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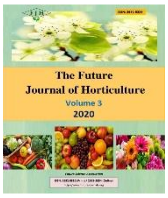

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EVALUATION OF SOME DIFFERENT ROOTSTOCKS ON VEGETATIVE GROWTH AND BIOCHEMICAL PARAMETERS OF H4 STRAIN AND SULTANA GRAPEVINES IN CLAY SOILS UNDER FLOOD-IRRIGATED SYSTEM

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Abstract: Recently, the demand for grafted grape on different rootstocks has been increased because of its benefit to combat the soil problems and enhancing productivity. Also, rootstocks are selected primarily based on their ability to withstand range of typical soil conditions, especially those that are important to maintaining vine vigor in the face of significant abiotic stresses. The current study was carried out during three years (2019, 2020, and 2021) to the effect of Freedom, Salt Creek, and SO4 rootstocks of H4 strain and Sultana grapevines on vegetative growth and biochemical parameters. The experiment was arranged in a randomized complete block design of eight treatments with three replicates with four trees each. The results indicate that H4 strain and Sultana grapevines grafted on the rootstock of Freedom enhanced the vegetative growth and biochemical parameters followed by grafted on Salt Creek and SO4 rootstock, respectively. Contrary, H4 strain and Sultana grapevines un-grafted (own rooted) recorded the least values of vegetative growth and biochemical parameters. In addition, the H4 strain grafted on studied rootstocks had superiority to grafted Sultana grapevines. The best results were achieved with the grafted grapevines on Freedom rootstock. Therefore, it can be recommended that graft H4 strain and Sultana grapevines on Freedom rootstock in clay soils under flood-irrigated system.

Key words: Grapevines, H4 Strain, Sultana, Rootstock, Freedom, Salt Creek, SO4, Vegetative Growth, Biochemical Parameters, Clay Soils.

INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the great significant fruit crops grown in Egypt's temperate zone, which has acclimated climate conditions. (**Ghule *et al.*, 021**). The total world largest yield of grape more than 73 million tons, in Egypt the grape is ranked fourth fruits next to citrus, mango and olive with a total cultivated area of 85240 ha producing 1435000 tons yielding an average of 16.83 ton/ha (**FAOSTAT, 2021**). The Sultana is a vigorously growing oval-fruited, seedless grape variety that is also known as Sultanina, oval-fruited Kishmish, Lady de Coverly and Thompson seedless in various parts of the world. Sultana is considering the one of the greatest cultivars that Egypt introduced for grapes production. It is large clusters, conical and compact with a small to medium berries and yellow-green color, so utilized for export, to produce raisins, and as a fresh the table grape. In addition, H4 is a strain of the sultana grape cultivar, which has been widely spread recently in Egypt due to its higher fertility rate than Thomson Seedless grapes, the higher production, and distinctive qualities of the cluster **(Belal, 2019).**

The most of vineyards are growing un-grafted on (own roots) that coming from cuttings. Recently, grapevine growers all over the world are interested in grafting grapevines on commercial rootstocks to obtain grapevines tolerant to certain adverse soil conditions and confer disease or pest resistance (**Gambetta *et al.*, 2009**). Furthermore, grafting grapevines on different rootstocks enhances plant physiology and nutritional status (**Upreti *et al.*, 2012**). Furthermore, rootstocks can be utilized to regulate the intake of water and the exclusion of nutrients in grapevines. (**Walker *et al.*, 2002**). Grafting grape on the rootstocks is a useful method for reducing biotic and abiotic stressors in vineyards. **(Walker *et al.*, 2014 and Jin *et al.*, 2016).** It's a widely used technique in viticulture around the world to defend against biotic stress factors like pathogen infections that impact the plant's roots and low fertility soils, excessive or inadequate water, salty soils, calcium carbonate soils, and other unfavorable conditions. **(Peterson & Walker, 2017).** In this regard, Freedom rootstock was created at the University of California-Davis, this rootstock is a hybrid among 1613 Couderc (*Vitis solonis* x Othello) and Dogridge (*Vitis* x *champini*). However, further research is needed testing on this rootstock, particularly on hardened soil areas that are regularly affected by drought conditions **(El-Gendy, 2013 and Hifny *et al.*, 2016)**. Salt creek ‘Ramsey’ **(***Vitis* × *Champini*) rootstock exhibits strong resistance to nematodes, performs well in light, sandy, low-fertility soils, has excellent tolerance to sodium chloride, and works well in moderately acidic and calcareous soil types **(Walker *et al.*, 2002; Goyzueta & Peniche, 2004 and El-Gendy, 2013)**. SO4 rootstock (*Vitis berlandieri* x *Vitis riparia*) it has a resistance to grape phylloxera (*Dactylosphaera vitifoliae*) and mild defense against various kinds of nematodes (**McCarthy & Cirami, 1990; Fraschini, 1990 and Wolpert *et al.*, 1991**). Scion vigor is considered moderate, and it seems to grant scion varieties "medium to short-cycling.", in terms of fruit and canopy maturity duration **(Howell, 2005).**

In Egypt, Nile River is the first source of water for irrigation, However, the main causes of soil degradation, especially in the North Delta region (i.e., clay soils near the Mediterranean Sea), were water scarcity brought on by scarce water supplies and insufficient rainfall. This increased soil salinity, which was brought on by high salt concentrations in groundwater, and limited agricultural productivity (Mohamed ***et al.*, 2019**). Additionally, rivers in arid and semi-arid areas typically get more salinized from their headwaters to their mouths, and too much salinity in the root zone reduces the growth of plants (**FAO, 2021**). Rootstocks perform differently in various soils and climates, so regional rootstock evaluations are necessary in determining which rootstock is best suited to a particular environment **(Shaffer, 2002).** The Egyptian Delta Region's grape production regulations demand the adoption of rootstock, in order to maintain vine vigor under significant abiotic challenges and to aid growers in ensuring uniform and early bud sprout as well as proper vine vigor. Farmers benefit economically from all of these causes **(Jogaiah *et al.*, 2013).** The production of grapevines has become reliant on the usage of rootstock due to declining irrigation water and soil conditions especially in the North Delta region (**Rizk-Alla *et al.*, 2011**). Therefore**,** the research aims to evaluate three commercial rootstocks (Freedom, Salt creek, and SO4) grafted on H4 strain and Sultana grapevines as compared with un-grafted (own rooted) with respect to vegetative growth and biochemical responses to identify the most promising and suitable rootstock in clay soil under flood-irrigated system of delta Egypt.

MATERIALS AND METHODS

1. Experimental Site, Design and Treatments

The trial planting was served in a private vineyard site at El-deer region near Aga town, Dakahlia Governorate, Egypt (30°34′29″ N, 31°26′81″ E and 15 m elevation above sea level) through three successive seasons (2019, 2020 and 2021) on H4 strain and Sultana grapevines grafted on Freedom, Salt Creek and SO4 rootstocks, additionally their own rooted. The seedlings under investigation were procured from the Horticultural Research Institute in Giza, the Agriculture Research Center, and the

Ministry of Agriculture and Land Reclamation, all located in Egypt. Weather conditions at the experimental location (**World Weather Online, 2021**) are displayed in Table 1. From the root zone (0–90 cm), soil samples were randomly taken for analysis in accordance with the approach used in the research of **Wilde *et al.* (1985)**. Grapevines were irrigated by the waters of the Nile River, and water samples were also taken for analysis (**Chapman & Pratt, 1961; Ali *et al.*, 2014; Abuzaid, 2018 and El-Sayed *et al.*, 2020**). Soil and water analysis values are shown in Table 2.

Table (1). Weather data of at El-deer region, Aga town, Dakahlia Governorate, from November 2019 to October 2021

Season		Temperature (ºC)	Humidity (%)	Rainfall (mm/month)	Wind speed (km/h)	Cloud (%)	Sun (h/month)	UV index
November	2019	24	57	0.0	11.2	9	358	6
	2020	22	63	9.7	10.4	28	339	6
December	2019	18	62	12.2	13.2	23	360	5
	2020	19	62	4.7	10.3	20	350	4
January	2020	14	70	9.6	14.0	32	349	4
	2021	17	64	3.7	13.1	20	365	5
February	2020	16	69	9.6	12.3	32	308	4
	2021	18	66	8.9	12.6	28	298	4
March	2020	19	63	20.6	14.7	24	345	7
	2021	20	60	0.6	14.3	15	368	5
April	2020	23	60	0.9	13.4	16	356	7
	2021	24	52	0.0	15.4	10	360	8
May	2020	28	51	0.6	14.3	11	367	7
	2021	30	46	0.0	13.4	4	372	8
June	2020	30	56	0.1	13.6	8	360	8
	2021	31	52	0.0	13.0	2	360	9
July	2020	33	63	0.0	12.6	9	372	8
	2021	34	54	0.0	13.3	3	372	9
August	2020	33	64	0.0	12.9	5	372	8
	2021	35	55	0.0	11.9	2	372	8
September	2020	32	65	0.0	12.2	6	359	7
	2021	30	60	0.0	13.3	6	358	7
October	2020	28	61	14.1	11.1	11	369	6
	2021	26	61	1.3	11.9	12	369	5

In March 2019, the seedlings were cultivated on the land at a 2 m × 3 m spacing in clay soil under flood-irrigated system. The grapevine trained on a pergola trellis system. The tested vines were loaded with 30 and 60 eyes per vine in the second and third seasons, respectively, after they were cane pruned in January of the respective growing seasons.

The experiments consisted of eight treatments and were arranged in a randomized complete block design (RCBD) with three replicates each, and four grapevines represented each replicate (Twelve vines per each treatment).

All grapevines under studied received the same common agricultural practices as the entire orchard throughout 2019, 2020 and 2021 seasons as recommended by the Ministry of Agriculture and land Reclamation of Egypt. The seasonal program of fertilization per hectare consisted of 173 kg calcium superphosphate ($CaH_6P_2O_9$) + 124 kg sulphate (SO_4^{2-}) used just once by the start of the vegetative stage; 173 kg potassium sulfate (K_2SO_4) + 370 kg ammonium nitrate (NH_4NO_3) + 124 kg magnesium sulfate ($MgSO_4$) + 124 kg of calcium nitrate ($Ca(NO_3)_2$ applied monthly from March to August. zinc sulphate ($ZnSO_4$) applied twice, after March and August and 1784 mg/L micronutrients (593 mg/L chelated-Fe, 593 mg/L chelated-Zn, and 593 mg/L Mn) applied directly at the onset of vegetative stage at 30-45 cm-shoot length, and 60-75 cm-shoot length. the flood irrigation system was operated at a total rate of around 10400 m3/ha, in accordance with the approved program used in the area for grape varieties. The total volume of water that was determined was based on a 2 L/h water flow rate for 55 minutes during each irrigation time frame. Throughout the course of the 13 irrigation times during the season, a total of 800 m^3/ha of water were used. With the exception of May, June, and July, when it was applied twice a month, irrigation occurred once per month. (**Myburgh, 2003**). All used chemicals in this experiment were imported from Sigma Aldrich, St. Louis, MO, USA.

Ninety-six grapevines of almost similar vigor were selected to present one of the following treatments: T1- H4 strain un-grafted (own rooted), T2- H4 strain grafted on Freedom rootstock, T3- H4 strain grafted on Salt Creek rootstock, T4- H4 strain grafted on SO4 rootstock, T5- Sultana un-grafted (own rooted), T6- Sultana grafted on Freedom rootstock, T7- Sultana grafted on Salt Creek rootstock and T8- Sultana grafted on SO4 rootstock.

Table (2). Soil and water analysis

Soil				Water	
Depth (cm)	**0–30**	**30–60**	**60–90**		
Clay (%)	49.25	50.55	51.15	Transparency (cm)	132.5
Silt (%)	27.69	26.72	26.11	Permeability index (%)	55.64
Sand (%)	23.06	22.66	21.55	Water quality index	21.54
Texture	Clay	Clay	Clay	pH	8.27
Field capacity (%)	45.8	44.7	44.3	Total dissolved salts (mg/L)	204.9
Permanent wilting point (%)	7.4	7.6	7.7	E.C. (μmhos/cm)	558.8
pH (1:2.5 extract)	7.7	7.11	7.11	O_2 (%)	95.8
Organic material (%)	2.3	0.55	0.35	$CaCO_3$ (mg/L)	100.6
E.C. (dS/m) [1:5 extract]	0.61	0.61	0.61	HCO_3^- (mg/L)	159.5
$CaCO_3$ (%)	1.83	1.41	1.88	CO_3^{2-} (mg/L)	7.0
HCO_3^- (meq/100 g)	0.30	0.37	0.40	SO_4^{2-} (mg/L)	15.13
CO_3^{2-} (meq/100 g)	0.0	0.0	0.0	SiO_2 (mg/L)	1.21
SO_4^{2-} (meq/100 g)	3.17	4.04	4.13	Cl^- (mg/L)	32.4
Cl^- (meq/100 g)	0.96	0.98	1.08	Na^+ (mg/L)	29.2
Na^+ (meq/100 g)	0.48	0.66	1.42	Ca^{2+} (mg/L)	27.8
Ca^{2+} (meq/100 g)	0.80	0.20	1.25	Mg^{2+} (mg/L)	14.7
Mg^{2+} (meq/100 g)	0.33	0.97	1.16	N (mg/L)	1.56
N (mg/kg)	32	24	18	P (mg/L)	0.094
P (mg/kg)	13	22	13	K (mg/L)	8.81
K (mg/kg)	271	240	230	Fe (mg/L)	0.23
Fe (mg/kg)	2.48	2.21	2.11	Mn (mg/L)	0.005
Mn (mg/kg)	4.10	3.50	3.21	Zn (mg/L)	0.60
Zn (mg/kg)	1.18	0.61	0.51	Cu (mg/L)	0.018
Cu (mg/kg)	4.24	2.10	0.75	Co (mg/L)	1.56
				Pb (mg/L)	0.77
				B (mg/L)	0.03
				Mo (mg/L)	0.009
				Al (mg/L)	0.03
				Ni (mg/L)	0.014
				Se (mg/L)	0.021
				As (mg/L)	0.044
				V (mg/L)	0.014

2. Studied Parameters

2.1 Vegetative growth

During the 2020 and 2021 seasons the following parameters were determined, the fully developed leaves (i.e., the ones located sixth and seventh from the branch tip) With each shoot that was chosen were taken and the leaf surface area (cm^2) determined using a leaf area meter Model LI-3100 (LI-COR, Inc., Lincoln, NE, USA), The method outlined by **Montero *et al.* (2000)** was used to compute the number of leaves per shoot and the leaf area per vine (m^2). and calculated as follows: (leaf surface area × number of leaves per shoot × number of shoots per vine). At the beginning 2020 and 2021 seasons before the bud burst, trunk thickness (cm) was determined and expressed in by using a digital caliper with 0.01 accuracy (Grizzly Industrial, Chicago, IL, USA), and the increment during 2019, 2020 and 2021 seasons was calculated according to (**El-kenawy, 2003**). In the middle of May, applying a wind-up measuring tape (1000 cm), the total length of four non-fruiting shoots off the renewal spurs was randomly marked (two shoots on each side of the vine), Fisher Scientific, Waltham, MA, USA. At dormant seasons, shoot diameter (cm) was measured from the third base internode by using a digital caliper with 0.01 accuracy (Grizzly Industrial, Chicago, IL, USA). Using a standard 30-cm metal ruler (Apuxon, Shenzhen, Guangdong, China), the internode length was measured from the third basal internode and expressed in (cm).

Coefficient wood ripening was recorded by labeling twelve shoots of the current seasons growth of each replicate to follow up the average of wood ripening (**Rizk & Rizk, 1994**). At dormant season, coefficient wood ripening was calculated as follows: length of the part ripened of shoot/total shoot length. The part of the shoot that ripened is changing in color from greenish to brownish (**Rizk & Rizk, 1994**).

2.2. Biochemical attributes on the leaves and canes

During the 2020 and 2021 seasons the following parameters were determined, the same leaves which were used for measuring leaf area, chlorophyll content and total carotenoids according to the protocol of **Wellburn (1994).** The absorbing substance being extracted was determined at 663 nm for chlorophyll ‘a’, 646 nm for chlorophyll ‘b’ and 470 for carotenoids using a UV/Vis spectrophotometer, Model UV-9100-B (LabTech Inc., Hopkinton, MA, USA). Chlorophyll and carotenoid contents (µg/ml) were calculated using the following equations:

Chlorophyll a = (12.21 E663 - 2.81 E646) (1)

Chlorophyll b = (20.13 E646 - 5.03 E663) (2)

Total chlorophyll = chlorophyll a + chlorophyll b (3)

Total carotenoids = [(1000 E470) - (3.27 × chlorophyll a + 104 × chlorophyll) b)]/198] (4)

where E is the optical density at the indicated wavelength.

Accordingly, chlorophyll and carotenoid contents (mg/g fw) was calculated as follows: [(value from each equation × volume extract)/(1000 × fw)].

To determine the content of N, P, K and Mg, twenty leaf petioles per each replicate were used for determination, leaf samples were collected at mid-May in mature leaves (6th and 7th leaf from the top) and dried at 65°C for 72 h until reaching a constant weight using a bench-top Heratherm GP oven (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, using a mortar and pestle set, dried leaves were ground into a powder, which was subsequently broken down using hydrogen peroxide and strong sulfuric acid **(Wolf, 1982).** With the spectrophotometer Model UV-120-20 (Shimadzu Corp., Kyoto, Japan), the total N and P colorimetric values were determined using the generated solution. (**Evenhuis & Dewaard, 1976 and Jones *et al.*, 1991**). The flame photometer was applied for measuring the content of potassium. (**Tendon, 2005**). The Mg content was also determined according to **Chang & Bray (1951**) using atomic absorption spectrophotometer Model UV-9100-B (LabTech Inc., Hopkinton, MA, USA). All values of N, P and K were expressed as a percentage (%) per dry weight (dw) of leaves, while Mg was expressed as a mg/1000 g dw of leaves.

Total carbohydrates, four non-fruiting canes of the renewal spurs, two canes at each side of the vine were randomly collected at the end of the growing season (late October) to determine total carbohydrates according to **Hodge & Hofreiter (1962).** Briefly, The middle portion of the cane was cutted into small pieces using a knife and heat-dried until a constant weight at 70°C for 72 h using a bench-top Herathermal GP oven (ThermoFisher Scientific, Waltham, MA, USA).

A bench-top Herathermal GP oven (ThermoFisher Scientific, Waltham, MA, USA) was used to heat-dry the middle section of the cane, which was cut into small pieces using a knife and heated to 70°C for 72 hours, or until a consistent weight was reached. A porcelain mortal and pistil set (Fisher Scientific, Waltham, MA, USA) were used to grind dried cane pieces. After that, 0.2 g powder sample was hydrolyzed for 6 hours using 15 ml of HCL (1 M). Total carbohydrates in the solution sampels were measured colorimetrically at 490 nm and calculated as a percentage of the dry weight.

Total proteins in the canes (mg/g dw), it was determined as described of **Lowry *et al.* (1951)**. Samples of the dry canes were extracted in a solution of 10% sodium dodecyl sulfate (SDS), 1% mercaptoethanol, 65 ml Tris/HCL (pH 6.8) and measured spectrophotometrically at 595 nm Using a UV/Vis spectrophotometer, Model UV-9100-B (LabTech Inc., Hopkinton, MA, USA). The result was expressed as mg of bovine serum albumin equivalent per gram of dry weight of the canes and was calculated from the standard curve using bovine serum albumin as standard **(Bradford, 1976)**.

Total free amino acid in the leaves, it was determined according to the method described by **Jayarman (1981)**. Briefly, 500 mg of dry leaves was extracted in 50 ml of ethanol 80%, then filtered to remove insoluble materials. A mixture of 0.5 ml of 0.07 mol/l phosphate buffer solutions (pH 8.04) and 0.5 ml of 2% ninhydrin solution containing 0.8 mg/ml of stannous chloride dihydrate ($SnCl_2–2H_2O$) was mixed with 1 ml of ethanol extract. The tubes were next heated to 100°C for 15 minutes in a "PrecisionTM General Purpose" water bath (Thermo Fisher Scientific, Waltham, MA, USA), cooled with cold water, and then diluted to 25 ml with distillated water. Following ten minutes of inactivity, the absorbance values of these blue-purple compounds were calculated as g/100 g fw using a UV/Vis spectrophotometer, Model UV-9100-B (LabTech Inc., Hopkinton, MA, USA), against a reagent blank at 550 nm.

The leaf proline content ($C_5H_9NO_2$) concentration was determined according to **Bates *et al.* (1973**). Briefly, A mixture of 0.5 ml of 0.07 mol/l phosphate buffer solutions (pH 8.04) and 0.5 ml of 2% ninhydrin solution containing 0.8 mg/ml of stannous chloride dihydrate ($SnCl_2–2H_2O$) was mixed with 1 ml of ethanol extract. The tubes were next heated to 100°C for 15 minutes in a "PrecisionTM General Purpose" water bath (Thermo Fisher Scientific, Waltham, MA, USA), cooled with cold water, and then diluted to 25 ml with distillated water. For one hour, the tubes were incubated at 100°C in a "PrecisionTM General Purpose" water bath (Thermo Fisher Scientific, Waltham, MA, USA). After that, they were allowed to cool for a full day at ambient temperature (about 22–23°C). Then, for twenty seconds, the solution was combined with 4 mL of toluene (C6H5CH3) using a Vortex-Genie 1 mixer (Scientific Industries, Inc., Bohemia, NY, USA). The tubes were kept upright for at least ten minutes to allow the toluene and the aqueous phase to separate. After that, the toluene phase was carefully pipetted out into a cuvette, and the absorbance was measured colorimetrically at 520 nm using a UV/Vis spectrophotometer, Model UV-9100-B (LabTech Inc., Hopkinton, MA, USA). Eventually, a proline standard curve was used to calculate the proline concentration as mg/g fw.

3. Statistical Analysis

Data were first examined utilizing the Shapiro-Wilk and Levene testing for numerical normality and homogeneity of variance, respectively. Before doing the analysis of variance (ANOVA), the percentage data were first converted to the values of the Arcsine square root. The outcomes were then shown as back-transformed means. The CoStat software packaging, version 6.311 (CoHort software, Monterey, CA, USA), was used for carrying out the ANOVA. Tukey's honestly significant difference (HSD) test was used to conduct mean comparisons at probability (p) < 0.05 **(Snedecor & Cochran, 1990**). Principal components analysis (PCA) was used to create the vegetative growth score and loading plot for biochemical parameters (**Jolliffe, 2011**). The means of the data matrices were used to generate two-way hierarchical cluster analysis (HCA) and heat map (**Michie, 1982**). Both PCA and HCA were performed using JMP Pro 16 (SAS Institute, Cary, NC, USA).

RESULTS

1. Effect of grafting H4 strain and Sultana grapevines on growth parameters

Vegetative growth parameters of leaf surface area, number of leaves/shoot, leaf area/vine, trunk thickness, shoot length, shoot diameter, internode length and coefficient wood ripping which have been proposed to be indices of grapevine vigour for H4 strain and Sultana grapevines are arranged in Figure 1A-D and Figure 2A-D. Results indicate grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 rootstocks were the better as compared to the un-grafted grapevines. However, H4 strain grafted on studied rootstocks recorded the maximum vegetative growth parameters comparable to Sultana grafted on the same rootstocks. Grafted H4 strain and Sultana grapevines on the rootstock of Freedom (T2,T6) significantly improved leaf surface area, number of leaves/shoot, leaf area/vine, trunk thickness, shoot length, shoot diameter, internode length and coefficient wood ripping followed by grafted on Salt Creek (T3,T7) and SO4 (T4,T8) rootstocks, respectively. On the contrary, un-grafted (own rooted) H4 strain and Sultana grapevines (T1,T5) recorded the significant lowest vegetative growth parameters.

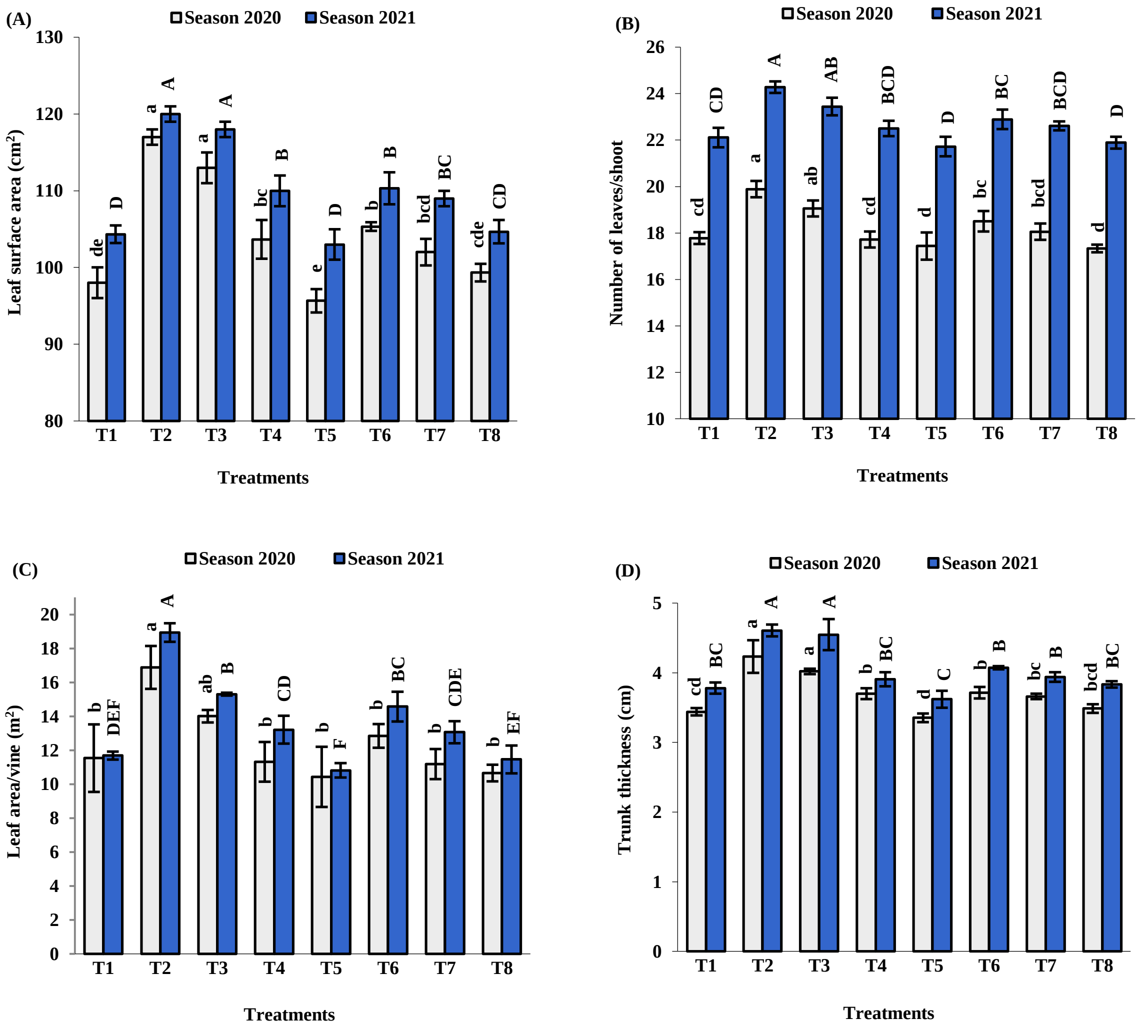


Fig. (1). Effect of grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on leaf surface area (A), number of leaves/shoot (B), leaf area/vine (C) and trunk thickness (D) during 2020 and 2021. T1 = H4 strain un-grafted, T2 = H4 strain grafted on Freedom, T3 = H4 strain grafted on Salt Creek, T4 = H4 strain grafted on SO4, T5 = Sultana un-grafted, T6 = Sultana grafted on Freedom, T7 = Sultana grafted on Salt Creek, and T8 = Sultana grafted on SO4. Values ± standard deviation (SD) are the means of three replicates (n = 3). The means in the 2020 or 2021 seasons, respectively, with the same lowercase or uppercase letters are not significant different at $p \leq 0.05$ using Tukey's HSD test.

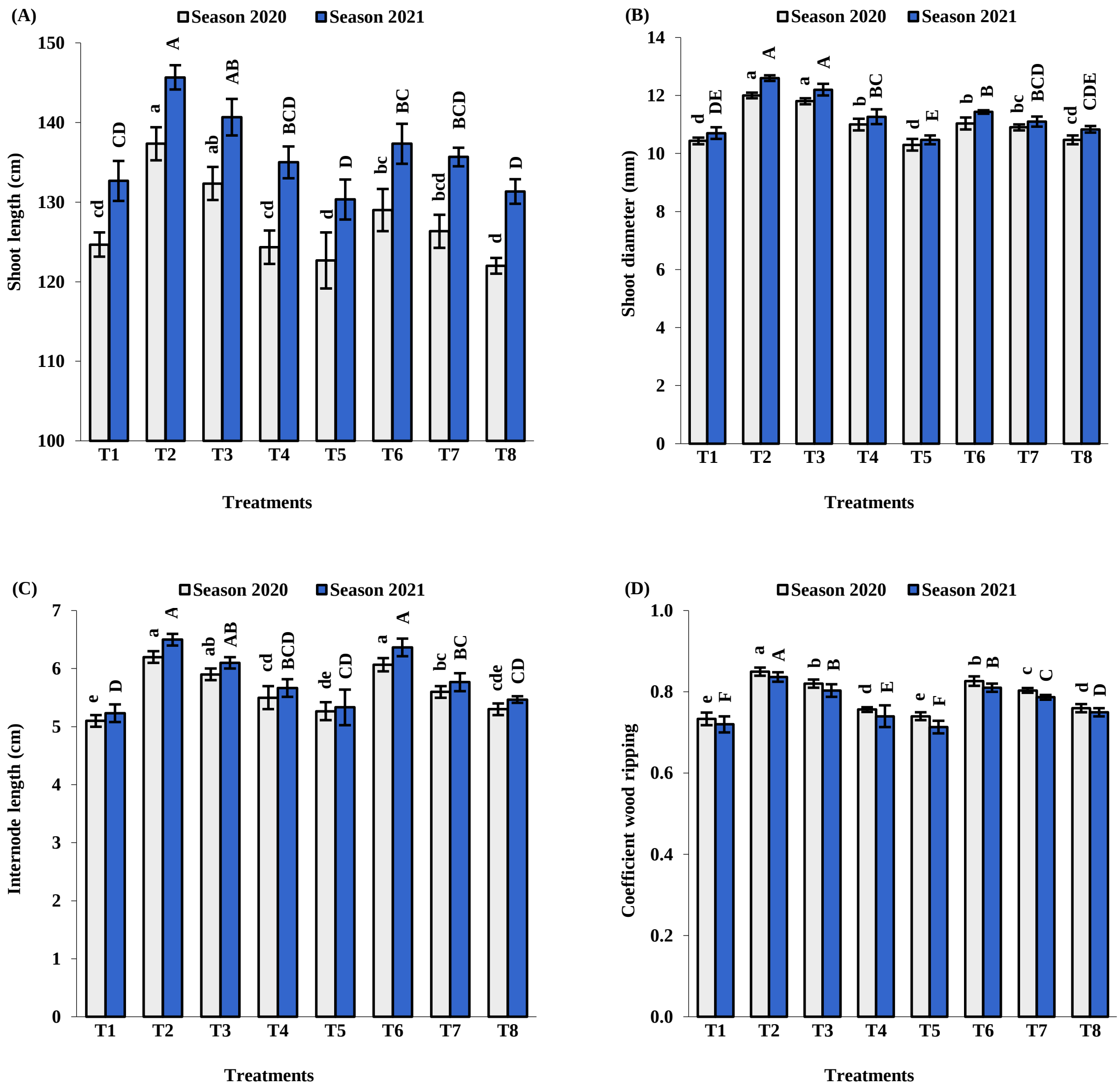


Fig. (2). Effect of grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on shoot length (A), shoot diameter (B), Internode length (C) and coefficient wood ripping (D) during 2020 and 2021. T1 = H4 strain un-grafted, T2 = H4 strain grafted on Freedom, T3 = H4 strain grafted on Salt Creek, T4 = H4 strain grafted on SO4, T5 = Sultana un-grafted, T6 = Sultana grafted on Freedom, T7 = Sultana grafted on Salt Creek, and T8 = Sultana grafted on SO4. Values ± standard deviation (SD) are the means of three replicates (n = 3). The means in the 2020 or 2021 seasons, respectively, with the same lowercase or uppercase letters are not significant different at *p* ≤ 0.05 using Tukey's HSD test.

2. Effect of grafting H4 strain and Sultana grapevines on biochemical attributes of the leaves and canes

Results in Figure 3A-D reveal that H4 strain and Sultana grapevines grafted on Freedom (T2,T6), Salt Creek (T3,T7) and SO4 (T4,T8) rootstocks significantly improved photosynthesis process in the leaves as compared to the un-grafted ones (T1,T5). Grafting H4 strain (T2,T3,T4) greatly increased photosynthesis pigments (chlorophyll a, chlorophyll b, total chlorophyll ,and total carotenoids) in most cases as compared to grafting Sultana grapevines on studied rootstocks (T6,T7,T8). In addition, Freedom rootstock recorded significantly higher leaf content of photosynthetic pigments then Salt Creek rootstock then SO4 rootstock, while un-grafted grapevines (T1,T5) recorded the lowest leaf contents of chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids.

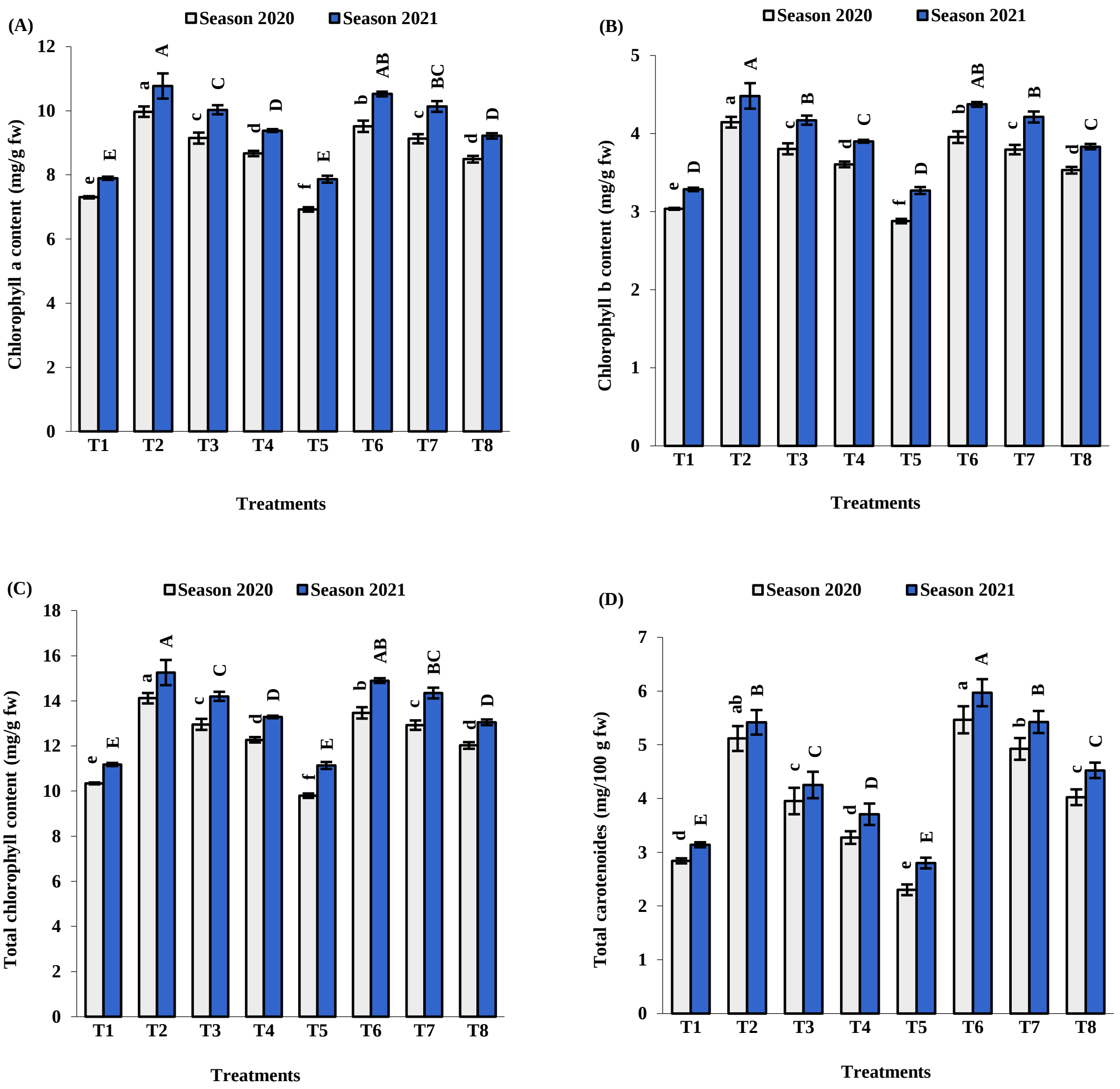


Fig. (3). Effect of grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on chlorophyll a content (A), chlorophyll b content (B), total chlorophyll content (C) and total carotenoides (D) during 2020 and 2021. T1 = H4 strain un-grafted, T2 = H4 strain grafted on Freedom, T3 = H4 strain grafted on Salt Creek, T4 = H4 strain grafted on SO4, T5 = Sultana un-grafted, T6 = Sultana grafted on Freedom, T7 = Sultana grafted on Salt Creek, and T8 = Sultana grafted on SO4. Values ± standard deviation (SD) are the means of three replicates (n = 3). The means in the 2020 or 2021 seasons, respectively, with the same lowercase or uppercase letters are not significant different at $p \leq 0.05$ using Tukey's HSD test.

The mineral content of the leaves suggests that both cultivars' nutrition levels were significantly impacted by the rootstocks. Grafting H4 strain and Sultana grapevines on Freedom, Salt Creek, and SO4 rootstocks significantly enhanced concentration of minerals in the leaves (N, P, K and Mg) as in comparison to the un-grafted grapevines (Figure 4A-D). Results also show that H4 strain grafted on Freedom rootstock (T2) gave the highest values of mineral leaf contents followed by Sultana grapevines grafted on Freedom rootstock (T6), then H4 strain grafted on Salt Creek rootstock (T3). In addition, results did not show any significant differences with either H4 strain grafted on Salt Creek or Sultana grafted on Freedom rootstocks in mineral content except P and Mg throughout the first and second years, respectively. On contrary, un-grafted Sultana (own rooted) (T5) recorded the significant lowest values of mineral contents in the leaves followed by un-grafted H4 strain (own rooted) (T1) without any significant differences between of them for P and Mg in 2020 season only.

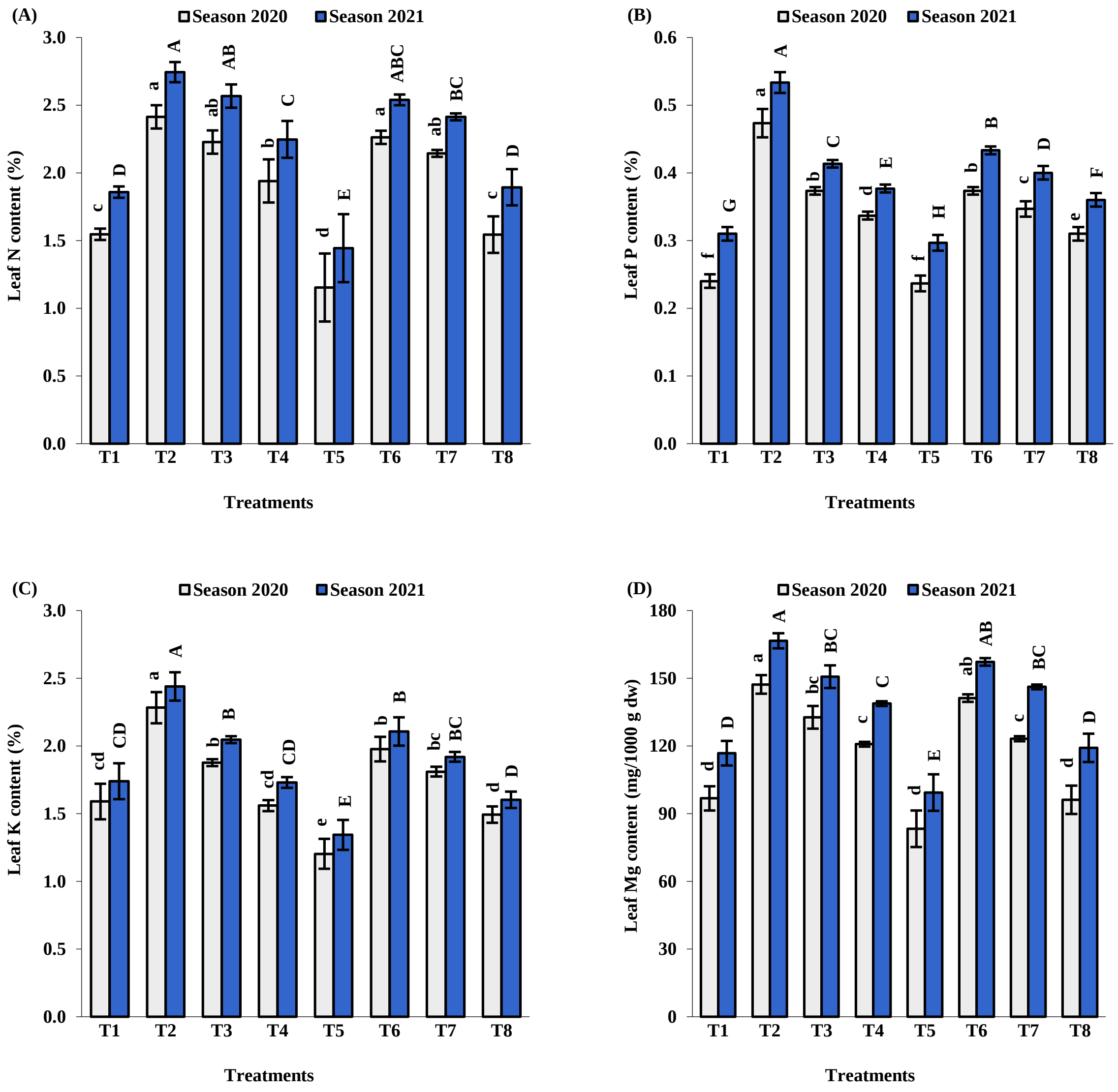


Fig. (4). Effect of grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on leaf N content (A), leaf P content (B), leaf K content (C) and leaf Mg content (D) during 2020 and 2021. T1 = H4 strain un-grafted, T2 = H4 strain grafted on Freedom, T3 = H4 strain grafted on Salt Creek, T4 = H4 strain grafted on SO4, T5 = Sultana un-grafted, T6 = Sultana grafted on Freedom, T7 = Sultana grafted on Salt Creek, and T8 = Sultana grafted on SO4. Values ± standard deviation (SD) are the means of three replicates (n = 3). The means in the 2020 or 2021 seasons, respectively, with the same lowercase or uppercase letters are not significant different at $p \leq 0.05$ using Tukey's HSD test.

Results in Figure 5A-D reveal that grafting H4 strain seedless with Freedom, Salt Creek, and SO4 rootstocks greatly improved biochemical attributes in the canes and leaves in most cases in comparison to Sultana grapevines grafted in the same rootstocks. Results indicate that H4 strain and Sultana grapevines grafted on rootstocks of Freedom (T2,T6), Salt Creek (T3,T7), and SO4 (T4,T8) significantly increased total carbohydrates and protein contents in the canes as well as leaf content of total free amino acids and proline as compared to un-grafted ones (own rooted) (T1,T5). Grafted H4 strain and Sultana grapevines on the rootstock of Freedom (T2,T6) recorded significantly higher contents of total carbohydrates, protein, total free amino acids and proline then by Salt Creek rootstock (T3,T7) next came SO4 rootstock (T4,T8). On opposite, the lowest total carbohydrates, protein, total free amino acids and proline contents were recorded with un-grafted (own rooted) (T1,T5).

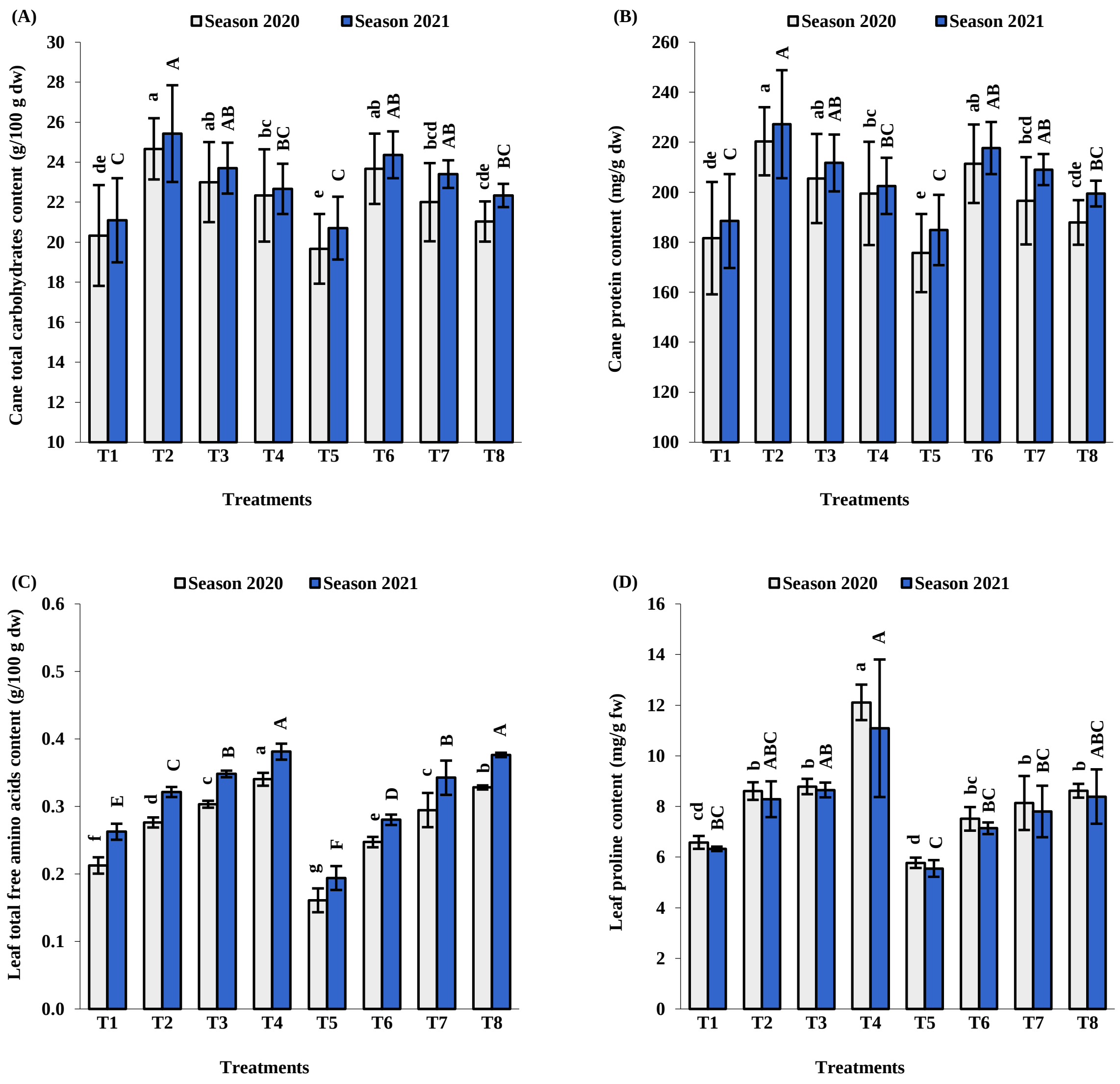
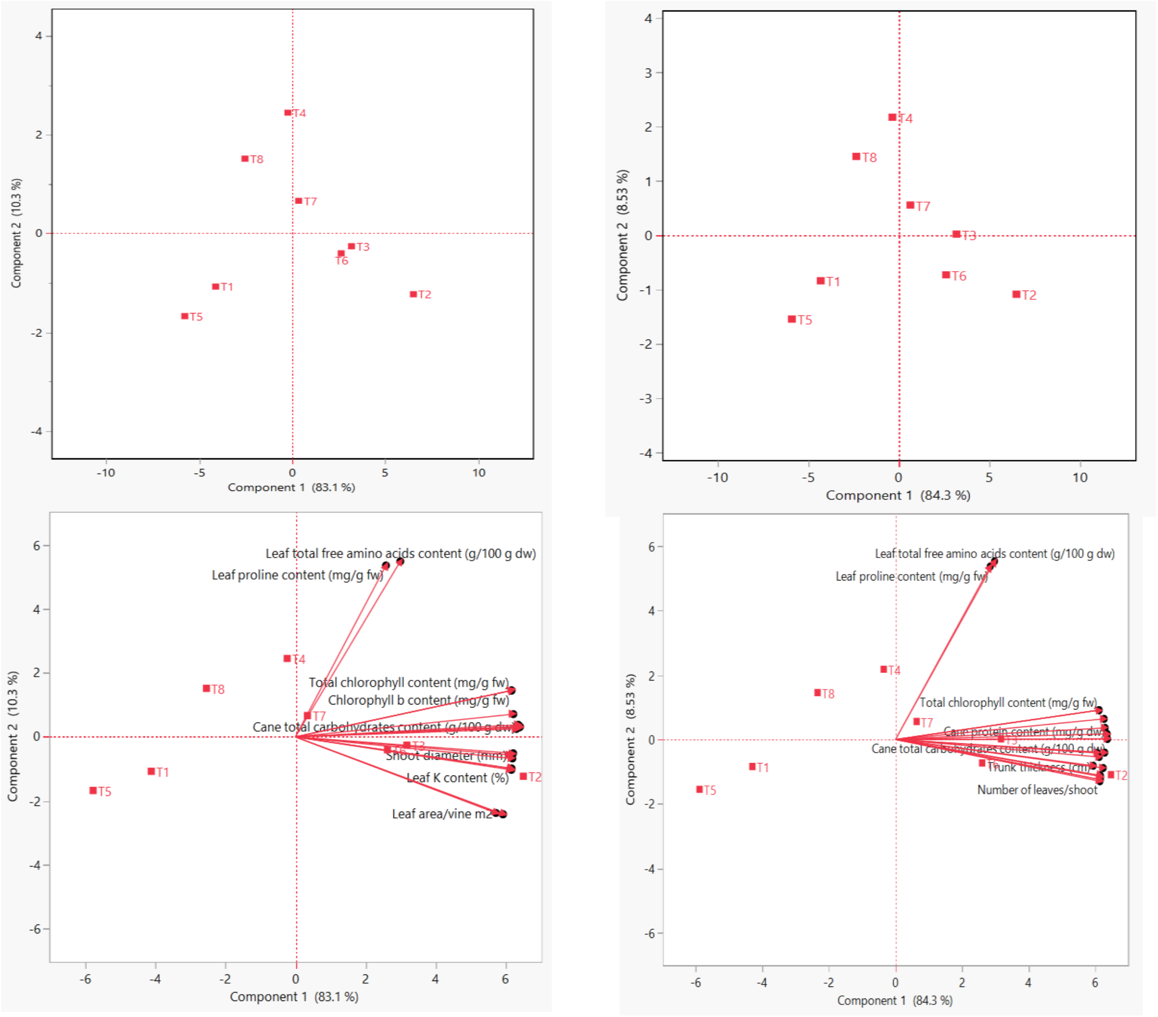


Fig. (5). Effect of grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on cane total carbohydrates content (A), cane protein content (B), leaf total free amino acids content (C) and leaf proline content (D) during 2020 and 2021. T1 = H4 strain un-grafted, T2 = H4 strain grafted on Freedom, T3 = H4 strain grafted on Salt Creek, T4 = H4 strain grafted on SO4, T5 = Sultana un-grafted, T6 = Sultana grafted on Freedom, T7 = Sultana grafted on Salt Creek, and T8 = Sultana grafted on SO4. Values ± standard deviation (SD) are the means of three replicates (n = 3). The means in the 2020 or 2021 seasons, respectively, with the same lowercase or uppercase letters are not significant different at *p* ≤ 0.05 using Tukey's HSD test.

3. Principal component analysis (PCA) and hierarchical cluster analysis (HCA)

The purpose of applying PCA and HCA was to give a more comprehensive image of the grafting impact. H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on qualities of vegetative growth parameters and biochemical attributes on the leaves and canes during the 2020 (A) and 2021 (B) seasons. As for the PCA (Figure 6), the score plot indicated this. H4 strain and Sultana grapevines grafted were different from the un-grafted affecting vegetative growth parameters and biochemical attributes on the leaves and canes in two years (Figure 6A,B). However, the more pronounced effect on all studied attributes was recorded for H4 strain and Sultana grapevines grafted on Freedom, and Salt Creek rootstocks (T2,T3,T6,T7) then H4 strain and Sultana grapevines grafted on SO4 rootstock (T4,T8). Principal components 1 and 2 accounted for 93.40 and 92.83 % of the total variance throughout the first and second years (Figure 6A,B), respectively.

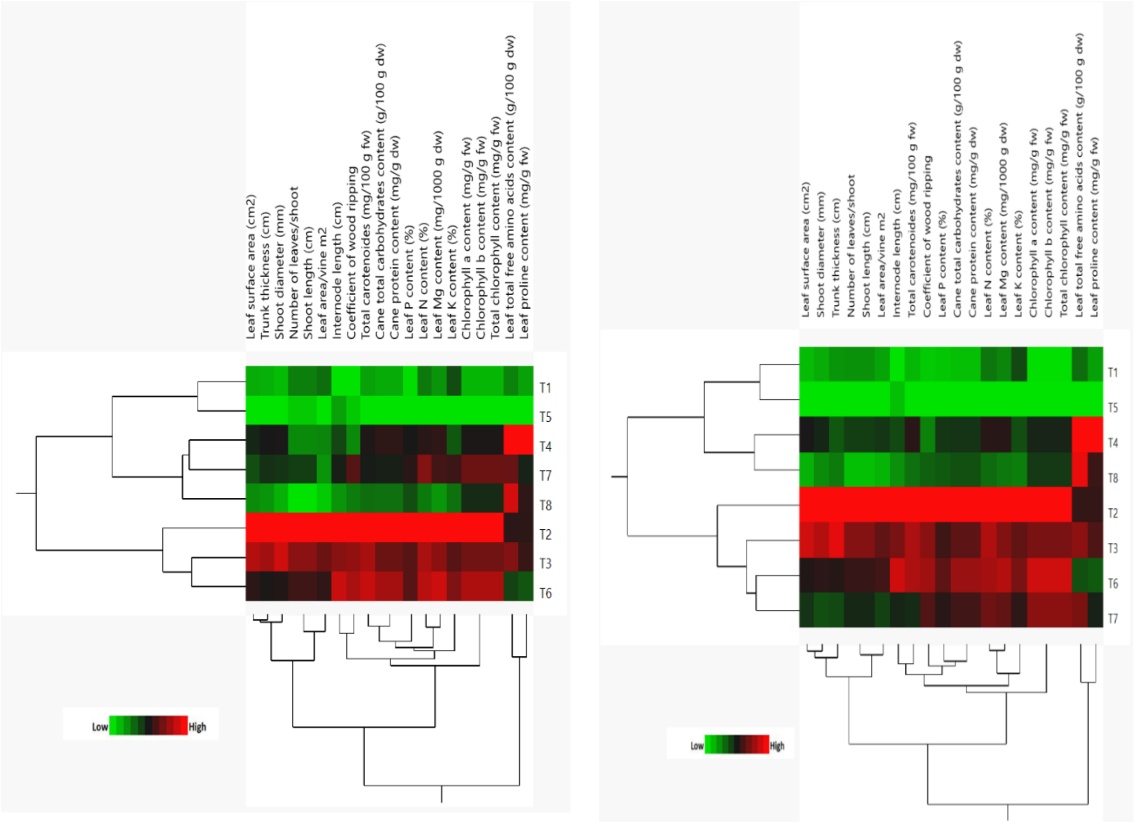


(A) Season 2020

(B) Season 2021

Fig. (6). Principal component analysis (PCA) showing the score and loading plots of grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on vegetative growth parameters and biochemical attributes on the leaves and canes during the 2020 (A) and 2021 (B) seasons. T1 = H4 strain un-grafted, T2 = H4 strain grafted on Freedom, T3 = H4 strain grafted on Salt Creek, T4 = H4 strain grafted on SO4, T5 = Sultana un-grafted, T6 = Sultana grafted on Freedom, T7 = Sultana grafted on Salt Creek, and T8 = Sultana grafted on SO4. Values are the means of three replicates (n = 3).

Likewise, the hierarchical cluster analysis (HCA) (Figure 7), where the highest and lowest values are represented with red and green colors, respectively. The results indicated H4 strain and Sultana grapevines grafted were different from the un-grafted affecting vegetative growth parameters and biochemical attributes on the leaves and canes in two years (Figure 7A,B). However, the most effective treatments on all studied parameters during both seasons was recorded for H4 strain and Sultana grapevines grafted on Freedom and Salt Creek rootstocks (T2,T3,T6,T7) next came by H4 strain and Sultana grapevines grafted on SO4 rootstock (T4,T8) (Figure 7A,B) which confirmed the PCA results (Figure 6). The un-grafted grapevines (own rooted) were represented in the 2020 and 2021 seasons (Figure 7). The HCA's heat map revealed that the un-grafted (T1,T5) recorded the lowest values in vegetative growth parameters and biochemical attributes on the leaves and canes in two years (Figure 6A,B), which support the earlier findings Figure 1, Figure 2, Figure 3, Figure 4 and Figure 5. H4 strain and Sultana grapevines grafted on Freedom and Salt Creek rootstocks (T2,T3,T6,T7) of the heat map also produced a superior result than the grafted H4 strain and Sultana grapevines on So4 (T4,T8) or un-grafted grapevines (own rooted) (T1,T5) in both seasons (Figure 7A,B).



(A) Season 2020

(B) Season 2021

Fig. (7). Two-way hierarchical cluster analysis (HCA) and heat map showing the effect of grafting H4 strain and Sultana grapevines on Freedom, Salt Creek and SO4 grape rootstocks on vegetative growth parameters and biochemical attributes on the leaves and canes during the 2020 (A) and 2021 (B) seasons. T1 = H4 strain un-grafted, T2 = H4 strain grafted on Freedom, T3 = H4 strain grafted on Salt Creek, T4 = H4 strain grafted on SO4, T5 = Sultana un-grafted, T6 = Sultana grafted on Freedom, T7 = Sultana grafted on Salt Creek, and T8 = Sultana grafted on SO4. Values are the means of three replicates (n = 3).

Discussion

Finer roots are essential for greater grapevine development and continued growth because they help with water and nutrient absorption from the soil, the synthesis and metabolism of plant growth chemicals, and the storage of carbohydrates. A higher root density facilitates the grapevines' ability to absorb as much nutrition as possible. **(Richards, 1983 and Somkuwar *et al.*, 2012)**. In the current research, Freedom was the better rootstock for grafting on H4 strain or Sultana grapevines to improves vegetative growth parameters (Figures 1 and 2) and biochemical attributes on the leaves and canes (Figures 3, 4 and 5), next came Salt Creek and SO4 rootstock, respectively, which confirmed the PCA and HCA results (Figures 6 and 7). Increasing the morphological features of vegetative development could be the result of a vigorous rootstock having a significant impact on scion growth **(Hartman *et al.*, 2002).** Regarding this matter, **Verma *et al.* (2010)** established that the influence of the rootstock qualities such as vigor growth and increment of root hairs density in the soil could be responsible for the noticeable difference in leaf area of the grafted "PusaUrvashi" grape on various rootstock. The high efficacy of the rootstocks in absorbing and transporting the water and minerals via the grafted union to the shoots of the scion as well as the favorable reciprocal relationship between stock and scion could be the cause of the grafting's beneficial effects on improvements in leaf surface area, number of leaves/shoot, leaf area/vine, trunk thickness, shoot length, shoot diameter, internode length, and coefficient wood ripping. (**El-Gendy, 2013 and Serra *et al.*, 2014**). **Somkuwar *et al.* (2015)** mentioned that the scion grafted on Freedom, Salt Creek and SO4 rootstocks exhibited higher vegetative growth parameters as compared to the un-grafted grapevines and this might be due to high N uptake. **Mohsen (2021**) discovered that, in comparison to ungrafted grapevines (own-rooted), grafted "Flame seedless" grapevines on Salt Creek, Richter, and Freedom rootstocks dramatically boosted vegetative growth metrics. The high efficacy of the rootstocks on enhancing the ripening of wood (Figure 2 D) may be attributed to the increases of N, P, K and Mg uptake and consequently increase in vigor of the grafted grapevines, thus, vegetative growth parameters (Figures 1 and 2), photosynthetic pigments (Figure 3), mineral leaf contents (Figure 4) were increased and thus raises the activation of the metabolism of carbohydrates, which enhances the coefficient of wood ripening. The favorable impact of grapevine rootstocks on the grafted cultivars increased mineral leaves concentrations and the variations in uptake of nutrients between rootstocks may be caused by the rootstocks' varying absorption capacities or tendencies for particular minerals **(Somkuwar *et al.*, 2014).** Accordingly, the variations in leaf nutrient content among the investigated rootstocks in this study can be explained by the fact that a rootstock with a larger root size may be more effective at absorbing nutrients from the soil (**Somkuwar *et al.*, 2015**). **Mervat *et al.* (2019)** found that grafted ‘Flame seedless’ grapevines on Freedom rootstock increased shoot length, total leaf surface area per vine and leaf content of N, P, and K followed by grafted on Salt Creek rootstock in comparison to the un-grafted (own-rooted) ones. The increase in the total carbohydrate content in the cane of H4 strain or Sultana grapes grafted on the studied rootstocks may be a result of improved vegetative growth parameters and increased content of photosynthetic pigments, as shown in Figures 1, 2 and 3 resulting from a higher rate of process of photosynthesis which aids in the production of increased carbohydrates **(Somkuwar *et al.*, 2014).** Accordingly, ‘Fantasy seedless’ grapevine grafted on the Freedom rootstock recorded high chlorophyll a content in the leaves, while the lowest content was observed in the grapevine grafted on the Salt Creek rootstock (**Somkuwar *et al.*, 2015**). In addition, the highest total carbohydrate content in the canes was recorded in grapevines grafted on St. George rootstock. **During (1994)** discovered that the impact of rootstock on gas exchange properties varies depending on the scion, perhaps as a result of the grafted grapevines' unique ability to carboxylate. In certain instances, grafting led to modifications in stomatal conductance, which improved the rate of photosynthesis. (**Jogaiah *et al.*, 2013**). As noted by **Southey (1992),** rootstocks have the tendency to preferentially absorb nutrients from the soil. These nutrients can then function as coenzymes in the synthesis of various secondary metabolites that are necessary for the synthesis of amino acids and proteins, including proline, which serves as a source of carbon, nitrogen, and energy for cellular metabolism **(Hare & Cress, 1997)**. Moreover, it might be supplying energy for the movement and build-up of sugars **(Kliewer, 1968)**. According to **Mervat *et al.* (2019)**, the ability of roots to absorb sufficient amounts of components like nitrogen, zinc, iron, and magnesium may have an impact on the quantity of photosynthesis pigments (carotenoids and chlorophyll). This promotes the synthesis of pigments in the leaves.

Conclusion

According to the results above, we might conclude that, the grafting of Sultana grapevines and the H4 strain on commercial rootstocks (Freedom, Salt Creek, and SO4) improved vegetative growth and biochemical parameters significantly when compared to ungrafted grapevines, according to the results above. Furthermore, this investigation demonstrated that Freedom rootstock produced the best outcomes with the two assessed cultivars, followed by Salt Creek rootstock and SO4. Finally, it can be recommended that Freedom is the more suitable rootstock for grafting H4 strain and Sultana grapevines in clay soil under flood-irrigated system of delta Egypt. Further study will be needed to know the effect of these rootstocks on the total yield, cluster quality and physicochemical properties of the berries when the grapevine reaches the productive stage.

Author Contributions

Conceptualization, A.F.A.E.-K., M.A.E.-K.; B.E.B. and M.N.M; methodology, A.F.A.E.-K., M.A.E.-K.; B.E.B. and M.N.M; software, A.F.A.E.-K; validation, A.F.A.E.-K., M.A.E.-K. and B.E.B.; formal analysis, A.F.A.E.-K. and M.A.E.-K.; investigation, A.F.A.E.-K., M.A.E.-K.; B.E.B. and M.N.M; resources, A.F.A.E.-K., M.A.E.-K.; B.E.B. and M.N.M; data curation, A.F.A.E.-K. and M.A.E.-K.; writing—

original draft preparation, A.F.A.E.-K., M.A.E.-K. and B.E.B.; writing—review and editing, A.F.A.E.-K.; visualization, A.F.A.E.-K., M.A.E.-K. and B.E.B.; supervision, A.F.A.E.-K. and M.A.E.-K.; project administration, A.F.A.E.-K.; funding acquisition, A.F.A.E.-K. All authors have read and agreed to the submitted version of the manuscript.

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Conflicts of Interest

No conflicts of interest are disclosed by the authors.

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