

## Alleviating the adverse effects of deficit irrigation in Flame seedless grapevine via Paulsen interstock

Mohamed Ahmed Fayek<sup>1</sup>, Ahmed Abdelhady Rashedy<sup>2</sup>, Amr Ebrahim Mohamed Ali<sup>3</sup>

**Abstract** - Using interstock with a potential genetic base is considered more recent and sustainable strategy for mitigating the water deficit. This investigation was carried out on transplant of Flame seedless (*Vitis vinifera*) grapevine grafted onto two rootstocks namely; Freedom (*Vitis champinii* x 1613C) and 1103Paulsen (*vitis berlandieri* x *Vitis rupestris*) with or without 1103Paulsen as interstock to determine its performance under deficit irrigation condition (50% of field capacity). The results indicated that Paulsen as rootstock or as interstock significantly increased the growth vigor of Flame seedless scion as well as the leaf content of total proline, phenols and sugars. Paulsen rootstock has decreased stomatal conductance, leaf transpiration rate and increased diffusion resistance under 50% deficit irrigation compared with grafting on Freedom rootstock. Moreover, Paulsen as interstock for Flame seedless grafted onto Freedom rootstock significantly increased relative water content accompanied by an increase in thickness of leaf anatomical characters such as midvein, lamina, palisade, xylem and phloem tissue under deficit irrigation compared with grafts without Paulsen interstock. This study suggests that using Paulsen as interstock, can be an adaptation strategy for water stress through controlling in some morphological, chemical physiological and anatomical responses of scion.  
**Index terms:** diffusion resistance; leaf anatomical; peroxidase; proline; stomatal conductance.

## Aliviando os efeitos adversos da irrigação deficitária na videira Flame sem sementes via enxerto intermediário de Paulsen

**Resumo** - A utilização de um enxerto intermediário com uma base genética potencial é considerada uma estratégia mais recente e sustentável para mitigar o déficit hídrico. Esta investigação foi realizada em transplante de videira Flame sem sementes (*Vitis vinifera*), enxertada em dois porta-enxertos denominados: Freedom (*Vitis champinii* x 1613C) e 1103Paulsen (*vitis berlandieri* x *Vitis rupestris*) com ou sem 1103Paulsen como enxerto intermediário para determinar seu desempenho sob condição de irrigação deficitária (50% da capacidade de campo). Os resultados indicaram que a Paulsen como porta-enxerto ou como enxerto intermediário, aumentou significativamente o vigor de crescimento do enxerto Flame sem sementes, bem como o conteúdo foliar total de prolina, fenóis e açúcares. O porta-enxerto Paulsen diminuiu a conduta estomática, taxa de transpiração das folhas, e aumentou a resistência à difusão sob 50% de irrigação deficitária, em comparação com o enxerto no porta-enxerto da Freedom. Além disso, a Paulsen como enxerto intermediário para Flame sem sementes, enxertada em porta-enxerto Freedom, aumentou significativamente o conteúdo relativo da água, acompanhada por um aumento da espessura de características anatômicas foliares, como a veia, a lamina, a paliçada, e o tecido do xilema e floema sob irrigação deficitária, em comparação com os enxertos sem o enxerto intermediário de Paulsen. Este estudo sugere que usar a Paulsen como enxerto intermediário pode ser uma estratégia de adaptação para o estresse hídrico através do controle em algumas respostas morfológicas, químicas, fisiológicas e anatômicas do enxerto.

**Termos para indexação:** resistência à difusão; anatômico foliar; peroxidase; prolina; condução estomática.

**Corresponding author:**

ahmed.rasheedy@agr.cu.edu.eg

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<sup>1</sup>Professor at the Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. E-mail: mohamed.fayk@agr.cu.edu.eg (ORCID 839 - 0000-0002-1427-6513)

<sup>2</sup>Assistant teacher at the Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. E-mail: amr.ali@agr.cu.edu.eg (ORCID 0000-0002-8472-692X)

<sup>3</sup>Assistant Professor at the Pomology Department, Faculty of Agriculture, Cairo University, Giza, Egypt. E-mail: ahmed.rasheedy@agr.cu.edu.eg (ORCID 0000-0002-0297-1594)

## Introduction

Grapevine (*vitis vinifera*) is one of the most important crops in the world (ALSTON and SAMBUCCI, 2019). Globally in terms of production, Egypt is ranked fourteenth with a production of 1641075 tons and the amount of exports is about 99001 tons (FAOSTAT, 2018). Flame seedless grapevine cultivar is considered one of the most important export table grape cultivars. But its production is affected by many stresses; it is sensitive to infection with nematodes (EL-NABI et al., 2013; ABOURAYYA et al., 2019) and soil water deficit (REZAEI et al., 2008). In recent years, the climate change forecast suggests that in the coming years, drought will be a more serious problem in the world. Drought stress is one of the foremost causes of viticulture production reduction worldwide under semiarid regions (CHAVES et al., 2007; MEGGIO et al., 2014). Water deficit can restrict plant water uptake, which ultimately results in disturbing plant functioning and declining productivity (KAPOOR et al. 2020). Many studies have shown that irrigated fields ranging from 30 to 50% of field capacity cause water stress and a significant decrease in viticulture growth (SATISHA et al., 2007; REZAEI et al., 2008; KOUNDOURAS et al., 2008; MEGGIO et al., 2014).

Selection of grapevine rootstock, not only for phylloxera and nematode resistance, but also for several other characteristics are also required, such as grafting compatibility and abiotic stress tolerance (COOKSON et al., 2013; TSEGAY et al., 2014). In this context, grapevine rootstocks exhibit differential degrees of stress tolerance. Freedom rootstock, for example, is resistant to nematode infection (EL-NABI et al., 2013), but it is sensitive to drought stress, whereas 1103Paulsen is more drought tolerant (KOUNDOURAS et al., 2008; FLEXAS et al., 2009; WILLIAMS, 2010; SERRA et al., 2014). Under drought stress, grapevine rootstock controls physiological and biochemical mechanisms which regulate water extraction capacity, stomatal conductance, transpiration rate, osmoregulator accumulation, and enzyme activity of the scion (SATISHA et al., 2007; TOUMI et al., 2007; VERMA et al., 2010; SERRA et al., 2014; TSEGAY et al., 2014; SUCU et al., 2018). Also, anatomical changes in plant leaves are stimulated, allowing plants to tolerate drought conditions by reducing water loss and maintaining photosynthetic rates (CHARTZOULAKIS et al., 2000; PATAKAS et al., 2003; DA SILVA et al., 2003; HAMEED et al., 2012). Plants can modify the properties of their anatomical leaves to adapt to drought stress in many fruit trees such as olive (ENNAJEH et al., 2010) and apple (LOCATELI et al., 2019). Interstock had a positive effect on solving grafting incompatibility (SOSNA and KORTYLEWSKA, 2013; GIRARDI and FILHO, 2006; AGUS and EKA, 2017), stimulating dwarfing (DI VAIO et al., 2009; AN et al., 2017) and

increased productivity (GIL-IZQUIERDO et al., 2004). More recently, interstock is able to increase tolerance to biotic stress (ERTI and AGUS, 2018; SHALTIEL-HARPAZ et al., 2018).

Few studies indicate the potential role of interstock for improving fruit trees' tolerance to abiotic stresses. Interstock of SH6 and M26 improved growth and drought tolerance of Fuji cv. apple under 30 and 60% of field capacity (LI et al., 2017). Moreover, "Salustiano" orange (*Citrus sinensis* L.) interstock improved salinity tolerance of Verna lemon (*Citrus limon* L.) trees (ZAPATA et al., 2003). Also, Valencia orange (*C. sinensis*) interstock improved salt tolerance and flooding tolerance and in some citrus trees (*Citrus sinensis* L.) (GIMENO et al., 2009; GIMENO et al., 2012). Many studies indicated that use of interstock with a length of 10cm had the best effect on tree performance (SOSNA and KORTYLEWSKA, 2013; DAS and DHAKAR, 2016) and noted that increasing the interstock length, caused reduction of this effect (DI VAIO et al., 2009).

There is a short knowledge on the effect of interstock on drought tolerance of grapevine. Under drought conditions Paulsen rootstock is more suitable than Freedom rootstocks but it is sensitive to nematodes infection. So, the hypothesis of this study is, does Paulsen as drought tolerance interstock to mitigate the adverse effect of deficit water conditions on Flame seedless grapevine?

## Materials and methods

### Plant material and growth condition

The pots experiment was carried out over two consecutive seasons in 2019 and 2020 at the net greenhouse of Pomology Department, Faculty of Agriculture, Cairo University (30°01'04"N31°12'30"E). The performance of Flame seedless grapevine cultivar grafted onto two rootstocks, Freedom and 1103Paulsen, with and without 1103Paulsen as interstock was evaluated under deficit water irrigation at 50% of field capacity. In early June, this experiment was performed on four graft combinations, namely; Flame seedless grafted directly onto both Freedom (FI/Fr) and Paulsen rootstocks (FI/P), or grafted indirectly using 1103Paulsen as interstock (FI/P/Fr and FI/P/P). The length of the interstock was 10 cm. All plants are grown in 10 L black plastic pots filled with washed sandy soil. The average height of the scion was 35 cm and contained 12 mature leaves. Plants were pruned by lateral shoot removal to provide uniform plants with a single main shoot. The electrical conductivity of the tap water was 0.48 ds/m. For three months, tap water was added early second day to adjust water irrigation at 50% of field capacity, which was computed by using the following equation:  $[(FW - DW) / DW \times 100]$ , where FW was the fresh weight of soil sample and DW was the

dry weight of soil sample after oven drying at 85°C for 3 days (COOMBS et al, 1987). During the experimental period water level were maintained by weighing pots and calculating the volume of water lost every two weeks. Macro and micro elements were added at weekly of 0.25 strength Hoagland for macronutrients and full strength for micronutrients (FOZOUNI et al., 2012). After three months of exposing plants to deficit water treatment, the following parameters were recorded.

### Morphological measurements

Increase in shoot length (cm), leaves number, shoot dry weight (g), and root dry weight (g) were measured. Leaf area (cm<sup>2</sup>) was calculated using the following equation: Leaf area (LA) = 0.465 + 0.914 \* W \* L where W is the leaf width (cm) and L is the leaf length according to (KHAN et al., 2016).

### Biochemical analysis

A sample of one half gram of fresh leaf (7<sup>th</sup> leave from the shoot apex) was used for the following chemical analysis. Free proline (µmole proline/g FW) using the ninhydrin method (BATES et al., 1973), total phenols (mg/g FW) using Folin ciocalteu method (MNG'OMBA et al., 2008), Peroxidase activity (mg/g FW) according to Hammerschmidt et al. (1982) and Ni et al. (2001) and total soluble sugars (mg/g FW) were determined by the phenol sulfuric acid method (DUBOIS et al., 1956). All procedures were carried out using a spectrophotometer (model WPA S2100) at wave lengths of 520, 765, 470 and 490 nm, for proline, total phenols, peroxidase activity and total soluble sugar, respectively.

### Physiological measurements

The following physiological measurements were measured at the end of the experiment on the 7<sup>th</sup> leaf from the shoot apex.

### Relative water content (RWC)

Ten discs of leaves were weighed to obtain fresh weight (FW). Then, the discs were placed in glass jars containing distilled water in the dark for a period of 24 hours to determine their turgid weight (TW). Finally, the discs were placed in an oven at 65°C until they reached constant dry weight (DW). Finally, RWC was calculated using the following formula: [(FW-DW)/(TW-DW) × 100] (MEDEIROS et al. 2012).

### Leaf gas exchange measurements

Transpiration rate (µg H<sub>2</sub>O/cm<sup>2</sup>.s), diffusion resistance(s/cm) and stomatal conductance (cm/s) of the 7<sup>th</sup> mature leaves of plant were determined according to Surendar et al. (2013) by using a portable steady-state porometer (LI-1600M, LI-COR, Nebraska, USA) during the period between 10:00 am and 12:00 pm with an air

temperature of 30°C, humidity of (29% - 30.4%) and photosynthetically active radiation of 1200 mmol m<sup>-2</sup> s<sup>-1</sup>.

### Anatomical studies

At the end of the experiment, a microscopic examination was performed on the 5<sup>th</sup> leaf from the shoot apex of the grafts combination, which demonstrated a remarkable response. Specimens (1 cm<sup>2</sup>) were taken from the centre of leaves and fixed for at least 48 hrs in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethyl alcohol 70%). The selected materials were washed in 50% ethyl alcohol, dehydrated in a normal butyl alcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 microns, double stained with crystal violet-erythrosin, cleared in xylene and mounted in Canada balsam (NASSAR and EL-SAHHAR, 1998). Photomicrographed sections were read to detect histological manifestations of noticeable responses by light compound microscope (LEICA DM750) and a LEICA ICC50 HD using the Leica Application Suite program.

### Statistical analysis

This experiment contains interaction between two grapevine rootstocks and presence and absent of interstock, including 4 treatments, each one divided into three replicates with total 15 plants for each one. This experiment followed a randomized complete block design and means of the treatments were compared by least significant difference (L.S.D.) at a significance level of 0.05 (DUNCAN, 1955).

## Results

### Morphological parameters

The results in table 1 revealed that Flame seedless grafted onto Paulsen rootstock (FI/P) through the first and second season achieved the highest significant value for shoot length (13.600 & 26.167cm), number of leaves (28.110 & 27.553), leaf area (77.090 & 57.057 cm<sup>2</sup>) and root dry weight (15.183 & 13.727g) as compared with grafts on Freedom rootstock. Also, it was clear that Paulsen interstock between Flame seedless scion and Freedom rootstock (FI/P/Fr) increased shoot length (12.733 & 25.333cm), and root dry weight (14.803 & 13.210g) in both seasons compared to grafts without Paulsen rootstock (FI/Fr). However, using a piece of Paulsen as interstock within the grafts of Flame seedless on Paulsen (FI/P/P) decreased all the tested morphological parameters.

**Table 1.** Effect of deficit water irrigation on morphological measurements of Flame seedless grapevine grafted onto Freedom and Paulsen rootstocks with and without Paulsen interstock.

	Rootstocks (A)	First season			Second season		
		Paulsen Interstock (B)		Mean (A)	Paulsen Interstock (B)		Mean (A)
		Without	With		Without	With	
<b>Increase in Shoot length(cm)</b>	<b>Freedom</b>	10.933 c	12.733 ab	11.833 B	23.917 c	25.333 ab	24.625 A
	<b>Paulsen</b>	13.600 a	12.653 b	13.127A	26.167 a	24.667 bc	25.417 A
	<b>Mean (B)</b>	12.267 A	12.693 A		25.042 A	25.000 A	
<b>Leaf number (n)</b>	<b>Freedom</b>	26.330 b	27.107 ab	26.718 A	21.170 c	25.667 b	23.418 A
	<b>Paulsen</b>	28.110 a	24.997 c	26.553 A	27.553 a	18.837 d	23.195 A
	<b>Mean (B)</b>	27.220 A	26.052 B		24.362 A	22.252 B	
<b>Leaf area (cm<sup>2</sup>)</b>	<b>Freedom</b>	56.143 c	62.277 b	59.210 B	45.970 b	49.550 b	47.760 B
	<b>Paulsen</b>	77.090 a	53.783 c	65.437 A	57.057 a	49.767 b	53.412 A
	<b>Mean (B)</b>	66.617 A	58.030 B		51.513 A	49.658 A	
<b>Shoot dry weight (g)</b>	<b>Freedom</b>	6.640 a	7.400 a	7.020 A	6.917 a	7.290 a	7.103 A
	<b>Paulsen</b>	7.947 a	4.380 b	6.163 A	7.517 a	6.707 a	7.112 A
	<b>Mean (B)</b>	7.293 A	5.890 B		7.217 A	6.998 A	
<b>Root dry weight (g)</b>	<b>Freedom</b>	12.603 b	14.803 a	13.703 A	12.107 b	13.210 a	12.658 B
	<b>Paulsen</b>	15.183 a	9.797 c	12.490 B	13.727 a	13.227 a	13.477 A
	<b>Mean (B)</b>	13.893 A	12.300 B		12.917 A	13.218 A	

Mean for each parameter within each season with the same letter were significantly equal at L.S.D. 5% level.

### Biochemical analysis

According to the results in table 2, Flame seedless grafted onto Paulsen rootstock without interstock (FI/P) significantly increased content of total proline (1.100 & 2.567  $\mu$ mole proline/g FW), phenols (8.273 & 10.643 mg/g FW), peroxidase activities (25.600 & 13.933 mg/g FW), and total soluble sugars (13.857 & 11.677 mg/g FW) in two seasons compared to Flame seedless grafted only on Freedom rootstock (FI/P). Furthermore, Paulsen interstock within Flame seedless grafts onto Freedom (FI/P/Fr) increased both proline content (1.100 & 2.850  $\mu$ mole proline/g FW) and phenol content (7.857 & 10.353) in addition to total soluble sugar (13.427 & 11.250 mg/g) but decreased significantly peroxidase activity (12.667 & 12.667) through first and second seasons compared to Flame seedless grafted onto Freedom without Paulsen interstock.

### Physiological parameters

Data presented in table 3 revealed that general grafting of Flame seedless on Paulsen rootstock (FI/P) significantly decreased its transpiration rate, stomatal conductance and increased diffusion resistance as compared with grafts of Flame seedless on Freedom rootstock (FI/Fr). Using of Paulsen as interstock between Flame seedless scion and Freedom rootstock (FI/P/Fr) significantly increased the relative water content of leaves, but did not show a significant effect on other parameters. However, Paulsen interstock between Flame seedless scion and Paulsen rootstock (FI/P/P) revealed some increase in leaf relative water content despite an increase in transpiration rate and stomatal conductance, there is a significant decrease in diffusion resistance.

**Table 2.** Effect of deficit water irrigation on chemical content of Flame seedless grapevine grafted onto Freedom and Paulsen rootstocks with and without Paulsen interstock.

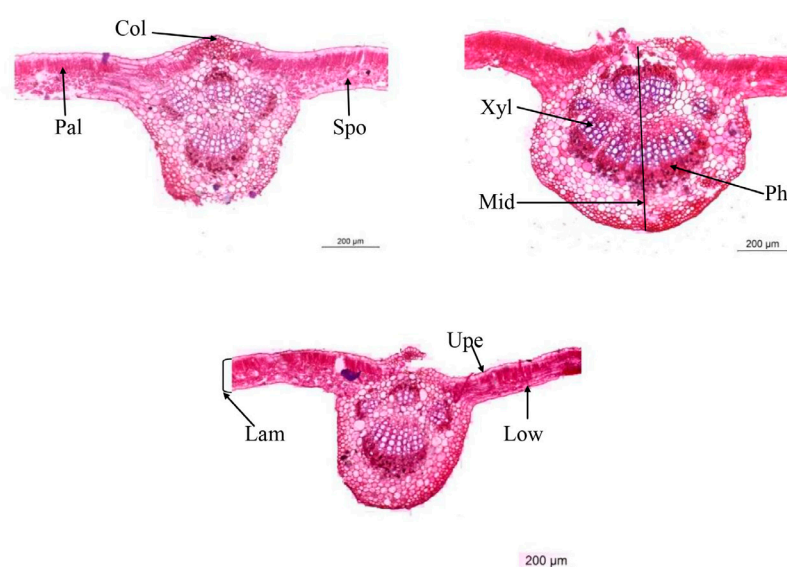
	Rootstocks (A)	First season			Second season		Mean (A)
		Paulsen Interstock (B)		Mean (A)	Paulsen Interstock (B)		
		Without	With			Without	With
Total Proline content $\mu\text{mole/g FW}$	Freedom	0.800 c	1.100 b	0.950 B	1.750 d	2.850 a	2.300 B
	Paulsen	1.100 b	1.400 a	1.250 A	2.567 b	2.467 c	2.517 A
	Mean (B)	0.967 B	1.267 A		2.158 B	2.658 A	
Total phenols content (mg/g) FW	Freedom	7.280 b	7.857 a	7.568 A	10.200 b	10.353 b	10.277 A
	Paulsen	8.273 a	7.307 b	7.790 A	10.643 a	10.207 b	10.425 A
	Mean (B)	7.777 A	7.582 A		10.422 A	10.280 A	
Peroxidase activity (mg/g) FW	Freedom	22.467 b	12.667 d	17.567 B	13.200 b	12.667 c	12.933 A
	Paulsen	25.600 a	18.500 c	22.050 A	13.933 a	10.117 d	12.025 B
	Mean (B)	24.033 A	15.583 B		13.567 A	11.392 B	
Total sugar content (mg/g) FW	Freedom	11.573 c	13.427 b	12.500 B	10.910 c	11.250 b	11.080 B
	Paulsen	13.857 a	13.827 a	13.842 A	11.677 a	11.387 b	11.532 A
	Mean (B)	12.715 B	13.627 A		11.293 A	11.318 A	

Mean for each parameter within each season with the same letter were significantly equal at L.S.D. 5% level.

### Leaf anatomical characters

Data presented in table 4 and figures 1 showed that using Paulsen as interstock between Flame seedless scion and Freedom rootstock (FI/P/Fr) increased the thickness of both Collenchyma, midvein, lamina, palisade, xylem and phloem tissue (73.1, 803.2, 173.3, 79.8, 81.9 and 90.4  $\mu\text{m}$ ) respectively compared to Flame seedless scion grafted onto Freedom rootstock without Paulsen interstock (FI/Fr). However, Paulsen as rootstock

recorded an increase in thickness of Collenchyma, midvein, lamina, palisade, spongy, xylem and phloem of leaves of Flame seedless grafted on it (73.3, 618.3, 185.7, 69.1, 75.6, 80.6 and 80.1  $\mu\text{m}$ ) respectively compared to Flame seedless grafted onto Freedom without interstock (FI/Fr) which recorded the lowest values.



**Figure 1.** Comparative leaf anatomy in blade cross-sections (100X) Flame seedless grafted onto Freedom (FI/Fr), Flame grafted onto Freedom with interstock (FI/P/Fr), Flame grafted onto Paulsen (FI/P) under deficit water (50 % field capacity). Upper epidermis (Upe), Lower epidermis (Low), Lamina thickness (Lam), Collenchyma thickness (Col), Midvein thickness (Mid), Palisade thickness (Pal), Spongy thickness (Spo), Xylem thickness (Xyl), Phloem thickness (Phl).

**Table 3.** Effect of water deficit on physiological response of Flame seedless grapevine grafted onto Freedom and Paulsen rootstocks with and without Paulsen interstock

Rootstocks (A)	Relative water content (%)		Transpiration rate ( $\mu\text{g H}_2\text{O}/\text{cm}^2\cdot\text{s}$ )				Diffusion resistance (s/cm)				Stomatal conductance (cm/s)					
	Interstock (B)		Mean (A)		Interstock (B)		Mean (A)		Interstock (B)		Mean (A)		Interstock (B)		Mean (A)	
	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With	Without	With
<b>Freedom</b>	53.357 b	58.847 a	56.102 A	1.687 a	1.703 a	1.695 A	14.780 b	14.927 b	14.853 B	0.070 a	0.070 a	0.070 A	0.070 a	0.070 a	0.070 A	0.070 A
<b>Paulsen</b>	53.750 b	54.647 b	54.198 B	1.170 b	1.563 a	1.367 B	20.170 a	14.893 b	17.532 A	0.053 c	0.067 b	0.060 B	0.053 c	0.067 b	0.060 B	0.060 B
<b>Mean (B)</b>	53.553 B	56.747 A		1.428 B	1.633 A		17.475 A	14.910 B		0.062 B	0.068 A		0.062 B	0.068 A		0.068 A

Mean for each parameter with the same letter were significantly equal at L.S.D., 5% level.

**Table 4.** Anatomical changes in cross-sections of leaves of grafts combination under deficit water (50 % field capacity).

	FI/Fr	FI/P/Fr	FI/P
<b>Upper epidermis thickness</b>	26.7	23.7	20.6
<b>Lower epidermis thickness</b>	21.8	20.7	20.4
<b>Collenchyma thickness</b>	70.6	73.1	73.3
<b>Midvein thickness</b>	605.7	803.2	618.3
<b>Lamina thickness</b>	169.	173.3	185.7
<b>Palisade thickness</b>	55.2	79.8	69.1
<b>Spongy thickness</b>	65.2	49.1	75.6
<b>Xylem thickness</b>	59.5	81.9	80.6
<b>Phloem thickness</b>	66.2	90.4	80.1

Flame grafted onto Freedom (FI/Fr), Flame grafted onto Freedom with interstock (FI/P/Fr), Flame grafted onto Paulsen (FI/P).

## Discussion

It is well known that grapevine rootstocks play a major role in the adaptive response of scions to water limiting conditions (KOUNDOURAS et al., 2008; TRAMONTINI et al., 2013). The present study indicated that Paulsen rootstock improved vigor growth of Flame seedless grapevine under deficit irrigation expressed as shoot length, leaf number and leaf area as well as root dry weight compared with grafts on Freedom rootstock. Previous researches on grapevines reported that deficit water reduce the whole plant growth sharply (LEBON et al., 2006; ARAN et al., 2017; SUCU et al., 2018; MOHSEN et al., 2020). This deficiency may be due to the reduction of cell division, elongation and expansion under water stress (ALVES and SETTER, 2004; FAROOQ et al., 2012). Moreover, as found in the present study, using Paulsen as interstock within Flame seedless scion onto Freedom rootstock (FI/P/Fr) improved its vigor growth and tolerance to deficit water. These findings agreed with those of Li et al. (2017), who reported that using SH6 and M26 rootstocks as interstock in apple improved the vigor growth of Fuji cv. apple under drought stress. Similarly, El-Tanany et al. (2019) found that using Flying Dragon as an interstock improved the growth and yield of Washington navel orange under deficit irrigation at 75% of field capacity. Also, The present study showed that the increase in shoot length in the second season was higher than in the first season. This may be due to the different climatic conditions between the two seasons.

Accumulation of compatible solutes such as proline, phenols, and sugars under water deficit conditions consider the important strategy for avoiding tissue dehydration and maintaining plant water status (FAROOQ et al., 2012, SANDERS and ARNDT, 2012, KAPOOR et al., 2020). Abdia et al. (2016) recorded an increasing WUE, as well as proline and soluble sugar concentrations in Rasheh grape cultivars as indicator for drought tolerant stress than was Bidane-Sefid cultivar.

In this context, the results showed that Paulsen rootstock increased significantly in proline content and total sugars of Flame seedless compared to grafts on Freedom rootstock. Furthermore, using Paulsen as interstock increased total proline content, total sugars, and total phenols accumulation while decreasing peroxidase activity of Flame seedless grafted onto Freedom rootstock (FI/P/Fr) compared to grafts without Paulsen interstock (FI/Fr). These results were in agreement with (EL-TANANY et al., 2019) as they found that using flying dragon (*Poncirus trifoliata*) as interstock increased leaf proline content of Washington navel Orange trees under (75%) deficit irrigation. Proline has a role in increasing the cellular solute content and thus maintaining higher water content of plants (MOHAMMAD et al., 2008; MAFAKHERI et al., 2010). In addition, the results were in line with Jogaiah et al. (2014) as they found accumulation of phenolic compounds under water stress with Paulsen rootstock which has shown tolerance to drought stress. Jogaiah et al. (2014) and Sharma et al. (2019) reported that Phenols may be play as antioxidants for scavenging the reactive oxygen species generated thus maintaining normal physiological and biochemical process of resistant cultivars. Similarly, Toumi et al. (2007) reported that increased total sugar accumulates in some grafts combinations of grapevine is indicator to tolerance of water stress. This may be due to the role of soluble sugar in maintaining the leaf water content, osmotic adjustment (MOHAMMADKHANI and HEIDARI, 2008), membrane protection and scavenging of reactive oxygen species (SAMI et al., 2016). Previous researches on grapevine studied the role of enzyme activity under dehydration stress and they suggested that enzyme activity is an indicator of drought tolerance (ARAN et al., 2017; SUCU et al., 2018). Peroxidase enzyme has a positive role of antioxidants in cellular redox homeostasis during drought stress (MAFAKHERI et al., 2011; ARAN et al., 2017). In our study the highest increase of peroxides activity recorded with Flame seedless grafted onto Paulsen rootstock compared with that grafted onto Freedom rootstock

Drought-tolerant rootstocks in grapevine can improve water extraction from the soil and transfer it to the scion, in addition to controlling stomatal conductance and transpiration rate of scion under water deficit condition (KOUNDOURAS et al., 2008; SERRA et al., 2014; TSEGAY et al., 2014) through chemical signaling such as ABA and compatible solute (SOAR et al., 2006; SERRA et al., 2014; PECCOUX et al., 2017). In the present study, using Paulsen as rootstock decreased stomatal conductance and transpiration of Flame seedless leaves and increased diffusion resistance of stomata. However, Paulsen as interstock with Flame seedless grafted onto Freedom significantly improved relative water content without a significant effect on transpiration

rate, diffusion resistance and stomatal conductance. These results were in agreement with Koundouras et al. (2008) as they noted that Paulsen is more water efficient rootstock through decreasing stomatal conductance and transpiration rate of scion grafted on it under water stress.

Many studies have indicated that increase in thickness of leaf anatomical characters such as collenchymas, midvein, lamina, palisade, xylem, phloem and others were related to drought tolerance in plants (CHARTZOULAKIS et al., 1999; NAWAZISH et al., 2006; DE MICCO and ARONNE, 2012; ZULFIQAR et al., 2020). This increase is greater with drought tolerant genotypes compared to sensitive genotypes (EL-AFRY et al., 2012; TOSCANO et al., 2019). This is consistent with our findings, which revealed an increase in the thickness of leaf anatomical characters with graft combinations (FI/P/Fr and FI/P) that were more tolerant to water stress than graft combinations (FI/Fr). A thicker palisade tissue may have a role in increasing the number of CO<sup>2</sup>-fixation sites. A thicker spongy parenchyma could result in easier diffusion of CO<sup>2</sup> to these sites, thus maintaining the photosynthesis rate of leaves under deficit water (ENNAJEH et al., 2010; LOCATELLI et al., 2019). The thickening of the vascular bundles of plant leaves allows for greater flows of water and mineral salts during the water deficit, thus adaptive it to water stress conditions (Queiroz-Voltan et al. 2014). On the other hand, Locatelli et al., (2019) reported that the xylem vessels of leaves were increased in number and were reduced in diameter under deficit water. Thus, it increases resistance to high pressures in the water column. Nawazish et al. (2006) and Taratima et al. (2019) reported that increasing lamina thickness is related to anatomical adaptation under drought. Taratima et al. (2020) indicated that increasing lamina thickness may expand bulliform cells.

## Conclusion

In conclusion, Paulsen as an interstock improved the tolerance of Flame seedless scion to deficit water stress via increasing proline and total sugar content as a biochemical mechanism. In addition, it had positive effects on the physiological response of the scion, such as increasing relative water content and stomatal conductance of the leaf. Furthermore, it caused thickening of midvein tissues, lamina, septum, xylem, and phloem in leaves as an anatomical mechanism

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