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Crop production

Water soaking and benzyladenine as strategy for improving grapevine grafting success

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Abstract -The grafting of grapevines has become essential to overcome biotic and abiotic stresses because permits access to the benefits of the agronomical characteristics of different rootstocks. Stimulating the rapid formation of the grafting union is the key to grafting success. This investigation studied the effect of removing growth inhibitors through soaking in water for 24 hours versus adding growth stimulate through fast dipping in 250 mg/L 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*). Water soaking came first and followed by treatment of 250 mg/L BA, which significantly improved the grafting success of both cultivars. Grafting success was positively associated with increasing callus formation at the grafting zone, which was accompanied with 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. In this study, it was suggested that the application of water soaking to grafted cuttings prior to grafting is an environmentally friendly and alternative practice for synthetic growth regulators to improve grafted cuttings success.
Index terms: Callus degree; Indols; Phenols; Peroxidase; *Vitis vinifera.*

Imersão em água e benziladenina como estratégia para melhorar o sucesso da enxertia em videira

Resumo -A enxertia de videiras tornou-se essencial para superar tensões bióticas e abióticas, pois permite acessar os benefícios de características agronômicas de diferentes porta-enxertos. Estimular a formação rápida da união do enxerto é a chave para o sucesso da enxertia. Esta investigação estudou o efeito da remoção de inibidores de crescimento através da imersão em água por 24 horas versus a adição de estimulador de crescimento através do mergulho rápido em 250 mg-1 de benziladenina (BA) por 30 segundos da estacas do enxerto antes da enxertia dos rebentos de sucesso Flame Seedless e Early Sweet (*Vitis vinifera*) enxertados no porta-enxerto Freedom (*Vitis champinii* x 1613C).A imersão em água veio primeiro, seguido do tratamento de 250 mg/L BA, o que melhorou significativamente o sucesso da enxertia de ambas as cultivares. O sucesso da enxertia foi positivamente associado ao aumento da formação de calos na zona de enxertia, que foi acompanhado com o maior índice total de indols, o menor índice total de fenóis e a atividade peroxidase acima e abaixo da zona de enxertia. No entanto, a imersão em água aumentou significativamente os indóis totais e diminuiu o conteúdo total de fenóis das estacas não enxertadas. Neste estudo, sugere-se que a aplicação de imersão em água para estacas enxertadas antes do enxerto é uma prática ambientalmente amigável e alternativa para os reguladores de crescimento sintético para melhorar o sucesso das estacas enxertadas.
Termos de indexação: grau de calosidade; indóis; fenóis; peroxidase; *Vitis vinifera.*

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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 a reduction in vine productivity (NICOL et al., 1999; BONA et al., 2007). So grafting vines on resistant American rootstocks to biotic and abiotic stresses is required for viticulture success (COOKSON et al. 2013; CORSO and BONGHI, 2014; RASHEDY, 2016; OPAZO et al., 2020; TEDESCO et al., 2020). Also, other characteristics are required for the selection of grapevine rootstocks, such as grafting compatibility (REYNOLDS; WARDLE, 2001; COOKSON et al., 2013; KÖSE et al., 2015; FAYEK et al., 2017). Also, the rootstock has an effect on the vegetative growth, nutrient content, quality, and production of grafted vines on it (RIZK-ALLA et al. 2011; EL-GENDY, 2013). 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 the new vascular connection regeneration between scion and rootstock, which is related to the grafting success of plants (KHILI et al., 1995; ÇELIK, 2000; ALONI et al., 2008; VRSIC et al., (2015); RASHEDY, 2016; TEDESCO et al., 2020). Previous studies have indicated the auxins and cytokines application increased the formation of callus and new vascular tissue via promoting cell division and development (RAVEN et al., 1992**;** ALONI et al., 2010; MAXWELL; KIEBER, 2010; YIN et al., 2012; AGHAEI et al., 2013). In this respect, auxins play a major role in regulating the growth and development of vascular tissues (xylem and phloem tissues), and their crosstalk with ether hormones further regulates the auxin cell signaling involved in the process of vascular tissue development (SHARMA; ZHENG, 2019), which is related to improving grafting success in citrus (SHINDE et al., 2008), almond (ISIKALAN et al., 2011), mulberry (KAKO, 2012; ZENGINBAL; EŞITKEN, 2016) and walnut trees (FARSI et al., 2018**)**. In grapevine, grafting success was improved by the application of hormones such as auxins and cytokinins (KÖSE; GÜLERYÜZ, 2006; BIDABADI et al., 2018) or the application of plant growth-promoting rhizobacteria (PGPR) (KÖSE et al., 2005; SABIR, 2013**)**. On the other side, many studies also indicated that soaking grapevine cuttings in water before propagation increases callus formation at the cutting base (WAITE; MAY, 2005; MOHAMED, 2017). Soaking cuttings in water leaches out growth inhibitors such as GA and ABA, which caused a decrease in cell division and the formation of callus tissue. Also, It has increased auxins like IAA levels in cuttings, which played a role in increasing cell division and developing callus tissue (KRACKE et al., 1981). However, Bazzi et al. (1991) found that soaking cuttings of grapevine in hot water before grafting improved callus formation and grafting success between four scions 'Albana', 'Lambrusco Grasparossa', 'Rulander' and 'Forttana' and four rootstocks 'Kober 5BB', '420A', 'Paulsen 1103' and '41B'. 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 (First season) and 2020 (second season)_to evaluate the effect of water soaking and benzyladenine treatments on the bunch 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 for the years (2019 and 2020) and cold stored for one month at 4° C and 70-80 RH before grafting (HALBROOKS, 1985; 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 25 cm 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 manual on 1st February (2019 and 2020). The following treatments were carried out: the cuttings were grafted without any application as control (T_1), the cuttings of both the scion and rootstock were soaked in running water for 24 hours before grafting (T_2), and the cut grafting surfaces of both the scions and rootstock were dipped in 250 mg/L 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 2000 mg/L 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 (2019 and 2020) and kept under greenhouse conditions up to the end of the experiment. The grafts were irrigated with tap water each two days. During the experiment period, the fertilizers were 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). Also, after four months of grafting (1st June) were recorded the grafting percentage, which was calculated by using the following equation: (total number of successful grafts/ total number of grafts x 100) 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).

Biochemical analysis

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

Total indols (mg/g FW)

Total indole 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%). An aliquot with 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 indole 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. Samples were 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 10 000 rpm for 10 minutes at 4 C. The supernatant was immediately frozen for future enzyme activity assays. Peroxidase activity was determined according to the procedure given by Hammerschmidt et al. (1982). To a spectrophotometer sample cuvette, 1.5 mL of pyrogallol (0.05 M) and 100 µl of enzyme extract were added. 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

This experiment contains interactions between two graft combinations and three treatments, including six treatments, each one divided into three replicates and 20 grafts per replicate. The data was analyzed using the MSTAT pocket program. The means of the treatments were compared using the L.S.D value at 5% (DUNCAN, 1955).

Results

Callus degree

The presented data in Table 1 showed that 'Flame Seedless' grafted onto 'Freedom' rootstock recorded an increase in callus degree at grafting union through the first and second seasons respectively, compared to 'Early Sweet' grafted onto 'Freedom' rootstock. As for the effect of treatments, it is clear that the BA treatment significantly increased the average callus degree at grafting union for both graft combinations through the first and second seasons compared to the control treatment, which gave the lowest significant values. Water soaking treatment increased callus degree compared to the control with a significant value in the first season.

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)	2019 Grafts combination (A)			2020 Grafts combination (A)		
	'Flame Seedless' / 'Freedom'	'Early Sweet' / 'Freedom'	Mean B	'Flame Seedless' / 'Freedom'	'Early Sweet' / 'Freedom'	Mean B
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 interactions (grafts combination x treatments) according to LSD value at 5%.

Grafting success

The results in Table 2 show a difference in grafting success percentage between the graft combinations and soaking treatments during the two seasons. 'Flame Seedless' grafted onto 'Freedom' rootstock achieved the highest significant grafting success percentage through the first and second seasons respectively, compared to 'Early Sweet' grafted onto 'Freedom' rootstock. With respect to 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 rather than the BA treatment through the first and second seasons respectively, compared to the control treatment, which recorded the lowest significant values. Concerning the interaction effects (grafts combination X treatments), soaking in water significantly increased grafting success percentage in both seasons for 'Flame Seedless' grafted onto 'Freedom' rootstock and 'Early Sweet' grafted onto 'Freedom' rootstock compared to control treatment for the same graft combinations. BA treatment significantly increased grafting success only for the graft combination 'Flame Seedless' onto 'Freedom' rootstock during the first and second seasons, respectively. But the increase was not significant for 'Early Sweet' grafted onto Freedom rootstock through the first and second seasons, respectively, compared to control treatment for the same graft combinations.

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

Treatments (B)	2019 Grafts combination (A)			2020 Grafts combination (A)		
	'Flame Seedless' / 'Freedom'	'Early Sweet' / 'Freedom'	Mean B	'Flame Seedless' / 'Freedom'	'Early Sweet' / 'Freedom'	Mean B
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 interactions (grafts combination x treatments) according to LSD value at 5%.

Total phenols and total indols content of ungrafting cuttings

According to the results in Table 3, soaking in water significantly decreased the average content of total phenols and increased total indols 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 phenols content compared to 'Early Sweet' and 'Freedom' cuttings which had the highest phenols content. Meanwhile, the highest total indole content was recorded with Freedom cuttings, followed by 'Flame Seedless' cuttings compared to 'Early Sweet' cuttings, which had the lowest content. Concerning the interaction effect (treatments X cuttings), the data showed that treatment of soaking in water for 'Flame Seedless', 'Early Sweet' and 'Freedom' cuttings significantly decreased total phenols content compared to non soaked control cuttings for each cultivar. Meanwhile, the total indols content of both ungrafted cuttings recorded no significant increase when soaked in water compared to the control treatment.

Table 3. Effect of soaking in water on total phenols content (mg/g F.W) and total indols content (mg/g F.W) of 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 cuttings, treatments, and interactions (grafts combination x treatments) according to LSD value at 5%.

Morphological parameters

The present data in Table 4 show a significant difference between the grafts combination through the first and second seasons for morphological measurements, except for the leaf numbers parameter. Grafts of 'Flame Seedless' on 'Freedom' rootstock achieved the highest significant shoot length, leaf area, shoot fresh weight, and root fresh weight through the first and second seasons respectively 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 and root dry weight in the second and first seasons respectively compared to 'Early Sweet' grafting on Freedom rootstock in the same seasons.

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

Parameters	2019		2020	
	Grafts combination		Grafts combination	
	'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 $p<0.05$.

Total phenols, total indols content, and peroxidase activity of grafts

After four months of grafting, the following total phenols, total indols, and peroxidase activity were determined at 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 beside, non-significant reduction in total phenols below the grafting side compared to 'Early Sweet' grafted onto 'Freedom' rootstock, which recorded the highest values at above and below grafting sides respectively. On the opposite, the results of total indols (table 5) revealed that a significant high content of these components in 'Flame Seedless' grafted onto 'Freedom' rootstock at above the grafting union besides non-significant high indols content at the below the grafting side compared to 'Early Sweet' grafted onto 'Freedom' rootstock, which recorded the lowest significant value at above and non-significant low indols content 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 compared to 'Early Sweet' grafted onto 'Freedom' rootstock at above and below the grafting sides.

Table 5. Chemical content at 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	At above the grafting zone		At below the grafting zone	
	Grafts combination		Grafts combination	
	'Flame Seedless' / 'Freedom'	'Early Sweet' / 'Freedom'	'Flame Seedless' / 'Freedom'	'Early Sweet' / 'Freedom'
Total phenols content (mg/g F.W)	11.790	12.250*	11.850	11.970^{ns}
Total indols content (mg/g F.W)	0.223*	0.157	0.223^{ns}	0.217
Peroxidase activity (mg/g F.W)	6.700	12.033*	5.747	7.373*

Means difference between grafts combination for each parameter within each side of grafting was significant at $p< 0.05$.

Discussion

Callus formation at the graft zone is an essential factor for grafting success in plants, by promoting the formation of new vascular connections between the scion and the rootstock (KHILI et al., 1995; ALONI et al., 2008). This is consistent with our findings, which revealed that the higher callus degree 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 as shown in Table 1. This is in agreement with our results in table 2, 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 control treatment (Table 1 & 2). This is in agreement with WAITE and MAY (2005) and MOHAMED (2017) 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 (KRACKE et al., 1981; WAITE and MAY, 2005; MOHAMED, 2017) or increasing IAA levels in cuttings by soaking water treatment (KRACKE et al., 1981). These findings are consistent with our results in table 3, We 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 control treatment. Yin et al. (2012) reported that an increase in auxins levels such as indole acetic acid (IAA) stimulated vascular differentiation, cell division, and differentiation at grafting union. Meanwhile, an increase in some phenols compounds such as Flavonol (catechins and proanthocyanidins) caused reducing cell division, development and differentiation (GAINZA *et al*., 2015) thus poor callus formation at the grafting union (MNG'OMBA et al., 2008). The present study revealed in table 1 and 2, that soaking in water treatment achieved the highest grafting success and callus degree of all graft combinations, as compared to the control treatment. Similar results were found by BazzI et al. (1991) in grapevine Basalo and Lina (2020) in jackfruit, as they found that soaking cuttings in water treatment before grafting improved callus degree and grafting success.

It is well known that hormones play an important role in cell division and differentiation, thus increasing the formation of callus and new vascular tissue (ALONI et al., 1990; RAVEN et al., 1992; MAXWELL; 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. These results are in agreement with those obtained by Köse and Guleryuz (2006) and Bidabadi et al. (2018) as they found that applications of cytokinin on cuttings of grapevines before grafting increased callus degree at the grafting zone and grafting success between scion cultivars and rootstocks. Also, Köse et al. (2005) and Sabir (2013) found that the application of plant growth-promoting rhizobacteria (PGPR) improved the grafting success of graft combinations of grapevine related to better callus degree at the graft union point.

It is well known too that the accumulation of some chemical compounds at 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 (MNG'OMBA et al., 2008; DARIKOVA et al., 2011; HUDINA et al., 2014). In our study, the lowest total phenols content at above and below the grafting zone (Table 5) were associated with high compatible grafts of 'Flame Seedless' on 'Freedom' rootstock, which gave the higher grafting success and morphological measurements (Tables 2 & 4) 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 were recorded with grafts of combination grapevines, which gave the highest grafting success. Also, Çölgeçen and Azimi (2015) found that phenolic compounds increased at the grafting union of Domat olive cultivar grafted onto Gemlik rootstock which recorded the lowest callus degree and grafting success percentage. Accumulation of phenols compounds at 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) suggest 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 indols content at above and below the graft zone (Table 5) was concomitant with graft combination of 'Flame Seedless' on 'Freedom' rootstock which recorded higher grafting success and morphological measurements (Tables 2 and 4) compared to 'Early Sweet' on the same rootstock. These results were in agreement with Stino et al. (2011) who reported that the highest grafting success in grafts combination grapevines was related to the highest total indols at the graft zone. Also, Aloni (1980) found that application of a low amount of auxin differentiates phloem in the callus of several plant species, while a high amount induces both phloem and xylem. 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 signaling 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 (PINA; ERREA, 2005; ZARROUK et al., 2010; GÜÇLÜ; KOYUNCU, 2012). It is clear from tables (2, 4, and 5) that the lowest peroxidase activity at above and below the grafting zone was obtained with the graft combination of 'Flame Seedless' on 'Freedom' rootstock which recorded the higher grafting success and morphological measurements compared to 'Early Sweet' on the same rootstock. These findings were consistent with those of Fayek et al. (2017) who found that the best grafting success was obtained with grapevine cultivars grafted onto 'Paulsen1103' 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 at above and below the grafting union because peroxidases are related to the oxidation of phenolic compounds (ZARROUK et al., 2010). However, Kawaguchi and Taji (2003) suggested that increased peroxidase activity in graft incompatible combinations may be due to the stress of a lack of water and mineral nutrients.

Conclusion

The soaking of grapevine cuttings in water for 24 hours or dipping in 250 mg/L BA for 30 seconds before grafting has a positive effect on the grafting success percentage of graft combinations via 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 at above and below the grafting zone, besides an increase in auxins content which is required for vascular reconnection between the two junctions rootstock and scion. Also, we believe that soaking grape grafted cuttings prior to grafting is an environmentally friendly, sustainable, and alternative practice for synthetic growth regulators to improve grafted cuttings' success.

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References

AGHAEI, P.; BAHRAMNEJAD, B.; MOZAFARI, A.A. Effect of different plant growth regulators on callus induction of stem explants in'Pistacia atlantica'subsp. kurdica. **Plant Knowledge Journal**, Brisbane, v.2 n.3, p.108-112, 2013.

ALONI, B.; COHEN, R.; KARNI, L.; AKTAS, H.A.K.A.N.; EDELSTEIN, M. Hormonal signaling in rootstock–scion interactions. **Scientia Horticulturae**, Wageningen, v.127, n.2, p.119-126, 2010.

ALONI, B.; KARNI, L.; DEVENTURERO, G.; LEVIN, Z.; COHEN, R.; KATZIR, N.; LOTAN-POMPAN, M.; EDELSTEIN, M.; AKTAS, H.; TURHAN, E.; JOEL, D.M.; HOREV, C.; KAPULNIK, Y. Physiological and biochemical changes at the rootstock-scion interface in graft combinations between Cucurbita rootstocks and a melon scion. **The Journal of Horticultural Science and Biotechnology**, Ashford, v.83, n.6, p.777-783, 2008.

ALONI, R. Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. **Planta**, Berlin, v.150, p.255-263, 1980.

ALONI, R.; BAUM, S.F.; PETERSON, C.A. The role of cytokinin in sieve tube regeneration and callose production in wounded Coleus internodes. **Plant Physiology**, Rockville, v.93, n.3, p.982-989, 1990.

BASALO, J.A.; LINA, D.P. Enhancing Graft-Take Success in Jackfruit (*Artocarpus heterophyllus Lam.)* Var.“EVIARC Sweet” Seedlings by Pre-Grafting Treatments. **Mindanao Journal of Science and Technology**, Cagayan de Oro, v.18, n.1, p.1-15, 2020.

BAZZI, C.; STEFANI, E.; GOZZI, R.; BURR, T.J.; MOORE, C.L. Hot-water treatment of dormant grape cuttings: Its effects on Agrobacterium tumefaciens and on grafting and growth of vine. **Vitis**, Davis, v.30, n.3, p.177-187. 1991.

BIDABADI, S.S.; AFAZEL, M.; SABBATINI, P. Iranian grapevine rootstocks and hormonal effects on graft union, growth and antioxidant responses of Asgari seedless grape. **Horticultural Plant Journal**, Amsterdam, v.4, n.1, p.16-23, 2018.

BONA, C.M.; GOULD, J.H.; CREIGHTON, J.; MILLER, JR.; MCEACHERN, G.R.; SETAMOU, M.; LOUZADA, E.S. In vitro micropropagation of nine grape cultivars. **Subtropical Plant Science**, Weslaco, v.59, p.56-63, 2007.

ÇELIK, H. The effects of different grafting methods applied by manual grafting units on grafting success in grapevines. **Turkish Journal of Agriculture and Forestry**, Ankaram v.24, p.499-504, 2000.

ÇÖLGEÇEN, H.; AZIMI, M. Assessment of graft compatibility of some olive cultivars on ‘gemlik’ rootstock by florescence microscopy. **Jordan Journal of Agricultural Sciences**, Amman, v.11, n.3, p.705-712, 2015.

COOKSON, S.J.; CLEMENTE MORENO, M.J.; HEVIN, C.; NYAMBA MENDOME, L.Z.; DELROT, S.; TROSSAT-MAGNIN, C.; OLLAT, N. Graft union formation in grapevine induces transcriptional changes related to cell wall modification, wounding, hormone signalling, and secondary metabolism. **Journal of Experimental Botany**, Oxford, v.64, n.10, p.2997-3008, 2013.

CORSO, M.; BONGHI, C. Grapevine rootstock effects on abiotic stress tolerance. **Plant Science Today**, Thiruvanthapuram, v.1, n.3, p.108-113, 2014.

DARIKOVA, J.A.; SAVVA, Y.V.; VAGANOV, E.A.; GRACHEV, A.M.; KUZNETSOVA, G.V. Grafts of woody plants and the problem of incompatibility between scion and rootstock (a review). **Journal of Siberian Federal University. Biology**, Krasnoyarsk, v.4, n.1, p.54-63, 2011.

DUNCAN, D.B. Multiple range and multiple F tests. **Biometrics**, San Francisco, v.11, n.1, p.1-42, 1955.

EL-GENDY, R.S.S. Evaluation of Flame Seedless grapevines grafted on some rootstocks. **Journal of Horticultural Science and Ornamental Plants**, Giza, v.5, n.1, p.1-11, 2013.

EL-NABI, A.H.; KHALIL, A.E.; EL-BASET, S.A.; MASSOUD, S. Screening of vineyards rootstock and cultivars for resistance to root-knot nematode (*Meloidogyne incognita*). **Journal of Plant Protection and Pathology**, Raleigh, v.4, n.1, p.23-34, 2013.

FARSI, M.; FATAHI MOGHADAM, M.R.; ZAMANI, Z.; HASSANI, D. Effects of scion cultivar, rootstock age and hormonal treatment on minigrafting of Persian walnut. **International Journal of Horticultural Science and Technology,** Tehran, v.5, n.2, p.185-197, 2018.

FAYEK, M.A.; RASHEDY, A.A.; MAHMOUD, R.A.; ALI, A.M.E. Biochemical indicators related to grafting compatibility in grapevine. Research **Journal of Pharmaceutical Biological and Chemical Sciences**, Jalpaiguri, v.8, n.3, p.574-581, 2017.

FOZOUNI, M.; ABBASPOUR, N.; BANCH, H.D.; Short term response of grapevine grown hydroponically to salinity: Mineral composition and growth parameters. **Vitis**, Davis, v.51, n.3, p.95-101, 2012.

GAINZA, F.; OPAZO, I.; MUÑOZ, C. Graft incompatibility in plants: Metabolic changes during formation and establishment of the rootstock/scion union with emphasis on Prunus species. **Chilean Journal of Agricultural Research**, Santiago, v.75, p.28-34, 2015.

GÜÇLÜ, S.F.; KOYUNCU, F. A Method for prediction of graft incompatibility in sweet cherry. **Notulae Botanicae Horti Agrobotanici Cluj-Napoca**, Cluj-Napoca, v.40, n.1, p.243-246, 2012.

HALBROOKS, M.C. Rapid and high volume grafting for florida viticulture. **Proceedings of the Florida State Horticultural Society**, Bradenton, v.98, p.170-171, 1985.

HAMMERSCHMIDT, R.; NUCKLES, F.; KUC, J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrchum lagenarium. **Physiological Plant Pathology**, Oxford, v.20, p.73-82, 1982.

HUDINA, M.; ORAZEM, P.; JAKOPIC, J.; STAMPAR, F. The phenolic content and its involvement in the graft incompatibility process of various pear rootstocks (*Pyrus communis* L.). **Journal of Plant Physiology**, Jena, v.171, p.76-84, 2014.

ISIKALAN, C.; NAMLI, S.; AKBAS, F.; EROL AK, B. Micrografting of almond (*Amygdalus communis*) cultivar'Nonpareil'. **Australian Journal of Crop Science**, Lismore, v.5, n.1 p.61-65, 2011.

KAKO, S.M. The effect of auxin IBA and kinetin in budding success percentage of mulberry (*Morus sp*.). **International Journal of Pure and Applied Sciences and Technology**, Nairobe, v.13, n.1, p.50-56, 2012.

KAWAGUCHI, M.; TAJI, A. Anatomy and physiology of graft incompatibility in sturt´ s desert pea (*Swainsona formosa*), an australian native plant. **Acta Horticulturae**, The Hague, v.683, n.249-258, 2003.

KHILI, B.D.; MICHAUX-FERRIERE, N.; GRENAN, S. Histochemical study on the incompatibility of micrografting and green grafting of grapevines. **Vitis**, Davis, v.34, n.3, p.135-140, 1995.

KORKUTAL, I.U.; KAYGUSUZ, G.; BAYRAM, S. Different effect of scion types on callusing in bench grafting. **African Journal of Biotechnology**, Nairobi, v.10, n.67, p.15123-15129, 2011.

KÖSE, B.; ÇELIK, H.; KARABULUT, B. Determination of callusing performance and vine sapling characteristics on different rootstocks of 'Merzifon Karası'grape variety (*Vitis vinifera* L.). **Anadolu Tarım Bilimleri Dergisi**, Istanbul, v.30, p.87-94, 2015.

KÖSE, C.; GÜLERYÜZ, M. Effects of auxins and cytokinins on graft union of grapevine (*Vitis vinifera*). **New Zealand Journal of Crop and Horticultural Science**, Wellington, v.34, n.2, p.145-150, 2006.

KÖSE, C.; GÜLERYÜZ, M.; ŞAHIN, F.; DEMIRTAŞ, İ. Effects of some plant growth promoting rhizobacteria (PGPR) on graft union of grapevine. **Journal of Sustainable Agriculture**, Binghamton, v.26, n.2, p.139-147, 2005.

KRACKE, H.; CRISTOFERI, G.; MARANGONI, B. Hormonal changes during the rooting of hardwood cuttings of grapevine rootstocks. **American Journal of Enology and Viticulture**, Davis, v.32, n2, p.135-137, 1981.

LARSEN, P.; HARBO, A.; KLUNGRON, S.; ASHEIN, T.A. On the biosynthesis of some indole compounds in *Acetobacter Xylinum*. **Physiologia Plantarum**, Lundi, v.15, p.552-65, 1962.

MAXWELL, B.B.; KIEBER, J.J. Cytokinin signal transduction. In: Davies PJ, editor. **Plant hormones**. Dordrecht: Springer, 2010. p.329-57.

MNG'OMBA, S.A.; DU TOIT, E.S.; AKINNIFESI, F.K. The relationshipbetween graft incompatibility and phenols in Uapaca kirkiana Müell Arg. **Scientia Horticulturae**, New York, v.117, n.3, p.212-8, 2008.

MOHAMED, G.A. Water soaking duration, indole butyric acid and rooting media and their effect on rooting ability of Ramsey grapevine rootstock cuttings. **Middle East Journal of Applied Sciences Sciences**, Giza, v.7, n.4, p.1080-1100, 2017.

NI, X.; QUISENBERRY, SS.; HENG-MOSS, T.; MARKWELL, J.; SARATH, G.; KLUCAS, R.; BAXENDALE, F. Oxidative responses of resistant and susceptible cereal leaves to symptomatic and non symptomatic cereal aphid (*Hemiptera:Aphididae*) feeding. **Journal of Economic Entomology**, Lanham, v.94, p.743-751, 2001.

NICOL, J.M.; STIRLING, G.R.; ROSE, B.J.; MAY, P.; VAN HEESWIJCK, R. Impact of nematodes on grapevine growth and productivity: current knowledge and future directions, with special reference to Australian viticulture. **Australian Journal of Grape and Wine Research**, Oxford, v.5, n.3, p.109-127, 1999.

OPAZO, I.; TORO, G.; SALVATIERRA, A.; PASTENES, C.; PIMENTEL, P. Rootstocks modulate the physiology and growth responses to water deficit and long-term recovery in grafted stone fruit trees. **Agricultural Water Management**, New Delhi, v.228, p.1-20, 2020.

PAUNOVIĆ, S.M.; MILETIĆ, R.; MITROVIĆ, M.; JANKOVIĆ, D. Graft-take success in walnut under controlled conditions and plant development in the nursery. **Notulae Botanicae Horti Agrobotanici Cluj-Napoca**, Cluj-Napoca, v.40, n.2, p.170-176, 2012.

PINA, A.; ERREA, P. A Review of new advances in mechanism of graft compatibility-incompatibility. **Scientia Horticulturae**, New York, v.106, p.1-11, 2005.

RASHEDY, A.A. Effect of pre-grafting incubation and grafted cuttings position on grape grafting success. **Egyptian Journal of Horticulture**, Aq Doqi, v.43, p.225-240, 2016.

RAVEN, P.H.; EVERT, R.F.; EICHHORN, S.E. **Biology of plants**. New York: Worth Publish; 1992.

REYNOLDS, A.G.; WARDLE, D.A. Rootstocks impact vine performance and fruit composition of grapes in British Columbia. **HortTechnology**, Alexandria, v.11, n.3, p.419-427, 2001.

RIZK-ALLA, M.S.; SABRY, G.H.; ABD EL-WAHAB, M.A. Effects of rootstocks on the performance of Red Globe grape cultivar. **Journal of American Science**, New York, v.7, n.4, p.71-81, 2011.

SABIR, A. Improvement of grafting efficiency in hard grafting grape Berlandieri hybrid rootstocks by plant growth-promoting rhizobacteria (PGPR). **Scientia Horticulturae**, New York, v.164, p.24-29, 2013.

SHARMA, A.; SHAHZAD, B.; REHMAN, A.; BHARDWAJ, R.; LANDI M.; ZHENG, B. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. **Molecules**, Berlin, v.24, p.1-22, 2019.

SHARMA, A.; ZHENG, B. Molecular responses during plant grafting and its regulation by auxins, cytokinins, and gibberellins. **Biomolecules**, Basileia, v.9, n.397, p.1-20, 2019.

SHINDE, E.D.; JOGDANDE, N.D.; AKHARE, A.A. Effect of different pre-treatments of plant growth regulators to shoot tips on in vitro shoot tip grafting in Nagpur seedless (*Citrus reticulate*, Blanco.). **Asian Journal of Horticulture**, Muzaffarnagar, v.3, n.1, p.98-99, 2008.

STINO, R.G.; GHONEIM, I.E.; MARWAD, I.A.; FADI, T.R. Performance of summer grafted superior seedlessgrape grafts on different rootstocks. **Jornal of Horticulture Science and Ornamental Plants**, Giza, v.3, n.1, p.86-90, 2011.

TEDESCO, S.; PINA, A.; FEVEREIRO, P.; KRAGLER, F. A phenotypic search on graft compatibility in grapevine. **Agronomy**, Madison, v.10, p.1-20, 2020.

VRSIC, S.; PULKO, B.; KOCSIS, L. Factors influencing grafting success and compatibility of grape rootstocks. **Scientia Horticulturae**, New York, v.181, p.168-173, 2015.

WAITE, H.; MAY, P. The effects of hot water treatment, hydration and order of nursery operations on cuttings of *Vitis vinifera* cultivars. **Phytopathologia Mediterranea**, Bologna, v.44, n.2, p.144-152, 2005.

WALLIS, C.M. Grapevine (*Vitis spp*.) rootstock stilbenoid associations with host resistance to and induction by root knot nematodes, *Meloidogyne incognita*. **BMC Research Notes**, London, v.13, p.1-7, 2020.

YIN, H.; YAN, B.; SUN, J.; JIA, P.; ZHANG, Z.; YAN, X.; CHAI, J.; REN, Z.; ZHENG, G.; LIU, H. Graft-union development: a delicate process that involves cell–cell communication between scion and stock for local auxin accumulation. **Journal of Experimental Botany**, Oxford, v.63, n.11, p.4219-4232, 2012.

ZARROUK, O.; TESTILLANO, P.S.; RISUEÑO, M.C.; MORENO, M.Á.; GOGORCENA, Y. Changes in cell/tissue organization and peroxidase activity as markers for early detection of graft incompatibility in peach/plum combinations. **Journal of the American Society for Horticultural Science**, Alexandria, v.135, n.1, p.9-17, 2010 .

ZENGINBAL, H.; EŞITKEN, A. Effects of the application of various substances and grafting methods on the grafting success and growth of black mulberry (*Morus nigra* L.). **Acta Scientiarum Polonorum Hortorum Cultus**, Lublin, v.15, n.4, p.99-109, 2016.