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Response of Grafted Olive (*Olea europaea* L. Cv. Coratina) to Water Deficit Conditions

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Abstract: Drought is one of the most damaging abiotic stresses; water deficit problem is increasingly occurring due to global climate change and negatively affects crop growth and productivity. Grafting on tolerate rootstocks is a promise approach to mitigate negative impacts of drought stress and ensure production sustainability. The present study was carried out to investigate the effect of water deficit stress on Coratina olive plants grafted on the following cultivars as rootstocks (Coratina, Koroneiki, Manzanillo, Picual and Sorani). Three water levels based on soil field capacity (FC) (100, 50% and 25% of FC) were used for water deficit treatments. Water deficit decreased shoot growth, stem diameter, leaves number and area, shoot and root weight. Leaf analysis showed marked decrease in total chlorophyll content while proline, total sugars and phenolic content increased with increasing water deficit level. The studied grafting combination differed in their response to water deficit treatments; Coratina grafted on Sorani and Koroneiki recorded higher values of growth parameters and accumulated higher amount of osmolytes (proline and total sugars) and phenolic compared to other grafted olive plants.

Keywords: Drought, Grafting, Olive, Rootstocks, Water stress.

Introduction

The Mediterranean ecosystem is characterized by multiple extreme climate conditions *i.e.* lack of rainfall, high evapotranspiration and extreme temperatures; due to the impacts of climate change, the Mediterranean region is expected to face a significant decrease in the total amount of precipitation, fluctuations in precipitation patterns and expand of drought-affected areas (De Ollas *et al.,* 2019). According to the global crop production statics, drought estimated to caused crop yield losses approximately of $30 billion (Gupta *et al.,* 2020; Yang *et al.,* 2022), the rapid population growth and increase in arable land, water demand could be increased (Gupta *et al*., 2020; Qadir *et al*., 2022). Under such conditions, it is crucial to choose drought-tolerant genotypes that can adapt to harsh environment conditions and maintain a normal growth and yield (Ennajeh *et al.,* 2010). Olive tree (*Olea europaea* L.) is a common crop in the Mediterranean agro-ecosystems, with great commercial and social importance (Loumou & Giourga, 2003). Olive is well-adapted to arid environment conditions, due to the unique morphological, physio-anatomical adaption mechanisms (Connor & Fereres, 2010; Fernandez, 2014). Responses of olive to drought include reducing leave area and number, slowing growth rate, increasing root density and increasing root-to-shoot ratio (Ben-Gal *et al.,* 2010; Abdallah *et al.,* 2018; Hegazi *et al.,* 2018). However, water is critical for sustainability of olive production, there are a lot of studies confirm that, tree growth, productivity, fruit quality are positively responds to availability of irrigation water (Pierantozzi *et al.,* 2020; Conde-Innamorato *et al.*, 2022). Water deficit significantly reduces cell water potential, transpiration rate, and inhibiting cell expansion which results in reducing leaf area, and photosynthetic rate (Farooq *et al.,* 2012; Karim *et al*., 2020). Water deficit, reduces carbon assimilation, gas exchange, nutrient uptake, biomass accumulation and stimulate accumulation of reactive oxygen species (ROS), which in turn causes poor tree growth and ultimately crop yield losses (Ben-Gal *et al.,* 2010; Perez-Martin *et al.,* 2014; Kumar *et al.,* 2017; Trabelsi *et al.,* 2019; Gupta *et al.,* 2020; Zia *et al.,* 2021). Recently much attention has been drawn to utilization drought-tolerant genotypes to achieve relatively high yields under drought conditions (Bolat *et al.,* 2014; Galindo *et al.,* 2018; Anđelković *et al.,* 2020). Interestingly, grafting has a potential to improve plant drought tolerance through the utilization of drought-tolerant rootstocks (Yang *et al.,* 2022). Rootstock can changes scion growth behavior, yield, fruit quality, nutritional status and (Mourão Filho *et al.,* 2007; Stegemann & Bock, 2009; Sharma *et al.,* 2016; Kumar *et al.,* 2017). Moreover, rootstocks are frequently used to improve crop tolerance to water deficit stress through regulating plant water relations (Trifilo *et al.,* 2007; Therios, 2009; Balal *et al.,* 2012), hence grafting is a potential approach that can mitigate negative stress effects, improve crop performance and ensure a sustainable crop production under stress conditions (García-Sánchez *et al.,* 2007; Nawaz *et al.,* 2016; Balfagón *et al.,* 2021). Rootstock may enhance stress tolerance of the grafted scions by regulating stress-responsive genes and activation antioxidant system (Balfagón *et al.,* 2021; Shehata *et al.,* 2022).

The aim of this study was to evaluate the usefulness of grafting of Coratina olive cv. on some olive genotypes as rootstocks to increasing drought stress resistance of the grafted olive plants.

Materials & Methods

Plant materials and growth conditions

This study was carried out at the experimental shade house (70 % shade-net) of Pomology Department, Faculty of Agriculture, University of Cairo (031°12'65"E longitude, 30°00'48"N latitude). Five olive cultivars with markedly different drought tolerance were selected; self-rooted transplants of olive cultivars namely Koroneiki, Picual, Sorani, Manzanillo and Coratina, were selected. One year old plants of the selected cultivars were transplanted into plastic bags filled with 20 kg of sand soil and received the recommended agriculture practices. The selected olive rootstocks were top grafted with scions of Coratina olives, during the 1st week of May. Soil and water samples were analyzed, at the Soil and Water Laboratory (Agricultural Research Centre, Egypt). Mechanical and chemical analysis of soil samples are illustrated in table (1).

Water stress treatments

A shade house experiment was performed to determine the response of grafted olive transplants cv. Coratina to water stress. Based on the soil field capacity, three different irrigation regimes were applied; the well-watered olive plants irrigated with 100% of field capacity, moderate drought stressed olive plants irrigated with 50% of field capacity and severe drought stressed olive plants irrigated with 25%. The olive transplanted were irrigated with tape water (EC = 0.48 dS. m^{-1}, pH= 7.53), for 90 days through drip irrigation system with one built in emitter (4.0L h^{-1}) per pot and located in a single line parallel to the transplants.

Table (1): Chemical analysis of experimental orchard soil samples.

Physical soil analysis			
Particle size distribution		Soil moisture constants	
Fine sand%	44.2	Saturation percentage (SP)	24
Coarse sand%	41.6	Field capacity% (FC)	15
Silt%	11.2	Wilting point% (WP)	6.2
Clay%	3	Available water% (AW)	8.8
Soil texture	Sandy soil		

Chemical soil analysis										
pH	EC (dS/m)	Cation (mM/L)				Anion (mM/L)				
		K+	Na+	Mg++	Ca++	SO4--	Cl-	HCO3-	CO3-	SO4--
7.30	1.21	0.3	60	1.9	3.8	1.63	10	0.37	-	1.63

Measurements

At the end of the experiment period (12 weeks), olive transplants were harvested, shoots and roots were separated after the cleaning of roots from the soil and the following measurements were recorded: stem diameter (mm), plant height (cm), leaves number, leaf area; leaf area was calculated using the formula L.A = 0.53 (L×W) + 1.66 (Hagagg *et al.,* 2020), total leaf area, root length, shoot and root fresh weight, shoot and root dry weight (after drying in a force-draft oven at 70ºC to a constant weight, total fresh biomass, total dry biomass, root-shoot ratio (Grotkopp & Rejmánek, 2007; James & Drenovsky, 2007). Total chlorophyll content was measured with a portable chlorophyll meter (Minolta- SPAD-502, Japan); the data were expressed as SPAD units (Markwell *et al.,* 1995). Proline content was determined according to Bates *et al.* (1973); 0.5g of fresh leaf sample was homogenized in 3% sulfosalicylic acid solution (w/v). 2 ml of leaf extract were mixed with 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid then placed in water bath for 1 h at 90 °C; a cooling bath of ice was used to terminate the reaction. The developed red color was extracted in 4 ml toluene, absorbance of the extracted was determined at wavelengths of 520 nm by spectrophotometer (JENWAY 6300, Staffordshire, UK), a standard curve with known concentration was generated using analytic grade proline, and proline concentration was calculated on a fresh weight basis according to Bates *et al.* (1973) equation. The total sugar content was determined utilizing the phenol–sulfuric acid method (Dubois *et al.,* 1956). One mL of leaf ethanolic extract was mixed with 1 mL of 5% phenol solution (w/v), followed by the addition of 5 mL of sulfuric acid (98%). The absorbance was measured at 490 nm by a spectrophotometer (JENWAY 6300, Staffordshire, UK); results were expressed in mg glucose on a fresh weight basis. Total phenols content were determined with the modified Folin–Ciocalteu method (Hmmam *et al.,* 2022). 1 mL of the leaf methanolic extract was mixed with 1 mL of Folin–Ciocalteu reagent, followed by the addition of 5 mL of 1M Na_2CO_3 (w/v), and 3 mL of distilled water. Samples were incubated at room temperature for 90 min in dark conditions, and the absorbance at 760 nm was measured by spectrophotometer (JENWAY 6300, Staffordshire, UK). The results were expressed as mg gallic acid (GAE) g^{-1} fw.

Statistical Analysis

The experiment was carried out in a randomized complete block design (CRBD) (Snedecor & Cochran, 1967); two-way analysis of variance (ANOVA) was performed using the Costat software package, to examine the effect of rootstock genotype and water regimes, and their interaction. The significant differences between treatments were assessed by means of multiple Duncan range test at significance level of 0.05 (Duncan, 1955). A hierarchical cluster analysis was performed using the ClustVis web tool (Metsalu & Vilo, 2015).

Results

Data presented in table (2) indicated that water level had an obvious effect on stem diameter of different grafted olive plants. In general decreasing water level gradually reduced stem diameter of the grafted olive plants. Coratina grafted on Sorani recorded the highest stem diameter (5.97mm), while Coratina grafted on Manzanillo recorded the lowest value (5.29mm). Also, data illustrated in table (2) showed that plant height was negatively affected by deficit water stress; shoot length declined in response to the reduction in water dose; Coratina grafted on Sorani recorded the highest plant height compared with other grafted olive plants, while Coratina grafted on Manzanillo recoded the lowest value. The water deficit treatments resulted statistically in a significant reduction in plant leaf area (Table, 3), the highest leaf area was recorded for the Coratina cv. grafted on Koroneiki (6.31 cm^2) followed by transplants grafted on Picual (6.08 cm^2), while grafting on Manzanillo rootstock recorded the lowest leaf area value (5.28 cm^2).

Table (2): Effect of water deficit level on stem diameter (mm) and plant height (cm) of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
Stem diameter (mm)						
100	6.92±0.31a	6.20±0.04b	5.92±0.07 bc	6.80±0.04 a	6.63±0.09 a	6.49 A
50	5.88±0.17c	5.67±0.04c	5.09±0.14 de	5.89±0.02 c	5.96±0.06 bc	5.70 B
25	5.08±0.18de	5.13±0.07de	4.87±0.27 ef	4.73±0.14 f	5.32±0.11 d	5.02 C
Mean B	5.96 A	5.66 B	5.29 C	5.81 AB	5.97 A	
Plant height (cm)						
100	101.0±0.0 b	107.3±3.68 a	95.5±1.22bc	93.3± 0.82c	108.6±3.30 a	101.16A
50	91.0±4.08c	79.6±1.70d	77.5±1.22 d	77.0±2.94 d	95.6±2.49 bc	84.16 B
25	79.0±6.53 d	75.3±0.94 d	65.5±0.41 e	64.0±4.03 e	74.6±4.99 d	71.70 C
Mean B	90.33 AB	87.44 B	79.50 C	78.11 C	93.00 A	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences. Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically different at $p < 0.05$.

Table (3): Effect of water deficit level on leaf area (cm^3), leaves number and total leaf area (cm^3), of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
Leaf area (cm^2)						
100	6.41± 0.22bc	6.88±0.03 a	5.66±0.11 de	6.56±0.09 ab	5.86±0.10d	6.27 A
50	6.06±0.47 cd	6.41±0.04 bc	5.35±0.02 e	6.02±0.14cd	5.73± 0.14de	5.91 B
25	4.82± 0.66f	5.64±0.02 de	4.83±0.15 f	5.66±0.06 de	5.62± 0.11de	5.31 C
Mean B	5.76 B	6.31 A	5.28 C	6.08 A	5.73 B	
Leaves number						
100	158.5±10.2 a	117.6±4.9 b	123.0±4.0 b	125.3± 2.0b	157.5±4.4 a	136.4 A
50	99.5±1.2 c	86.0±1.6 d	87.0±1.6d	64.0±2.4 ef	94.0± 3.2cd	86.1 B
25	71.5±2.8 e	70.0±3.8 e	73.0± 12.2e	54.5±3.0 f	65.5±4.4 e	66.9 C
Mean B	109.83 A	91.22 B	94.33 B	81.27 C	105.66 A	
Total leaf area (cm^2)						
100	1015.7±55.0 a	809.6±26.1 c	697.0±13.6 d	822.5±7.2 c	923.7±26.1 b	853.77 A
50	602.6±44.2 e	551.2± 9.17ef	465.1±10.4 fg	385.4±14.3 gh	539.0±28.0 ef	508.70 B
25	345.2±108.9h	394.7±24.2gh	352.6±70.5 h	308.1±27.5h	368.0±43.4 h	353.76 C
Mean B	654.54 A	585.21 B	504.96 C	505.37C	610.32 AB	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences. Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically different at $p < 0.05$.

Also, data presented in table (3) indicated that, significant differences were observed between the grafted olive plants in response to water deficit level; self-grafted transplants and Coratina grafted on Sorani recorded the highest leaves number and total leaf area, while Coratina grafted on Picual recorded the lowest leaves number. Regarding the effect of water stress, general decreasing water level gradually reduced leaf area of different grafted olive plants. The adverse effect of water deficit on shoot fresh and dry biomass production, as shown in table (4) olive shoot weight varied significantly according to the grafting combination and water deficit level; the self-grafted Coratina transplants recorded the highest significant value of shoot fresh and dry weight followed by Coratina grafted on Sorani with non-significant differences between them, while Coratina grafted on Manzanilo recorded the lowest values. In general decreasing water level gradually reduced shoot fresh and dry weight of different grafted olive plants. Data in presented in table (5) indicated the effect of water deficit level on root fresh and dry weight of grafting combinations; root fresh and dry weight was significantly higher in the transplants irrigated with 100% of FC compared to those irrigated with 50 or 25% of FC. Among the tested olive genotypes, Coratina transplant grafted on Koroneiki recorded the highest significant value (27.15 and 14.85g for root fresh and dry weight respectively), while Coratina transplant grafted on Manzanillo recorded the lowest value (18.89 and 11.4g for root fresh and dry weight respectively).

Table (4): Effect of water deficit level on shoot fresh and dry weight (g) of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
Shoot fresh weight (g)						
100	56.5±0.0 a	40.0±3.1 b	31.6±1.3 c	40.2±0.9 b	53.3± 2.6a	44.35 A
50	39.2±2.2 b	29.6±1.8 cde	25.7±0.2 fg	30.7±1.8cd	42.0± 0.3b	33.48 B
25	32.1±2.0 c	27.3±1.7 def	26.3±0.0efg	23.6± 0.8g	29.6± 2.2cde	27.81 C
Mean B	42.61 A	32.37 B	27.90 C	31.54 B	41.65 A	
Shoot dry weight (g)						
100	29.4±2.1 a	18.7± 1.1bc	18.3±0.4 bc	16.9±0.6 cde	28.8±1.2 a	22.50 A
50	17.6± 0.5bcd	15.6± 0.1ef	12.0±0.8 i	13.5±0.4 ghi	19.2±1.7 b	15.62 B
25	16.4±0.2 def	14.5± 0.3fgh	11.5±0.4 i	12.7±0.7 hi	14.8±0.5 fg	14.01 C
Mean B	21.19 A	16.31 B	13.98 C	14.40 C	20.99 A	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences. Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically different at $p < 0.05$.

Table (5): Effect of water deficit level on root fresh and dry weight (g) of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
Root fresh weight (g)						
100	21.9±1.0 def	35.2±1.2 a	20.8±1.4 ef	27.1±1.5b	23.8±2.0 cde	25.79 A
50	19.8±0.1 fg	26.7±1.6 bc	19.0±2.4 fgh	24.4±0.8bcd	20.9±0.3ef	22.18 B
25	16.3±0.8 h	19.4±3.0 fgh	16.8±2.0 gh	22.0±0.8def	16.9±0.8 gh	18.32 C
Mean B	19.37 C	27.15 A	18.89 C	24.52 B	20.55 C	
Root dry weight (g)						
100	10.3±0.1 gh	19.9±0.3 a	12.7±0.3 de	17.8±1.6 b	14.0± 0.6cd	14.96 A
50	10.5±0.2 gh	14.8±0.8 c	11.9±0.8 ef	14.9±0.8 c	12.3±0.7ef	12.93 B
25	8.68±0.4 i	9.7±1.2 hi	9.5±0.8 hi	12.2±0.8 ef	11.1± 0.8fg	10.26 C
Mean B	9.85 D	14.85 A	11.40 C	14.99 A	12.49 B	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences. Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically different at $p < 0.05$.

Data in presented table (6) indicated that Coratina transplant grafted on Koroneiki recorded the significantly highest root length value (50.77cm) followed by the self-grafted Coratina transplant (42.5cm), while Coratina transplant grafted on Manzanillo recorded the lowest value (34.05 cm). Root length was negatively affected with water deficit treatments. The recorded data showed that, exposure of olive transplants to water deficit stress significantly reduced shoot/root ratio of all grafting combinations; transplants irrigated with 100% of the FC recorded significantly higher values of shoot/root ratio (Table 6). However, transplants grafted on Sorani showed values similar to those of the self-grafted transplants. In general decreasing water level gradually reduced root length of different grafted olive plants. Data presented in table (7) indicated that the self-grafted Coratina transplants recorded the highest significant total biomass value followed by Coratina grafted on Sorani, while Coratina grafted on Manzanilo recorded the lowest value. In general decreasing water level gradually reduced plant total biomass of different grafted olive plants. In general decreasing water level gradually reduced total biomass of different grafted olive plants.

Table (6): Effect of water deficit level on root /shoot ratio, and root length of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
			Root length (cm)			
100	52.5±1.6 b	59.0±0.8 a	38.6±1.4fg	36.3±2.1 ghi	40.3±0.4 ef	45.36 A
50	42.0±1.6 de	48.3±2.6 c	32.5±0.4 kl	36.0±0.8 g-j	37.3± 1.8fgh	39.23 B
25	33.0±0.4 jkl	45.0±0.8 d	31.0±3.0 l	34.0±1.2 i-l	34.6±1.2 h-k	35.53 C
Mean B	42.50 B	50.77 A	34.05 D	35.44 D	37.44 C	
			Root/shoot ratio			
100	2.57±0.13 a	1.14±0.2 g	1.53±0.05 e	1.48±0.03 e	2.23±0.09 b	1.79 A
50	1.98±0.13 c	1.11± 0.01g	1.37±0.19 ef	1.26±0.07 fg	2.01±0.02 c	1.54 B
25	1.97±0.09 c	1.41± 0.08ef	1.56±0.15 de	1.07±0.01 g	1.75±0.02 d	1.55 B
Mean B	2.17 A	1.22 D	1.49 C	1.27 D	2.00 B	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences. Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically

Table (7): Effect of water deficit level on total plant biomass of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
			Total biomass (g FW^{-1})			
100	78.46±1.0 a	75.31±3.0 a	52.5±2.8 ef	67.31±2.5 b	77.13±4.5 a	70.14 A
50	59.03±2.1 cd	56.44±3.5 de	44.77±2.2 g	55.16±2.3 de	62.92± 0.7bc	55.66 B
25	48.45±2.5 fg	46.81±4.3 fg	43.15±1.9 g	45.74 ±1.6g	46.57±3.0 g	46.14 C
Mean B	61.98 A	59.52 A	46.80 C	56.07 B	62.20 A	
			Total biomass (g DW^{-1})			
100	39.80±2.2 b	38.73±0.9 b	31.12±0.3 d	34.81±2.2 c	42.86 ±0.9a	37.46 A
50	28.27±0.8 ef	30.53± 0.9de	23.95±1.6 g	28.46± 0.4ef	31.59±0.9 d	28.56 B
25	25.09±0.7 g	24.24±1.1 g	21.10± 0.7h	24.91±0.7 g	26.01±1.1 fg	24.27 C
Mean B	31.05 B	31.17 B	25.39 D	29.39 C	33.49 A	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically different at $p < 0.05$.

It has been reported in many studies that drought treatments reduced plant biomass in a number of plant species, this decrease could be related to stomatal closure during the high levels of stress. Exposure of olive transplants to water deficit stress significantly reduced plant photosynthetic pigments in all grafting combinations (Table 8). However, there were slight non-significant differences between the studied olive rootstocks in their behavior under water deficit conditions. Olive transplants grafted on Koroneiki and Picual recorded higher values of total chlorophyll than the other grafting combinations. Also, data presented in table (8) indicated that, proline content was increased linearly with increase water deficit treatments Coratina transplant grafted on Koroneiki recorded the highest proline followed by the self- grafted Coratina transplant, while Coratina transplant grafted on Manzanillo recorded the lowest value. According to data presented in table (9), differences in total sugar content was statistically significant; total soluble sugars accumulated gradually by increasing water deficit intensity, particularly of Coratina grafted on Koroneiki and Sorani compared with transplants grafted on Coratina and Manzanillo cvs. During exposure to water deficit, olive accumulate higher amount of total phenolic content, the highest significant total phenolic content was observed with Coratina scions grafted on Koroneiki and the lowest significant was observed with scions grafted on Manzanillo cv.

Table (8): Effect of water deficit level on total chlorophyll content (SPAD value) and proline concentration of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
Total chlorophyll content (SPAD value)						
100	73.4±2.6 abc	77.60±1.1 a	76.06±3.9 ab	76.73 ±0.6a	75.03±1.9 ab	75.76 A
50	68.45±3.9 c-f	69.63± 2.3b-e	71.4±5.7 a-d	71.36±2.6 a-d	68.5±0.9 c-f	69.87 B
25	64.6±1.3 efg	66.66± 5.7d-g	62.43±4.5 fg	65.16±0.3 d-g	61.13±0.6 g	64.00 C
Mean B	68.81 A	71.3 A	69.96 A	71.08 A	68.22 A	
Proline (µmoles g FW^{-1})						
100	12.71±0.8d	14.10±1.6 [illegible]	5.710±0.08 g	8.787±0.64 e	8.46±0.32 ef	9.953 C
50	13.09±0.81 d	15.20±0.81ab	5.130±0.08g	9.920±0.73 e	12.99±0.81 d	11.27 B
25	14.72±0.57 bc	16.28±0.08 a	7.170±0.13 f	13.29±0.16cd	13.10±0.8 d	12.91 A
Mean B	13.51B	15.19 A	6.003 E	10.67 D	11.52 C	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences. Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically different at $p < 0.05$.

Table (9): Effect of water deficit level on total phenolic and soluble sugars concentration of the grafted olive transplants.

Water level	Rootstock					Mean A
	Coratina	Koroneiki	Manzanillo	Picual	Sorani	
			Phenolic (mg g FW^{-1})			
100	50.33±4.19 ef	51.09± 0.8 de	45.67±0.24 gh	43.19±1.2 hi	42.15±0.08 i	46.48 C
50	54.13±1.63cd	54.61±0.46 c	45.14±0.27 ghi	53.85±3.7 cd	47.42±0.89 fg	51.03 B
25	54.04± 0.81cd	63.84±0.8 a	46.52±0.32 gh	54.99±0.69 c	59.56±0.32 b	55.79 A
Mean B	52.83 B	56.51 A	45.78 D	50.68 C	49.71 C	
			Total sugars (mg. g FW^{-1})			
100	103.7±0.8 i	109.6±5.6 gh	87.41±0.8 j	107.0±0.8 h	113.7±0.8 ef	104.3 C
50	88.14±0.8 j	123.7±1.3 c	100.6±0.8 i	117.1±1.6 d	114.5±0.7 def	108.8 B
25	112.9±1.1 ef	143.7± 1.2 a	112.1±0.81 fg	116.1±1.63 de	128.0±0.8 b	122.6 A
Mean B	101.6 D	125.6 A	100.0 D	113.4 C	118.7 B	

Interaction values (treatment ×rootstock genotype) followed by different lowercase letters indicate statistical differences. Mean values of treatment or rootstock genotype followed by different uppercase letters are statistically different at $p < 0.05$

Heat-map and hierarchical clustering

The hierarchical clustering and heat map (Fig. 1), showed the change in the measured parameters of different genotypes under both normal (100%) and sever water deficit (25%). Based on the observed variations, the measured traits were grouped into number of row clusters; cluster-A includes total sugars, proline and total phenolic; in comparison with non-stressed condition, parameters of cluster-A increased in water deficit treatments, a positive correlations were found between different shoot growth parameters (plant height, stem diameter, leaf area, number of leaves shoot and root weight and total plant biomass), which were assembled in the cluster-B. Finally, cluster-C comprises root length, root fresh and dry weight, and total leaf area. Parameters of cluster-B and C showed a decreasing trend in water-stressed transplants. Heat map represent the performance of olive genotypes in both normal and water deficit conditions; olive genotypes were grouped into two main column-clusters each represents water level treatment; each column-cluster divided into sub-clusters consisted of cultivars with closely associated behavior, in comparison with non-stressed condition, olive genotypes behavior were changed dramatically under water deficit condition; Picual, Koroneiki and Sorani showed similar behavior, the higher tolerance was reflected by maintenance of relatively higher values of the measured parameters, while Manzanillo showed lower values of most measured parameters.

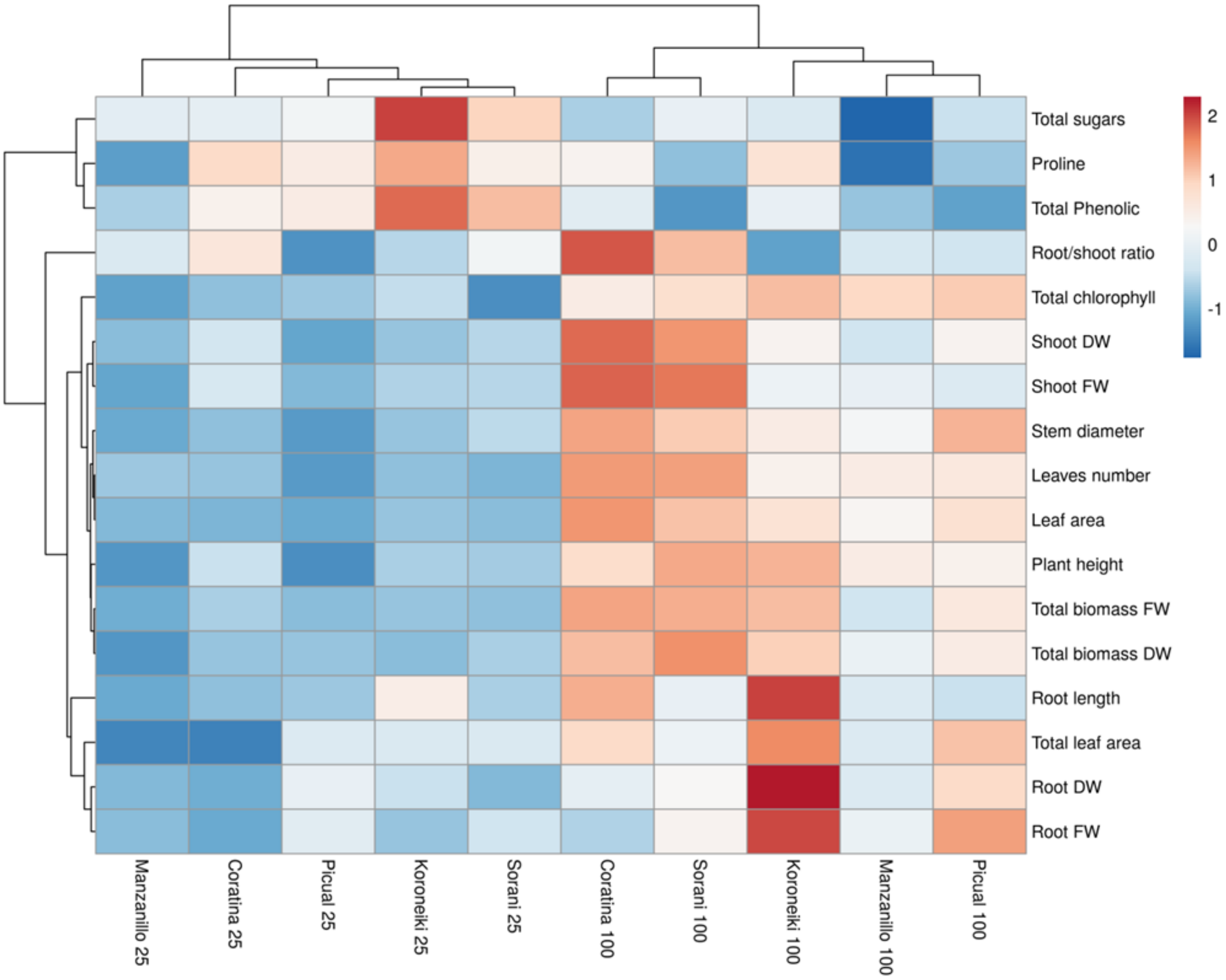

Fig. (1): Heat map and hierarchical clustering illustrating the associations among olive genotypes and different measured variables under different water regimes. Columns represent olive genotypes and water deficit, whereas each row represents a measured variable; red color indicates a higher relative value, and blue color indicates a lower one.

Discussion

This study was conducted to characterize the change in growth parameters of grafted olive transplants in response to water deficit stress. The obtained results reveal a significant reduction in different growth parameters *i.e.* shoot length, shoot fresh and dry weight, and root fresh and dry weight under water deficit conditions, the reduction was gradually in response to severity of water stress. Moreover the variations in growth measurements of olive shoots and roots were highly dependent on rootstock genotype. Drought stress leads to a series of morphological, physio-chemical, and molecular changes that affecting plant growth and productivity (Ahmad *et al.,* 2014). Previous studies showed that, responses of olive to drought include reducing leave area and number, slowing growth rate, increasing root density and increasing root-to-shoot ratio (Ben-Gal *et al.,* 2010; Abdallah *et al.,* 2018; Hegazi *et al.,* 2018). It is known that drought stress suppresses shoot growth in different crop plants, which is primarily ascribed to the reduction in moisture content, cell expansion, carbon assimilation and biomass accumulation. Plant growth depends on both of cell division and cell enlargement, which are highly sensitive to the reduction in turgor pressure; cell elongation can be inhibited by interruption of water flow from the xylem to the surrounding tissues. The reduction in cell elongation result in reduced plant height, leaf area and shoot growth under drought. (Ahmad *et al.,* 2014; Kumar *et al.,* 2017; Hegazi *et al.,* 2018; Trabelsi *et al.,* 2019; Gupta *et al.,* 2020; Zia *et al.,* 2021). Moreover, analyses of transplant growth behavior showed that the tolerant olive rootstocks (Picual and Koroneiki cvs.) tend to increase the root system growth at the expense of shoot mass under water deficit condition, the relatively expanded root system, enable the plants to absorb higher amount of water from the soil (Eziz *et al.,* 2017). Most research on how olive trees respond to water shortage stress has focused on growth-related aspects, photosynthetic activity and accumulation of osmolytes such as sugars and proline (Ahmed *et al.,* 2009; Dichio *et al.,* 2009; Abdallah *et al.,* 2018). There are reports showing the decrease in chlorophyll content under drought stress (Abdallah *et al.,* 2018; Hegazi *et al.,* 2018). Under drought stress, proteolytic enzymes *i.e.* chlorophyllase, showed higher activity, which is responsible for the chlorophyll degradation (Baccari *et al.,* 2020; Boussadia *et al.,* 2023). Moreover, the maintenance of relatively higher chlorophyll content may represent an adaptive feature to cope with drought stress in some olive genotypes (Boussadia *et al.,* 2023). Picual, Koroneiki and Sorani cvs., tended to accumulate higher amount of total sugar, phenols, and proline content; differences in total sugar content of olive genotypes under different water regime treatments were significant; total sugars accumulated gradually by decreasing the water amount. Generally, plants accumulate osmolytes, such as soluble sugars and amino acids, in response to water deficit stress to maintain cellular osmotic balance (Rhodes *et al.,* 2002). Proline is commonly accumulated in plants in response to water stress (Reddy *et al.,* 2004). Proline plays an important role in osmotic regulation, reduces oxidative damage and maintaining of water uptake and photosynthetic activity (Hasegawa *et al.,* 2000). More recently, it has been demonstrated that the accumulation of phenolic compounds is a well-known adaptive mechanism of olive tree against drought conditions (Petridis *et al.,* 2012; Cetinkaya *et al.,* 2016). The accumulation of phenolic compounds induced by water stress in plants has been shown to play an important role in cellular protection and scavenging of ROS that are produced under stress conditions (Nakabayashi & Saito, 2015; Falahi *et al.,* 2018). Recently much attention has been drawn to utilization drought-tolerant genotypes to achieve relatively high yields under drought conditions (Bolat *et al.,* 2014; Galindo *et al.,* 2018; Anđelković *et al.,* 2020). Interestingly, grafting has a potential to improve plant drought resistance through the use of drought-resistant rootstocks (Yang *et al.,* 2022), hence grafting is a potential approach that can mitigate negative stress effects and improve crop performance and ensure a sustainable crop production under stress conditions (García-Sánchez *et al.,* 2007; Nawaz *et al.,* 2016; Balfagón *et al.,* 2021). Rootstocks may significantly influence the tree performance by affecting water balance and influencing the eco-physiological behavior of the grafted scion (Marguerit *et al*., 2012). The obtied results showed that, Picual, Koroneiki and Sorani have higher tolerance to water deficit stress which was reflected by maintenance of relatively higher values of the measured parameters, while Manzanillo showed lower values of most measured parameters.

Conclusion

It could be concluded from the obtained results, that water deficit had an adverse effects on different grafted olive plants under investigation Grafting could be an effective tool to mitigate negative impacts of drought stress on olive transplants growth. Coratina transplants grafted on Sorani and Koroneiki may be considered as relatively tolerant to water deficit compared with transplants grafted Manzanillo. Our research results shed light on the importance of grafting on tolerate rootstocks is a potential approach to mitigate negative impacts of drought stress, which has become a significant problem in the arid and semi-arid regions.

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Contributions of authors

E.S.H., Supervision, conceptualization, reviewing.

A.M.A.A., Visualization, investigation, review and editing.

A.A.A., Resources, Methodology, Investigation, Formal Analysis, Writing original draft. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

The authors declare no conflict of interest

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استجابة شتلات الزيتون المطعم (*Olea europaea* L. Cv. Coratina) لظروف نقص المياه

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قسم بساتين الفاكهة، كلية الزراعة، جامعة القاهرة، مصر

المستخلص: يعد اجهاد الجفاف اكثر الاجهادات الغير حيوية ضررًا للمحاصيل الزراعية وخاصة مع تزايد نقص المياه بسبب التغيرات المناخية العالمية والتى تؤثر سلبًا على نمو المحاصيل وإنتاجيتها. يعد التطعيم على الأصول المتحملة أحد الحلول الواعدة للتخفيف من الآثار السلبية لإجهاد الجفاف وضمان استدامة الانتاج بشكل جيد. أجريت هذه الدراسة لمعرفة تأثير الإجهاد المائي على نباتات الزيتون صنف كوراتينا المطعمة على الأصناف التالية كأصول (كوراتينا، كوروناكي، منزانيللو، بيكوال وصوراني) مع استخدام ثلاثة مستويات للري وهى كالتالى (١٠٠ ٪ و٥٠ ٪ و٢٥٪) طبقا للسعة الحقلية للتربة لتقييم أثر الاجهاد المائى على النباتات المختبرة. أدى نقص الماء إلى انخفاض نمو المجموع الخضري وقطر الساق وعدد الأوراق ومساحتها ووزن المجموع الخضري والجذري. أظهر تحليل الأوراق انخفاضاً ملحوظاً في محتوى الكلوروفيل الكلي بينما زاد محتوى البرولين والسكريات الكلية والمواد الفينولية مع زيادة الاجهاد المائي. النباتات المطعومة أظهرت استجابات مختلفة تحت تأثير معاملات الاجهاد المائى المختلفة حيث سجلت نباتات الزيتون المطعومة على كلا من أصل الصوراني وأصل الكورونيكي قيم أعلى لمؤشرات النمو وتراكمت كمية أعلى من (البرولين والسكريات الكلية) والمواد الفينولية مقارنة بنباتات الزيتون المطعومة الأخرى.

الكلمات المفتاحية: الزيتون، الجفاف، الاجهاد المائي، التطعيم، الاصول.