measurements were recorded after four months of grafting (1st June). The grafting success percentage was calculated using the following equation: (total number of successful grafts/total number of grafts) x 100. Data on shoot length of the scion (cm), leaves number, leaf area (cm^2), shoot and root fresh weight (g) and shoot and root dry weight (g) were recorded.

Biochemical analysis: The bark samples were taken with a sharp knife from ungrafted cuttings of scions and rootstock before and after soaking in water treatment (1st February). Also, after four months of grafting (1st June), it was taken at 4 cm above and below the grafting zone of all graft combinations. These samples were used to determine the total phenols, total indols and peroxidase activity.

Total phenols (mg/g FW): Total phenols content was determined according to the Folin-Ciocalteu method (Sharma *et al.*, 2019). The samples (0.5 g FW) were extracted for three days in the dark in 20 mL of methanol (80 %). One mL of extract was mixed with 1 mL of Folin 10 %, 5 mL of sodium carbonate (20 %) and the final volume was adjusted to 10 mL with distilled water. The mixture remained for one h and then absorbance at 765 nm was determined by a spectrophotometer. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of fresh bark weight.

Total indols (mg/g FW): Total indols content was determined according to (Larsen *et al.*, 1962). The samples (0.5 g FW) were extracted for three days in the dark in 20 mL of methanol (80 %). 1 mL of extract was mixed with 4 mL of P-dimethyl amino benzaldehyde (1 g of P-dimethyl amino benzaldehyde dissolved in 50 mL of HCL and 50 mL of ethanol 95 %). The mixture remained for 1.30 h at 30°C and absorbance at 530 nm was determined by a spectrophotometer. The total indols content was expressed as indole acetic acid (IAA) in milligrams per gram of fresh bark weight.

Peroxidase activity (mg/g FW): The samples (0.5 g FW) were stored at -20°C and then processed as described in Ni *et al.* (2001). The enzymes from the frozen plant samples were extracted using cold potassium phosphate buffer (0.1M, pH 7.0) containing 1 % (w/v) polyvinylpyrrolidone and 1 % (v/v) Triton X-100. The samples were macerated with 1 mL of the extracting buffer and further ground with another 1 mL of the extracting buffer. In total, 2 mL of the extracting buffer was used for each sample. An aliquot (1.5 mL) of the extract was centrifuged at 10000 rpm for 10 minutes at 4 ºC. The supernatant was immediately frozen for future enzyme activity assays. Peroxidase activity was determined according to the procedure given by Hammerschmidt *et al.* (1982). 1.5 mL of pyrogallol (0.05 M) and 100 µL of enzyme extract were added to a spectrophotometer sample cuvette. The readings were adjusted to zero at 420 nm. To initiate the reaction, 100 µL of hydrogen peroxide (1%) was added to the sample cuvette. The enzyme activity was expressed as a change in absorbance/min/g sample.

Statistical analysis: The experiment was designed in randomized complete blocks with three replications and 20 grafts per replicate. The data was analyzed using the MSTAT pocket program. The means of the treatments were compared using the LSD value at 5 % (Duncan, 1955).

Results

The study's results revealed intriguing findings across various aspects of grafting and growth in Flame Seedless and Early Sweet grape cultivars on Freedom rootstock. Callus formation and grafting success significantly differed due to rootstock, treatments, and their interaction. Soaking impacted phenols, indols, and ungrafted cuttings.

Table 1. Effect of soaking in water and BA on callus degree at grafting zone of Flame Seedless and Early Sweet grafted onto Freedom rootstock

Treatments (B)	First season			Second season		
	Grafts combination (A)		Mean B	Grafts combination (A)		Mean B
	Flame Seedless / Freedom	Early Sweet / Freedom		Flame Seedless / Freedom	Early Sweet / Freedom	
Control	3.000 cd	2.160 e	2.580 C	3.160 a	2.660 a	2.910 B
Soaking in water	3.330 b	2.887 d	3.108 B	3.163 a	2.997 a	3.080 B
Dipping in BA	3.660 a	3.110 bc	3.385 A	3.387 a	3.497 a	3.442 A
Mean A	3.330 A	2.719 B		3.237 A	3.051 A	

Means difference within grafts combination, treatments and interaction (grafts combination x treatments) according to LSD value at 5 %.

Callusing: The presented data in Table 1 show that Flame Seedless grafted onto Freedom rootstock recorded an increase in callus degree at grafting union (3.330 and 3.237) through the first and second seasons, respectively, compared to Early Sweet grafted onto Freedom rootstock (2.719 and 3.051). As for the effect of treatments, it is clear that the BA treatment significantly increased the average callus degree at the grafting union for both graft combinations through the first and second seasons (3.385 and 3.442) compared to the control treatment, which gave the lowest significant values (2.580 and 2.910). Also, water soaking treatment increased callus degree compared to the control with a significant value in the first season. Notably, this effect was observed in the first season, with BA-treated grafts showing higher callus formation than the control, which resulted in the lowest callus values. This suggests that BA treatment positively influenced callus formation for both graft combinations in the first season of the study.

Grafting success (%): The results in Table 2 show a difference in grafting success percentage between the graft combinations and soaking treatments during the two study seasons. Flame Seedless grafted onto Freedom rootstock achieved the highest significant grafting success percentage (62.217 and 71.944 %) through the first and second seasons, respectively, compared to Early Sweet grafted onto Freedom rootstock. Concerning the effect of soaking treatments on grafting success, it is clear that soaking in water significantly increased the average grafting success percentage for both graft combinations (54.440 and 79.167 %) rather than the BA treatment (49.992 and 68.333 %) through the first and second seasons respectively, compared to the control treatment, which recorded the lowest significant values (35.182 and 62.500 %). Concerning the interaction effect (grafts combination X treatments), the data revealed that soaking in water significantly increased grafting success percentage in both seasons for Flame Seedless grafted onto Freedom rootstock (71.107 and 79.167 %) and Early Sweet grafted onto Freedom rootstock (37.773 and 79.167 %) compared to control treatment for the same graft combinations. Also, BA treatment significantly increased grafting success only for the graft combination Flame Seedless onto Freedom rootstock (71.103 and 73.333) during the first and second seasons, respectively. But the increase was not significant for Early Sweet grafted onto Freedom rootstock (28.880 and 63.333 %) through the first and second seasons, respectively, compared to control treatment for the same graft combinations.

Content of total phenols and indols (mg/g FW) before grafting: According to the results in Table 3, soaking in water significantly decreased the average content of total phenols (4.591 mg/g FW) and increased total indols (0.173 mg/g FW) for both ungrafted cuttings compared to non-soaked control cuttings. Regarding the cultivar of ungrafted cuttings, Flame Seedless cuttings recorded, on average, the lowest significant value for total phenol content (3.400 mg/g FW) compared to Early Sweet (3.918 mg/g FW) and Freedom cuttings, which had the highest phenols content (8.928 mg/g FW). Meanwhile, the highest total indol content was recorded with Freedom cuttings, followed by Flame Seedless cuttings (0.175 and 0.168 mg/g FW), respectively, compared to Early Sweet cuttings, which had the lowest content (0.161 mg/g FW). Concerning the interaction effect (treatments X ungrafted cuttings), the data showed that treatment of soaking in water for Flame Seedless, Early Sweet and Freedom cuttings significantly decreased total phenols content (3.164, 3.331 and 7.278 mg/g FW), respectively, compared to nonsoaked control cuttings for each cultivar. Meanwhile, the total indol content of both ungrafted cuttings recorded no significant increase when soaked in water compared to the control treatment.

Vegetative growth of grafts: The present data (Table 4) shows a significant difference between the grafts combination through the first and second seasons for morphological measurements, except for the number of leaf parameters. Grafts of Flame Seedless on Freedom rootstock achieved the highest significant shoot length (68.333 and 68.333 cm), leaf area (67.163 and 70.773 cm^2), shoot fresh weight (22.997 and 24.193 g), and root fresh weight (6.007 and 5.823 g) through the first and second seasons respectively

Table 2. Effect of soaking in water and BA on grafting success (%) of Flame Seedless and Early Sweet grafted onto Freedom rootstock

Treatments (B)	First season			Second season		
	Grafts combination (A)		Mean B	Grafts combination (A)		Mean B
	Flame Seedless / Freedom	Early Sweet / Freedom		Flame Seedless / Freedom	Early Sweet / Freedom	
Control	44.440 b	25.923 d	35.182 C	63.333 b	61.667 b	62.500 C
Soaking in water	71.107 a	37.773 c	54.440 A	79.167 a	79.167 a	79.167 A
Dipping in BA	71.103 a	28.880 d	49.992 B	73.333 a	63.333 b	68.333 B
Mean A	62.217A	30.859 B		71.944 A	68.056 B	

Means difference within grafts combination, treatments and interaction (grafts combination x treatments) according to LSD value at 5 %.

Table 3. Effect of soaking in water on total phenols content (mg/g F.W) and total indols content (mg/g F.W) of ungrafted cuttings of Flame Seedless, Early Sweet cvs. and Freedom rootstock

Ungrafted cuttings (A)	Total phenols (mg/g F.W)			Total indols (mg/g F.W)		
	Treatments (B)			Treatments (B)		
	Control	Soaking in water	Mean A	Control	Soaking in water	Mean A
Flame Seedless cv.	3.636 d	3.164 e	3.400 C	0.161 a	0.175 a	0.168 AB
Early Sweet cv.	4.505 c	3.331 de	3.918 B	0.161 a	0.162 a	0.161 B
Freedom rootstock	10.578 a	7.278 b	8.928 A	0.167 a	0.183 a	0.175 A
Mean B	6.240 A	4.591 B		0.163 B	0.173 A	

Means difference within ungrafted cuutings, treatments and interaction (grafts combination x treatments) according to LSD value at 5 %.

Table 4. Morphological measurements of the grafts combination Flame Seedless and Early Sweet onto Freedom rootstock

Parameters	First season		Second season	
	Flame Seedless / Freedom	Early Sweet / Freedom	Flame Seedless / Freedom	Early Sweet / Freedom
Shoot length (cm)	68.333 *	52.333	68.333 *	55.107
Leaf number (n)	27.000 ns	22.000	22.890 ns	20.000
Leaf area (cm^2)	67.163 *	36.630	70.773 *	46.653
Shoot fresh weight (g)	22.997 *	15.000	24.193 *	17.757
Root fresh weight (g)	6.077 *	4.143	5.823 *	4.527
Shoot dry weight (g)	8.553 ns	6.383	9.457 *	6.353
Root dry weight (g)	2.670 *	1.937	2.737 ns	2.197

Means difference within grafts combination for each parameter was significantly at $P<0.05$.

compared to grafts of Early Sweet on Freedom rootstock which recorded the lowest significant values. Moreover, Flame Seedless grafting onto Freedom rootstock showed a significant increase in shoot dry weight (9.457 g) and root dry weight (2.670 g) in the second and first seasons, respectively, compared to Early Sweet grafting on Freedom rootstock in the same seasons.

Biochemical analysis of grafts: After four months of grafting, the phenols, total indols and peroxidase activity were determined above and below the grafting union of the grafts combination under study. Data presented in Table 5 showed that Flame Seedless grafted onto Freedom rootstock showed a significant decrease in total phenols content above the grafting union (11.790 mg/g FW) besides nonsignificant reduction in total phenols at below the grafting side (11.850 mg/g FW) compared to Early Sweet grafted onto Freedom rootstock, which recorded the highest values (12.250 and 11.970 mg/g FW) at above and below grafting sides respectively. On the opposite, the results of total indols (Table 5) revealed that a significantly high content of these components in Flame Seedless grafted onto Freedom rootstock at above the grafting union (0.223 mg/g FW) besides nonsignificant high indols content at the below grafting side (0.223 mg/g FW) compared to Early Sweet grafted onto Freedom rootstock, which recorded the lowest significant value (0.157 mg/g FW) at above and nonsignificant low indols content (0.217 mg/g FW) at below grafting union respectively. As for peroxidase activity, it showed a significant decrease with Flame Seedless grafted onto Freedom rootstock at above and below the grafting union (6.700 and 5.747 mg/g FW), respectively, compared to Early Sweet grafted onto Freedom rootstock at above and below the grafting sides (12.033 and 7.373 mg/g FW).

Discussion

Callus formation at the graft zone is essential for grafting success in plants by promoting new vascular connections between the scion and the rootstock (Aloni *et al.*, 2008). The report is consistent with our findings, which revealed that the higher degree of callusing at the grafting zone was recorded with Flame Seedless onto Freedom rootstock, which achieved the highest grafting success rather than Early Sweet on the same rootstock. Soaking scion and rootstock cuttings in water before grafting increased the callus degree at the grafting zone and the grafting success of Flame Seedless and Early Sweet grafted onto Freedom rootstock compared to the control treatment. This is in agreement

Table 5. Chemical content above and below the grafting union of the grafts combination of Flame Seedless and Early Sweet onto Freedom rootstock after four months from grafting.

Parameters	Above the graft union		Below the graft union	
	Flame Seedless/ Freedom	Early Sweet / Freedom	Flame Seedless / Freedom	Early Sweet / Freedom
Total phenols content (mg/g FW)	11.790	12.250*	11.850	11.970ns
Total indols content (mg/g FW)	0.223*	0.157	0.223ns	0.217
Peroxidase activity (mg/g FW)	6.700	12.033*	5.747	7.373*

The difference between grafts combination for each parameter within each grafting side was significant at $P< 0.05$.

with (Mohamed, 2017), who reported soaking grapevine cuttings in water before propagation enhanced callus formation at the base of the cuttings. This may be due to leaching out of growth inhibitors (Mohamed, 2017) or increasing IAA levels in cuttings by soaking water treatment (Gökbayrak *et al.*, 2010). We also found that soaking scions and rootstock cuttings in water before grafting decreased total phenol content (as inhibitors) and increased total indols content of ungrafted cuttings compared to the control treatment. Yin *et al.* (2012) reported that increased auxin levels stimulated vascular differentiation, cell division and differentiation at grafting union.

Increased total phenols caused reduced cell division, development and differentiation (Gainza *et al.*, 2015), thus, poor callus formation at the grafting union (Mng'omba *et al.*, 2008).

It is well known that hormones play an important role in cell division and differentiation, thus increasing callus formation and new vascular tissue (Maxwell and Kieber, 2010; Aghaei *et al.*, 2013). Moreover, as found in the present study, cytokinin treatment (BA) increased callus degree at the grafting zone and grafting success of Flame Seedless and Early Sweet cultivars grafted onto Freedom rootstock compared to the control treatment. Bidabadi *et al.* (2018) also found that application of cytokinins on cuttings of grapevines before grafting increased callus degree at the grafting zone and grafting success between scion cultivars and rootstocks. Also, Sabir (2013) found that the application of plant growth-promoting rhizobacteria (PGPR) improved the grafting success of graft combinations of grapevines related to better callus degree at the graft union point.

It is well known that the accumulation of some chemical compounds above and below the grafting union plays a major role in the success and compatibility of grafting between scion and rootstock in fruit tree species (Darikova *et al.*, 2011; Hudina *et al.*, 2014). In our study, the lowest total phenols content above and below the grafting zone was associated with highly compatible grafts of Flame Seedless on Freedom rootstock, which gave the higher grafting success and morphological measurements compared to Early Sweet on the same rootstock. These results agree with those of Stino *et al.* (2011) and Fayek *et al.* (2017), as they found that the lowest total phenols content was recorded with grafts combination of grapevines, which gave the highest grafting success. Also, Çölgeçen and Azimi (2015) found that phenolic compounds increased at the grafting union of the Domat olive cultivar grafted onto Gemlik rootstock, which recorded the lowest callus degree and grafting success percentage. Accumulation of

phenols compounds above and below the graft zone may cause a decrease in cell division and development, resulting in poor callus formation at the grafting union (Mng'omba *et al.*, 2008). Gainza *et al.* (2015) suggested that phenolic compounds disrupt xylem and phloem tissue growth and cause hormonal imbalances at the grafting union.

Furthermore, the current study revealed that increasing total indol content above and below the graft zone was concomitant with the graft combination of Flame Seedless on Freedom rootstock, which recorded higher grafting success and morphological measurements compared to Early Sweet on the same rootstock. Stino *et al.* (2011) also reported that the highest grafting success in graft combination grapevines was related to the highest total indols at the graft zone. In this respect, Sharma and Zheng (2019) demonstrated that auxins are the main hormones that regulate the growth and development of vascular tissues, and their crosstalk with ether hormones further regulates the auxin cell signalling involved in the process of vascular tissue development.

Also, the highest peroxidase activity level was related to the lowest success and compatibility of grafting between scion and rootstock in many plants (Zarrouk *et al.*, 2010; Güçlü and Koyuncu, 2012). Lowest peroxidase activity above and below the grafting zone was obtained with the graft combination of Flame Seedless on Freedom rootstock, which recorded higher grafting success and morphological measurements compared to Early Sweet on the same rootstock. Fayek *et al.* (2017) found that the best grafting success was obtained with grapevine cultivars grafted onto Paulsen rootstock, which had the lowest peroxidase activity above and below the grafting union. Accumulation of total phenols in the less compatible grafts may explain the increase in peroxidase activity above and below the grafting union because peroxidases are related to the oxidation of phenolic compounds (Zarrouk *et al.*, 2010).

Soaking grapevine cuttings in water for 24 hours or dipping in 250 ppm BA for 30 seconds before grafting improved the grafting success percentage of graft combinations by improving callus formation at the grafting union zone. Compatible grafting of Flame Seedless rather than Early Sweet on Freedom rootstock was concomitant with a decrease in phenolic compounds and peroxidase activity above and below the grafting zone, besides an increase in auxin content which is required for vascular connection between rootstock and scion.

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