



ORIGINAL ARTICLE

Changes in Drought Tolerance Mechanism at Different Times of Stress and Re-hydration in Hybrid Pistachio Rootstock

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ABSTRACT

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Drought is one of the main important adverse environmental events, certainly has an impact on plant growth and development. Pistachio is cultivated in areas where soil water deficits and salinity conditions are higher than normal. In most orchards, deficit irrigation is a common practice. There is only limited understanding of the physiological mechanisms pistachio uses to survive in drought. Many adaptive strategies have been developed in plants for dealing with water stress. The objective of the present study was to evaluate the effect of drought stress on the photosynthetic, physiological and biochemical parameters in one-year-old seedlings of 12 pistachio hybrids. Therefore, a greenhouse experiment was carried out to assess the effects of two drought stress treatments (drought stress and full-irrigation) for 40 days with a subsequent two weeks' recovery period and several parameters (pigments (total chlorophyll, anthocyanins and carotenoids), internal CO₂ concentration (Ci), chloride (Cl⁻) ions and fluorescence parameter) were evaluated at four different times (beginning, middle and end of stress and then recovery). After the end of the stress period, the seedlings were irrigated for two weeks. Results revealed that the drought stress treatments led to a change in the studied parameters and the mechanism of drought tolerance was variable at different times. *Pistacia atlantica* hybrid, *P. vera* "Sarakhs" and *P. vera* "Shasti" hybrid rootstocks had the highest water use efficiency; *P. vera* "Sarakhs" hybrid, *P. vera* "Khanjari" hybrid and *P. vera* "Badami" hybrid had the highest Cl⁻ and mesophyll efficiency. *P. atlantica* and *P. vera* "Khanjari" had the highest anthocyanins and carotenoids. *P. vera* "Shasti", *P. vera* "badami", *P. mutica* and *P. mutica* hybrid rootstocks have been able to withstand drought stress by increasing the amount of K⁺ ion and maintaining gas exchanges. The results also showed that the response of the rootstocks to rehydration was different. Seedlings that recovered well after rehydration had a higher tolerance threshold. The "Khanjari" cultivar was recovered better than the others.

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Introduction

Drought stress is one of the general environmental factors limiting plant growth and development, especially in arid and semi-arid areas, which leads to many morphological, physiological, biochemical and metabolic changes in plants (Gorai *et al.*, 2015). Bagheri *et al* (2012) reported plant water stress may happen as a consequence of a cyclic reduction in available soil water, developing in the long term, or may result from drought spells. The timing, intensity and duration of water delicate are fundamental factors to assign the effects produced by drought (Lotfi *et al.*, 2019).

Plants have developed several apparatuses to prevent or partially reduce the destructive effects of water stress (Kalaji and Loboda, 2009). On the other hand, plants can respond to drought stress by morphological and physiological changes (Zokaee-Khosroshahi *et al.*, 2014). In fact, the plant responds to water deficit with a rapid closure of stomata to avoid further loss of water through transpiration (De Souza *et al.*, 2013; Asayesh *et al.*, 2021) and then photosynthesis reactions occur (Zlatev and Yordanov, 2004). The reduction in rate of photosynthetic under drought stress may result from biochemical and stomatal limitations (Flexas and Medrano, 2002; Babaei *et al.*, 2021). Esmailpour *et al* (2016) reported that the number, length and width of stomata were strongly affected by drought stress and the response of different cultivars was different.

There are many studies that confirm chlorophyll fluorescent characteristics are advisable to determine the performance of plant photosynthetic system under water deficit conditions (Hailemichael *et al.*, 2016; Ebrahimpour *et al.*, 2020). Under drought stress, the leaf water status affects photosynthesis rate by demolition of the efficiency of the photosystem II (PSII) function (Fini *et al.*, 2013; Afrousheh and Javanshah, 2020). Chlorophyll fluorescence studies have long been used as an indicator of functional changes of PSII photochemistry under drought stress (Ranjbar-Fordoei,

2019). For example, the intensity of drought stress in most studied plants was significantly related to the maximum quantum efficiency of PSII (Fv/Fm) and effective quantum yield of PSII (Φ_{PSII}) (Šajbidorova *et al.*, 2015). Fv/Fm is the most important property used for the evaluation of plant's response to water deficient (Umar *et al.*, 2019). Ranjbar (2017) described that in response to water deficit, the maximum quantum yield of PSII photochemistry and quantum yield baseline of both rootstocks (*P. atlantica* and *P. khinjuk*) showed a clear gradient from low values in without water deficit to high values in severe water deficiency.

Growth, development and water regulation are the main functions of mineral nutrients in plants (Goldstein *et al.*, 2013; Behrooz *et al.*, 2019). Long-distance water transport in plants can be adjustment by changing the concentration of cations in the xylem sap (Nardini *et al.*, 2011). The ionic effect also has a valuable function in adjusting the transport of nutrients and water to different plant parts and in regulating resistance to drought and saline stress (Sellin *et al.*, 2010; Karimi and Rahemi, 2012). Mineral deficiency due to physiological and biochemical functions leads to growth inhibition and reduced crops (Liang *et al.*, 2018).

The amount of both chlorophyll (a and b) changed under-water stress (Farooq *et al.*, 2009). The carotenoids play vital functions and help plants to resist drought stress (Ranjbar, 2017). Ranjbar (2017) reported that in all studied seedlings, water deficit induced a dramatic lessening in pigments (chlorophyll and carotenoids).

Several researches have exposed that after re-irrigation, leaf water potential recovers before the physiological components (Gorai *et al.*, 2015). Although stressed plants have different mechanisms to resistant stress, the potentiality to recovery is a vital factor of plant's response (Miyashita *et al.*, 2005). Even in irrigated orchards, some periods of water stress could

occur due to water scarcity or deficit irrigation schedules (El Mokh *et al.*, 2021). The selection of suitable rootstock is important and necessary, especially in an arid and semi-arid region; however, our knowledge about the impact of rootstocks on the water relations of pistachio trees is limited. Pistachio is an important crop in Iran, which recently; its cultivated area has exceeded 500,000 hectares in Iran (Agri-Jihad, 2019). Pistachio is considered as a drought-resistant crop (Rieger, 1995), but in recent years, declining water has reduced crops. The purpose of this study was the investigation of twelve pistachio rootstocks (two equal groups including open (O) and close (H) pollination) response to water stress.

Materials and Method

Plant material and experimental set-up

The experiments were performed in a greenhouse of the Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran (35°44'27.70" N 51°09'55.79" E) from

March 2017 to July 2019. 12 one-year-old pistachio hybrid seedlings (*P. atlantica* × *P. integrima*; *P. vera* L. cv. Badami–Rize–Zarand × *P. integrima*; *P. mutica* × *P. integrima*; *P. vera* L. cv. Khanjari × *P. integrima*; *P. vera* cv. Sarakhs × *P. integrima*; *P. vera* cv. Shasti × *P. integrima* and open pollination with *P. vera* L.) were used (Table 1). We performed this hybridization in the crossbreeding program (Close and Open Pollination) at the Iranian Pistachio Research Institute (IPRI) in 2017. The seeds (20 seeds per treatment) sown in a plastic pot (30 cm upper diameter, 20 cm lower diameter, and 40 cm high) containing perlite and 3 months' later seedlings were transplanted into 11L Pots. The pots were filled with a loamy soil, (silt, sand and clay) with the ratio of 40:40:20 respectively in Table 2. The main factor was the rootstock and the secondary and third factors were irrigation regime and times (start, middle, end of stress and 2 weeks after rehydration). The irrigation regime treatments were full irrigation (Control) and no irrigation (40 days) and then recovery (from 40th to 60th day).

Table 1. Abbreviation of studied pistachio rootstock.

No.	Open Pollination with <i>P. vera</i> pollen	Abbreviation	No.	Close Pollination with <i>P. integrima</i> pollen	Abbreviation
R1	<i>P. atlantica</i>	atlO	R2	<i>P. atlantica</i> × <i>P. integrima</i>	atlH
R3	<i>P. vera</i> L. cv. Badami–Rize–Zarand	badO	R4	<i>P. vera</i> L. cv. Badami–Rize–Zarand × <i>P. integrima</i>	badH
R5	<i>P. mutica</i>	mutO	R6	<i>P. mutica</i> × <i>P. integrima</i>	mutH
R7	<i>P. vera</i> L. cv. Khanjari	khaO	R8	<i>P. vera</i> L. cv. Khanjari × <i>P. integrima</i>	khaH
R9	<i>P. vera</i> cv. Sarakhs	sarO	R10	<i>P. vera</i> cv. Sarakhs × <i>P. integrima</i>	sarH
R11	<i>P. vera</i> L. cv. Shasti	ShaO	R12	<i>P. vera</i> L. cv. Shasti × <i>P. integrima</i>	ShaH

Table 2. Analysis of soil used in 11liter pots.

EC (μmoscm^{-1})	pH	O.M %	O.C %	Clay %	Silt %	Sand %	Texture	N (%)
5.9	7.3	43.01	15.37	20	40	40	SL	0.12
P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	B (ppm)
5	150	6.4	6.1	2.9	1.1	0.7	2.5	65

Greenhouse conditions

Plants were located in the greenhouse with a relative humidity of $45 \pm 5\%$ and temperature of $30/25^\circ\text{C}$ (day/night). During this period, seedlings were irrigated with a Hoagland solution for 60 days. The nutrient solution ($\text{pH } 6.5 \pm 0.1$) was renewed every 3 days and the substrate was moderately rinsed with distilled water to prevent accumulation of nutrients.

Irrigation regimes

The canopy volume of assessed seedlings was not measured, but based on our observations; seedling's canopies volume was very similar. The water stress treatments were started 60 days after transplanting and maintained for 40 days and rehydration for 2 weeks. Control seedlings were irrigated until slight drainage occurred. Trees under drought stress treatment were not irrigated until 40 days. After the stress period, plants were re-irrigated up to field capacity. 4 replications (each rep. including 10 Samples) were used for each treatment (drought stress and full irrigated).

Chlorophyll fluorescence

The chlorophyll fluorescence parameters including, minimum fluorescence (F_0); maximum fluorescence of the dark-adapted leaf (F_m), variable fluorescence (F_v), and the maximum quantum yield of primary photochemistry (F_v/F_m) were recorded using a Handy Plant Efficiency Analyzer (PEA, Hansatech, USA) according to the method described by Havaux (1993). The selected mature leaves from the middle of the shoot were dark-adapted using leaf clips. After preconditioning of leaves in the dark for 20 min, a saturating flash-light of $3,000 \mu\text{mol m}^{-2} \text{s}^{-1}$ (photon) was applied to achieve the maximum fluorescence.

Gas exchange analysis

Gas exchange analysis was carried out on the five youngest, fully expanded and mature leaves, from the middle of the main shoot, using the LCI portable device of photosynthesis (ADC Bio Scientific Ltd., USA). Measurements were performed during the time period from 10:00 to 12:00 o'clock, at natural full light intensity. Finally, the rate of net photosynthesis (P_n , $\text{mol m}^{-2} \text{s}^{-1}$), the stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), the transpiration rate (E , $\text{mol m}^{-2} \text{s}^{-1}$), internal CO_2 concentration (C_i , $\text{mol m}^{-2} \text{s}^{-1}$) were Measured. Instantaneous water uses efficiency (A/E), intrinsic water uses efficiency (A/g_s), mesophyll efficiency (A/C_i), were calculated as fractions of net photosynthesis rate and transpiration rate, net photosynthesis rate and stomatal conductance, net photosynthesis rate and internal CO_2 concentration, respectively (Maseyk *et al.*, 2008).

Mineral nutrients

To determine the concentration of minerals in a plant, 1g of frozen samples was completely pulverized and ashed at 550°C for 10 h and HCl was used to completely dissolve the ash. Inductively coupled plasma atomic emission spectrometry (ICP-AES; Maxim-III, Applied Research Laboratories, Switzerland) was used to determine the concentration of potassium (K^+), sodium (Na^+), magnesium (Mg^{2+}) in digest solutions (Dahlquist and Knoll, 1978) and Cl^- by precipitation titration with silver nitrate by Mohr's method (Azevedo Neto and Tabosa, 2001).

Pigments

Total chlorophyll (TChl: Chla+b), carotenoid (carts) and anthocyanin (anths) were measured spectrophotometrically (Itd T80+ UV/VIS; PG Instruments, Leicestershire, UK). TChl and carotenoid contents were determined according to Arnon (1967)

and the determination of anthocyanin contents was carried out using the method of Wagner (1979). The absorbance reading was taken at 663 nm for chlorophyll a, 645 nm for chlorophyll b, 470 nm for carotenoid and 510 nm for anthocyanin.

Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) procedures according to a completely randomized split plot design with 4 replicates with the SPSS (Statistical Package for the Social Sciences, SPSS Institute Inc., Cary, NC, USA) base 25 software. Mean comparisons were made using Duncan's multiple range test. Pearson correlation coefficient, dendrogram clustering and principal component analysis (PCA) were performed using R v3.4.3 (www.r-project.org). Means are presented with the standard errors of the mean and significance expressed at $p < 0.05$.

Results

Gas exchange and net photosynthesis rate

Net photosynthesis rate

The results showed that interaction between water treatment and rootstocks was significant during the time ($P < 0.05$). Significant differences were observed between stress and control treatments and the photosynthesis rate (Pn) was lower in pistachio under-drought stress at the end of stress period but after 15 days of rehydration, the improvement in Pn was accompanied especially in *khaO* ($P < 0.01$). The Pn in hybrid pistachio was 10% and 37% higher than non-hybrid at the end of stress and recovery, respectively (Fig.1).

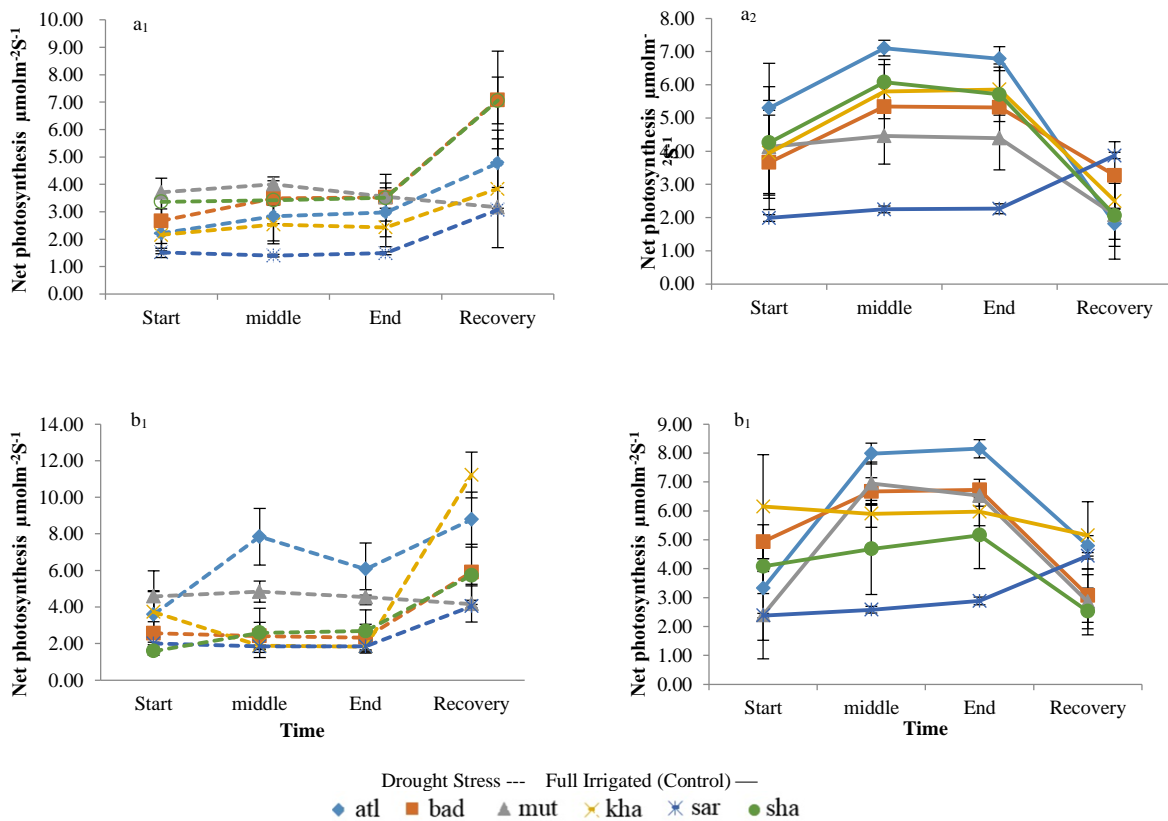


Fig. 1. Effect of water stress and rehydration on net photosynthesis (A) of irrigated (—) and stressed (---) for 40 days period on hybrid (a₁ and a₂) and non-hybrid (b₁ and b₂) pistachio Seedling.

Transpiration

Among all treatments, significant differences were found in the interaction of time and drought stress treatment. Leaf transpiration (E) was also measured on the same dates in Pn. The results showed that the amount of E also decreased with increasing in measurement times. The minimum E values during the

stress period were observed at the end of the water stress in *KhaO*. During recovery, E values in drought-stressed treatments were significantly lower compared to their respective controls, whereas no significant differences were found between hybrids and non-hybrids (Fig.2)

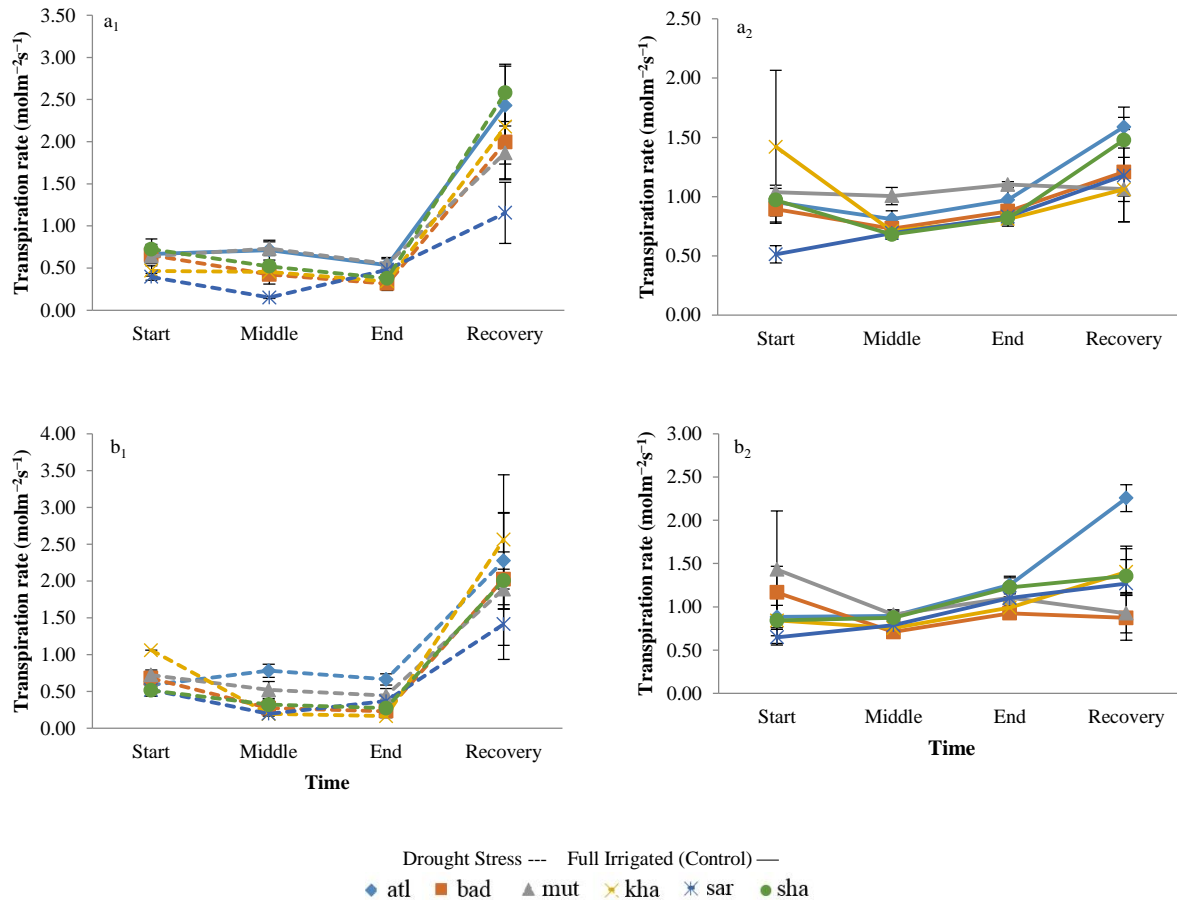


Fig.2. Changes in transpiration (E), under irrigated (—) and stressed (---) for 40 days period on hybrid (a₁ and a₂) and non-hybrid (b₁ and b₂) pistachio Seedling.

Stomatal conductance (gs)

For stomatal conductance (gs), a significant difference was observed between rootstocks and water treatment (full irrigation and drought stress) in all of the time. The patterns of gs rate were in some way similar to those of E. According to the mean comparison (Fig.3), the amount of gs was reduced with induction of drought treatments compared to the control condition in all studied pistachio rootstocks. The lowest values had

reached the end of drought stress, and *khaH* and *sarO* showed the least values but after rehydration, an appreciable recovery of gs rate was observed in all rootstocks especially *mutO*. However, for stomatal conductance rate in the control plant did not reach the same value as the drought stress plants upon rehydration and the highest gs were obtained in *mutO* and *badH*.

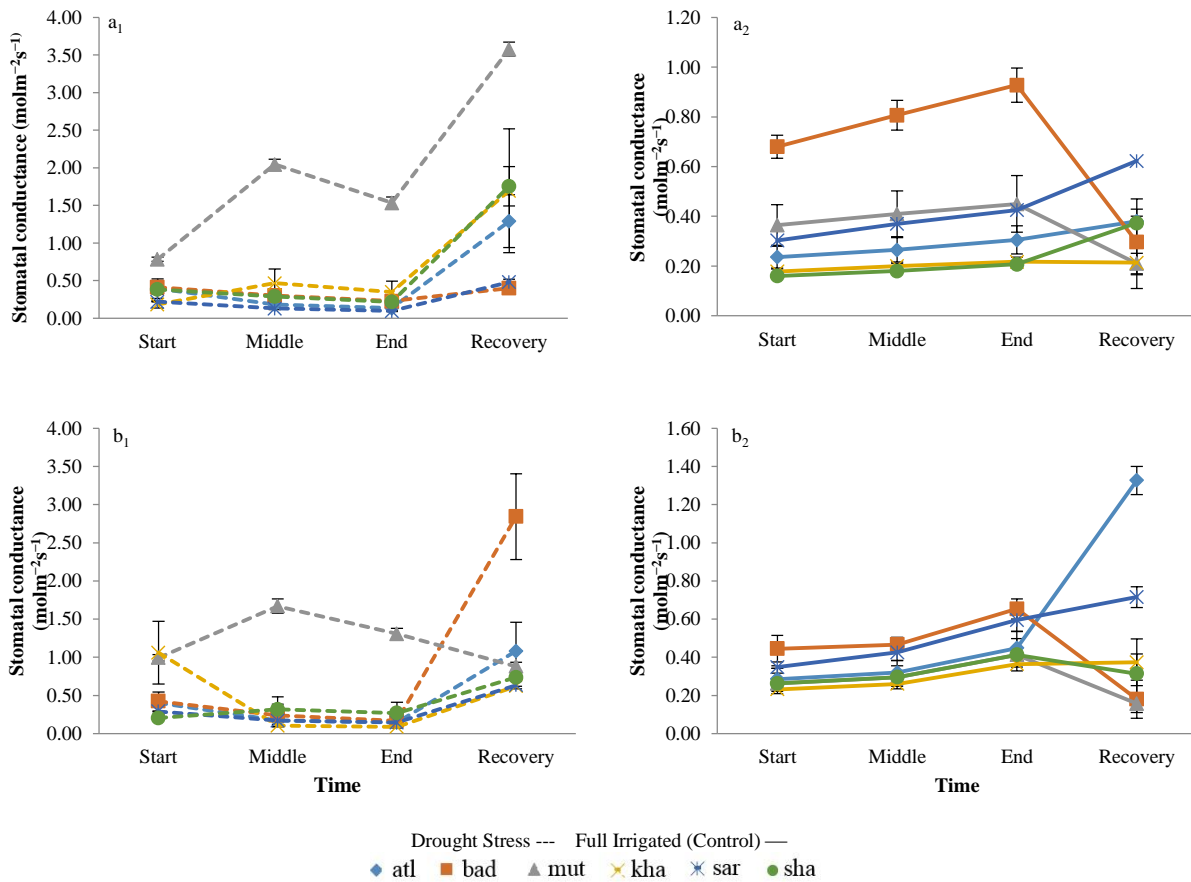


Fig.3. Amount of stomatal conductance at different drought stress after a 40-days period followed by a two weeks' recovery period in both irrigated (—) and stressed (---) on hybrid (a₁ and 2) and non-hybrid (b₁ and 2) pistachio Seedling.

Intrinsic and instantaneous water use efficiency (WUE)

Both intrinsic (Pn/gs) and instantaneous (Pn/E) WUE were significantly affected by the interaction of rootstocks and drought stress ($P < 0.01$). Under drought stress both WUE (intrinsic and instantaneous) increased for most rootstocks (Fig 4). In drought stress conditions, increased percentages in WUE (Pn/E) were 12 % and

44 % in hybrids compared to non-hybrids at the end days and after recovery, respectively. After 15 days of recovery there was still a significant difference between plants that had been subjected to the drought stress so that the highest and lowest WUE values were recorded in *KhaH* and *sarO*, respectively (Fig.4).

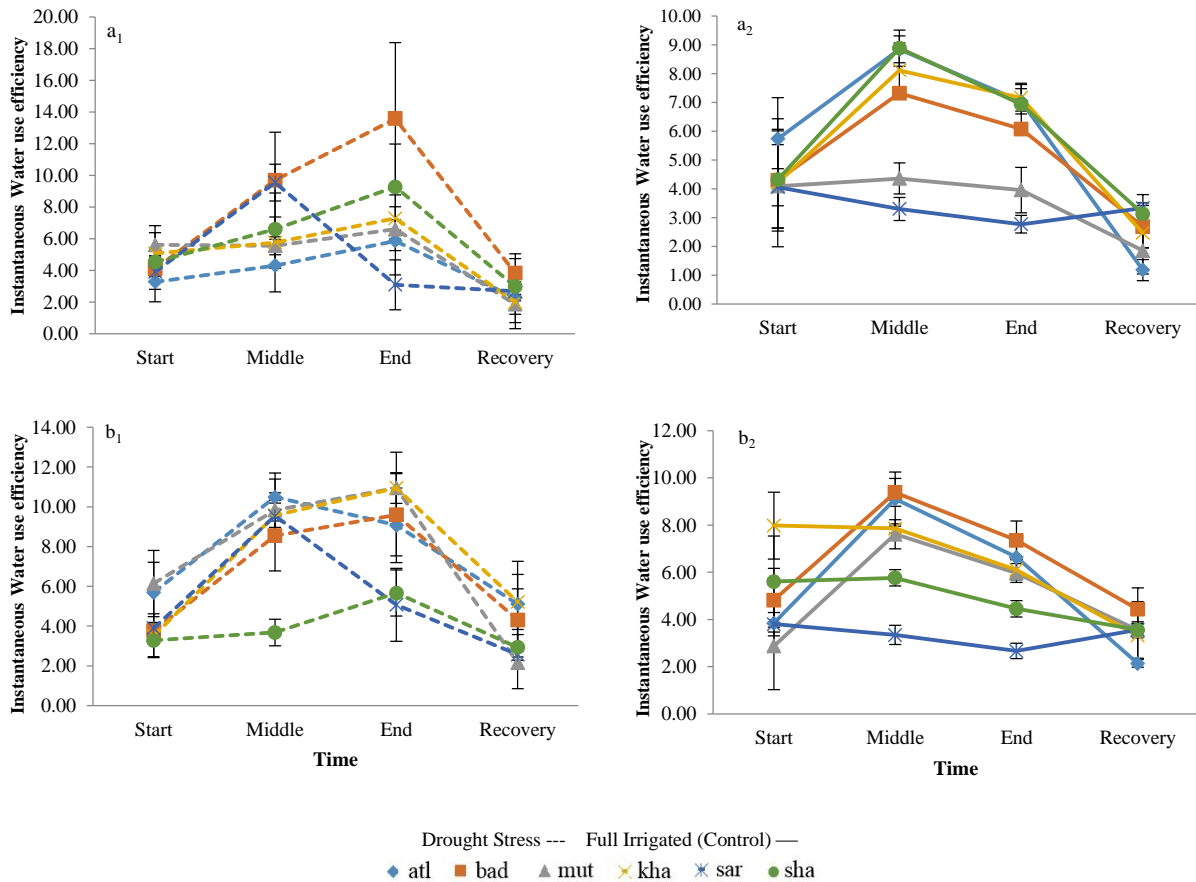


Fig.4. Change in instantaneous water use efficiency of hybrid (a₁ and a₂) and non-hybrid (b₁ and b₂) pistachio rootstock subjected to a 40-days period of stressed (---) and full irrigated (—) and a two weeks' period of recovery.

Mesophyll efficiency and internal CO₂ Concentration (Ci)

In the most studied species, water stress-induced a decrease in mesophyll efficiency. However, mesophyll efficiency at different measurement times showed significant differences between species and water stress treatment ($p < 0.01$). The most important reductions

were observed at the end of the water stress period while after rehydration, mesophyll efficiency reached values maximum rate except *mutO*. The highest recovery was in *khaO* (Fig.5).

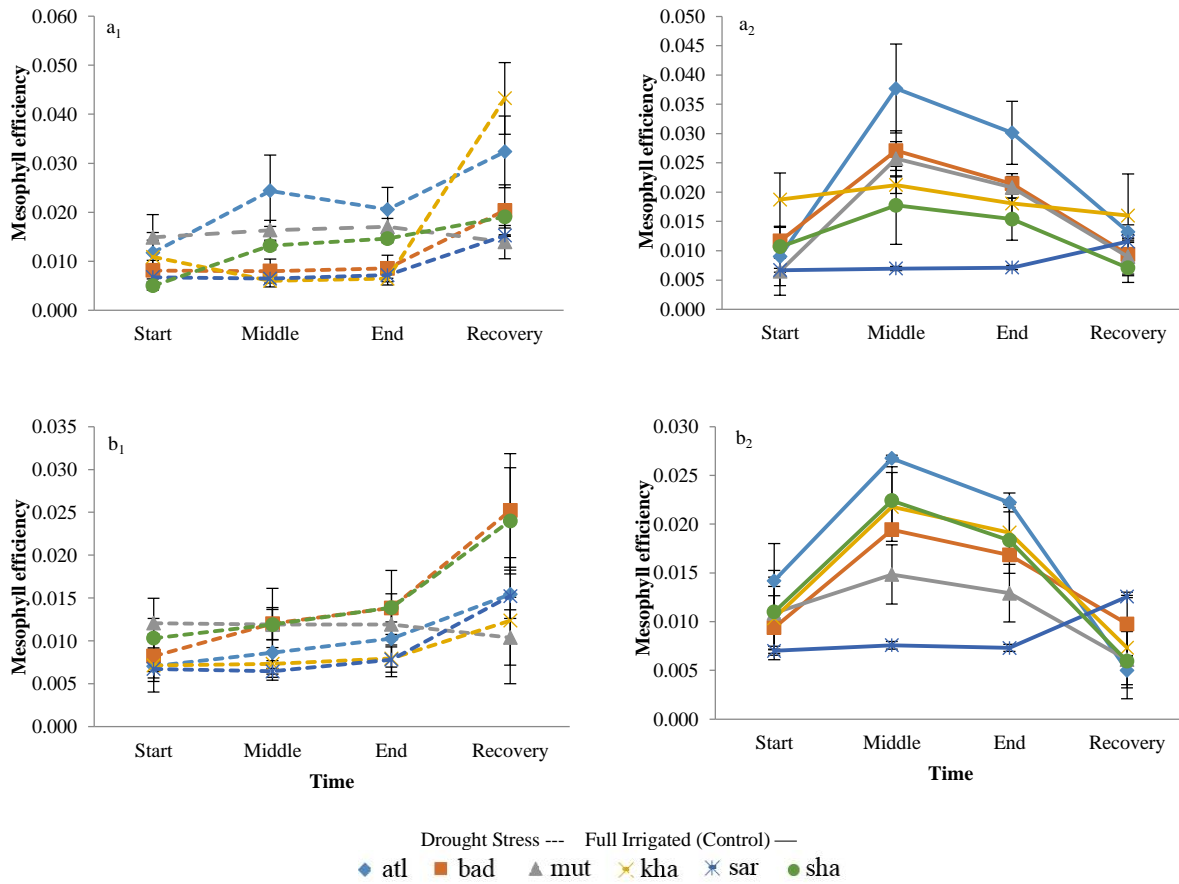


Fig.5. Changing ratio of A/Ci (mesophyll efficiency) for 12 pistachio rootstock under drought stress (---) and full irrigation (—). a₁ and a₂: hybrid and b₁ and b₂: non-hybrid;

Fluorescence parameters

In response to water stress, the maximum quantum yield of PSII (F_v/F_m) of some rootstocks showed a clear gradient from low values in middle drought stress to high drought stress. However, the development of F_v/F_m in response to the water deficit was quite similar

between the three rootstocks. In drought stress, in all rootstocks except *atlO*, the amount of F_v/F_m decreased with increasing stress. After recovery in rootstocks *khaO*, *sarO* and *shaO* this amount increased, but decreased in others (Fig.6 and Table 3).

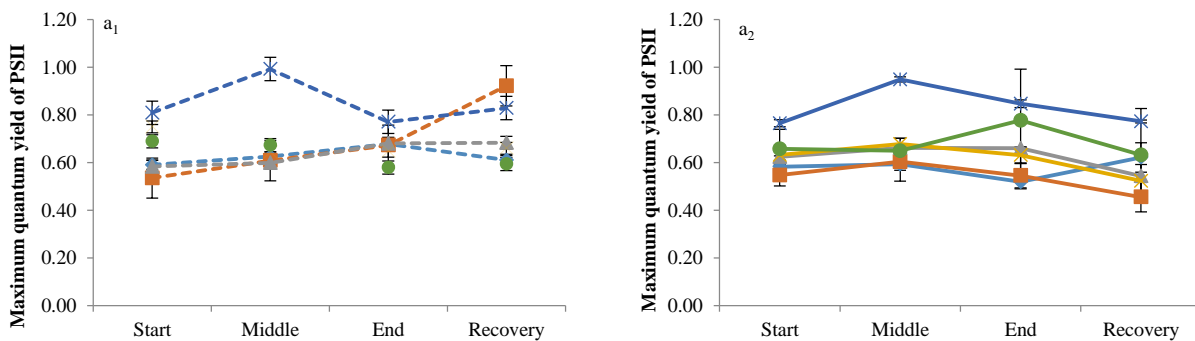


Fig.6. Trend of Maximum quantum yield of PSII (F_v/F_m) of hybrid (a₁ and a₂) and non-hybrid (b₁ and b₂) pistachio rootstock under drought stressed (---) and full irrigated (—) for 40 days and a two weeks' period of rehydration.

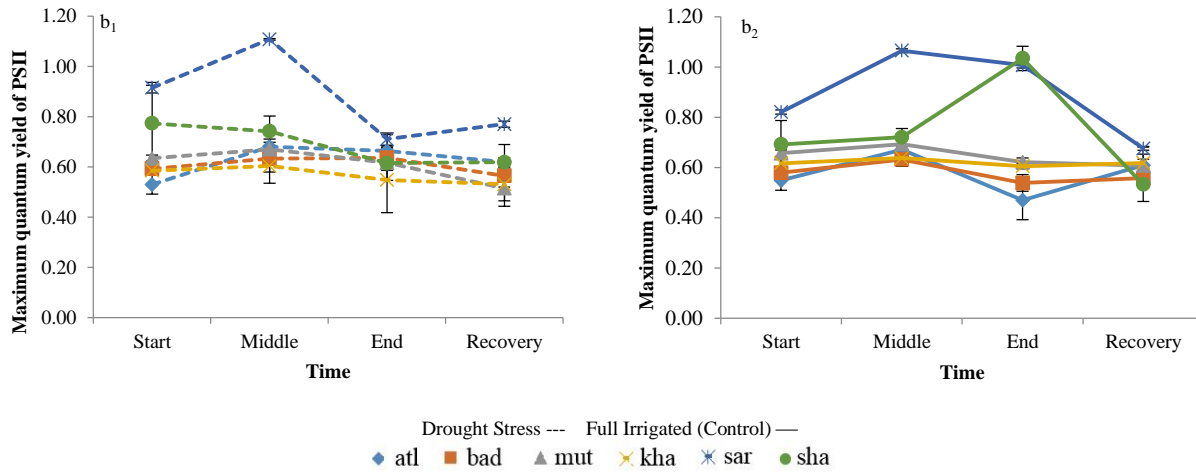


Fig.6. Contineud.

Table 3. Mean of some fluorescence parameters of 12 studied pistachio rootstock under normal (full irrigation) and drought condition.

Rootstocks	Treatment	Fv	Fm	F ₀	Fv/F ₀	F ₀ /Fm
<i>atlO</i>	Stress	17.9± 0.99 abc	27.9± 1.2 ab	10.1± 0.60 abcd	1.8± 0.07 b	0.39± 0.02 ab
<i>atlH</i>		17.4± 0.54 abc	27.5± 0.7 ab	10.0± 0.37 abcd	1.8± 0.05 b	0.37± 0.01 ab
<i>badO</i>		17.2± 0.50 abc	28.3± 0.7 ab	11.3± 0.65 abcd	1.6± 0.05 b	0.40± 0.01 ab
<i>badH</i>		17.8± 0.72 abc	27.4± 1.0 ab	10.2± 0.51 abcd	1.8± 0.06 b	0.41± 0.02 ab
<i>banO</i>		15.0± 0.50 abc	24.0± 0.5 ab	9.0± 0.23 bcd	1.7± 0.05 b	0.39± 0.02 ab
<i>banH</i>		18.3± 0.81 abc	28.3± 1.0 ab	9.6± 0.49 abcd	1.9± 0.06 b	0.35± 0.01 b
<i>khaO</i>		17.8± 0.97 abc	30.2± 1.3 ab	11.7± 0.66 abcd	1.5± 0.06 b	0.42± 0.02 ab
<i>khaH</i>		24.7± 1.43 a	35.1± 1.6 a	11.4± 0.53 abcd	2.1± 0.09 a	0.35± 0.01 b
<i>sarO</i>		23.6± 1.13 ab	25.7± 0.7 ab	12.2± 0.43 abcd	1.9± 0.09 b	0.50± 0.02 ab
<i>sarH</i>		25.2± 0.81 a	29.2± 0.7 ab	13.2± 0.33 a	1.9± 0.04 b	0.47± 0.02 ab
<i>shaO</i>		20.7± 1.53 abc	28.4± 1.6 ab	10.3± 0.74 abcd	2.0± 0.04 a	0.40± 0.02 ab
<i>shaH</i>		20.3± 1.29 abc	30.8± 1.6 ab	11.4± 0.67 abcd	1.7± 0.07 b	0.40± 0.02 ab
<i>atlO</i>	Control	13.1± 0.62 bc	22.1± 0.8 ab	10.0± 0.55 abcd	1.3± 0.05 bc	0.48± 0.03 ab
<i>atlH</i>		15.0± 0.82 abc	25.2± 1.1 ab	10.1± 0.78 abcd	1.5± 0.06 b	0.41± 0.02 ab
<i>badO</i>		15.2± 0.43 abc	26.3± 0.7 ab	11.0± 0.67 abcd	1.4± 0.03 b	0.42± 0.01 ab
<i>badH</i>		11.3± 0.45 c	20.6± 0.6 b	9.3± 0.39 abcd	1.2± 0.04 bc	0.47± 0.02 ab
<i>banO</i>		14.7± 0.61 abc	22.4± 0.8 ab	7.9± 0.38 d	1.8± 0.04 b	0.36± 0.01 b
<i>banH</i>		14.8± 0.49 abc	23.6± 0.6 ab	8.3± 0.37 cd	1.8± 0.05 b	0.36± 0.01 b
<i>khaO</i>		19.6± 0.58 abc	31.6± 0.9 ab	12.0± 0.80 abc	1.7± 0.04 b	0.38± 0.01 ab
<i>khaH</i>		17.1± 0.75 abc	27.0± 1.0 ab	9.9± 0.59 abcd	1.7± 0.05 b	0.39± 0.02 ab
<i>sarO</i>		21.9± 1.02 abc	24.1± 0.8 ab	12.9± 0.19 ab	1.7± 0.07 b	0.60± 0.04 ab
<i>sarH</i>		17.0± 0.54 abc	20.2± 0.5 b	11.0± 0.50 abcd	1.5± 0.03 b	0.55± 0.01 ab
<i>shaO</i>		17.6± 1.03 abc	24.9± 1.2 ab	11.6± 1.12 abcd	1.6± 0.08 b	0.63± 0.15 a
<i>shaH</i>		14.4± 0.55 abc	21.9± 0.7 b	9.5± 0.66 abcd	1.6± 0.05 b	0.45± 0.02 ab

Values are means ± SD of four replicates. Bars with different letters show significant differences at P ≤ 0.05 (LSD).

Mineral Nutrients

Drought stress had a significant effect on sodium (Na^+) concentration at a 5% probability level. The lowest sodium concentration (0.07 mgKg^{-1} dry weight) at the beginning and end of drought stress was in rootstocks *SarH* and *SarO*, respectively. The highest concentration at the end was in *atlH* (0.41 mgKg^{-1} dry weight). 15 days after the beginning of the rehydration, some rootstocks recovered well and the *SarO* had the lowest amount (0.05 mg Kg^{-1} dry weights) of Na^+ concentration in its tissue (Fig.7).

Drought stress and rootstocks were no significant for magnesium (Mg^{2+}) but interaction of drought stress, rootstocks and time were significant difference (1% probability level). An increase in drought stress decreased the Mg^{2+} concentration in the leaves. The highest Mg concentration was in *ShaO* at middle and end of drought stress, while the lowest was in *SarO* (Fig.8).

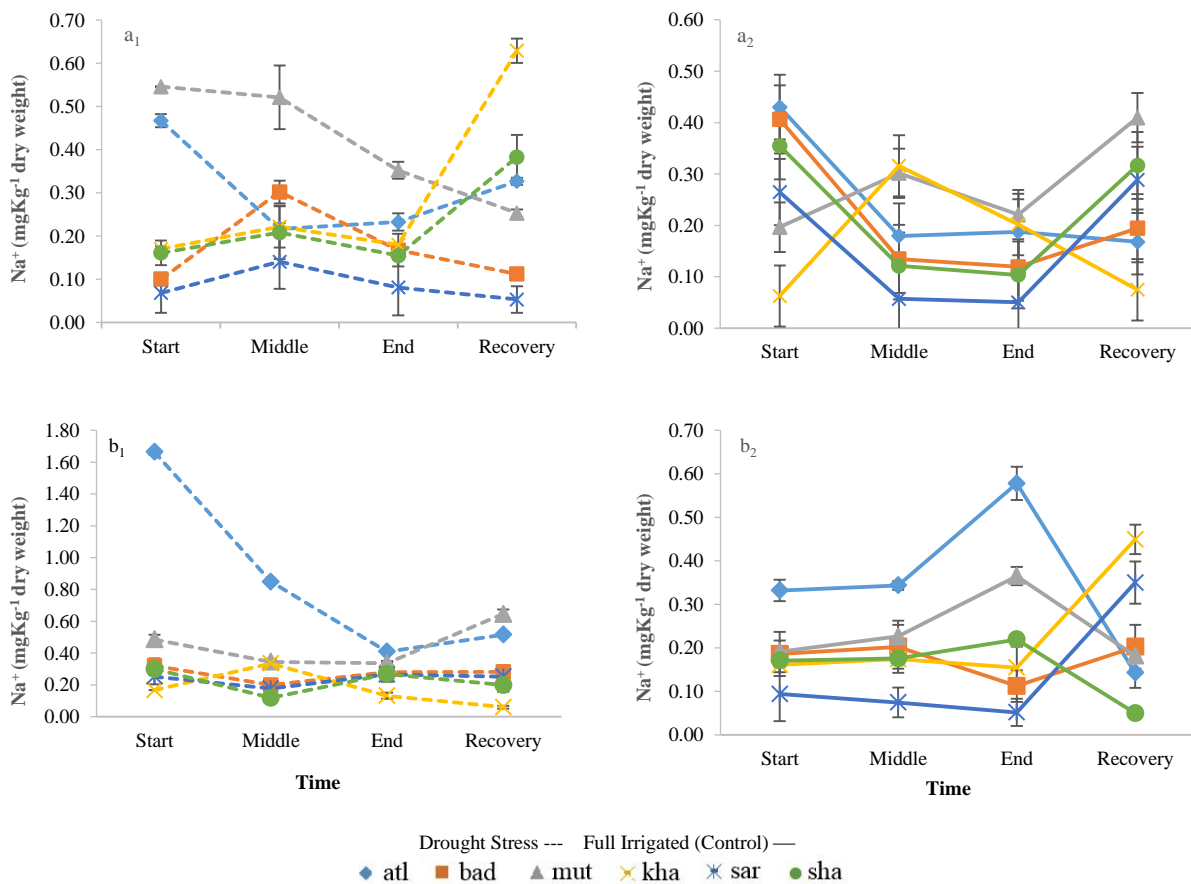


Fig.7. Changes of sodium (Na^+) ions concentration in drought stressed (---) and full irrigated (—) at different times for 12 pistachio rootstock (a: hybrid and b: non-hybrid).

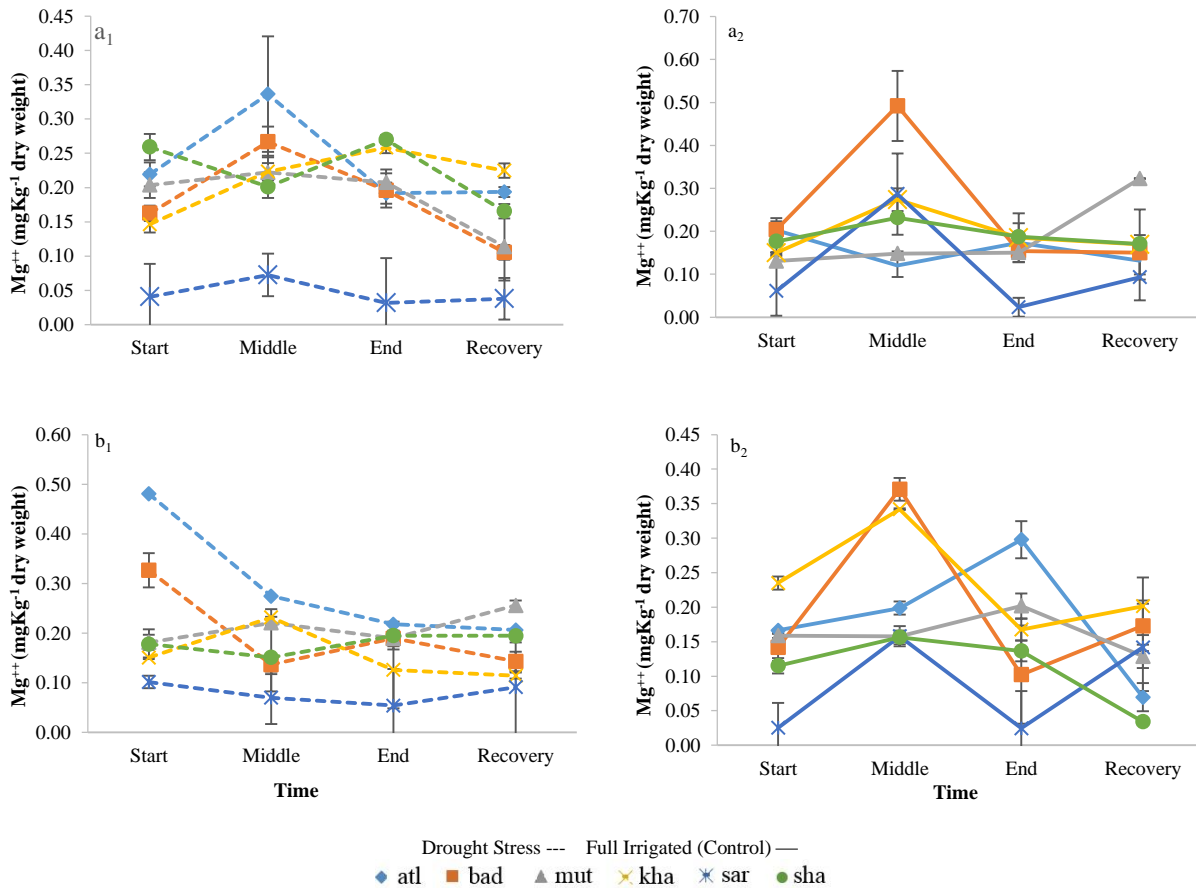


Fig.8. Changes of magnesium (Mg²⁺) ions concentration in drought stressed (---) and full irrigated (—) at different times for 12 pistachio rootstock (a: hybrid and b: non-hybrid).

Although drought stresses did not significantly affect the potassium concentration (K⁺) of rootstocks, but showed a significant difference on triple interactions. The K⁺ concentration in the plant tissue increased as the severity of the drought stress increased and it reached its maximum on the twentieth day in *mutH*. At the end of the drought stress period, the highest and lowest K⁺ concentrations were in the *mutO* and *atlO*, respectively (Fig. 9).

The concentration of chloride (Cl⁻) was measured at the beginning, end of the experiment and rehydration. The highest concentrations of Cl⁻ were found in the *atlH* (0.15 mg Kg⁻¹ dry weight) and *badH* (0.08 mg Kg⁻¹ dry weights) at the beginning and end of drought stress respectively. 15 days after the beginning of the rehydration, the lowest Cl⁻ (0.03 mg Kg⁻¹ dry weight) was in *sarO* and the highest (0.06 mg Kg⁻¹ dry weight) were in *atlO*, *badO*, *khaO* and *badH* (Fig.10).

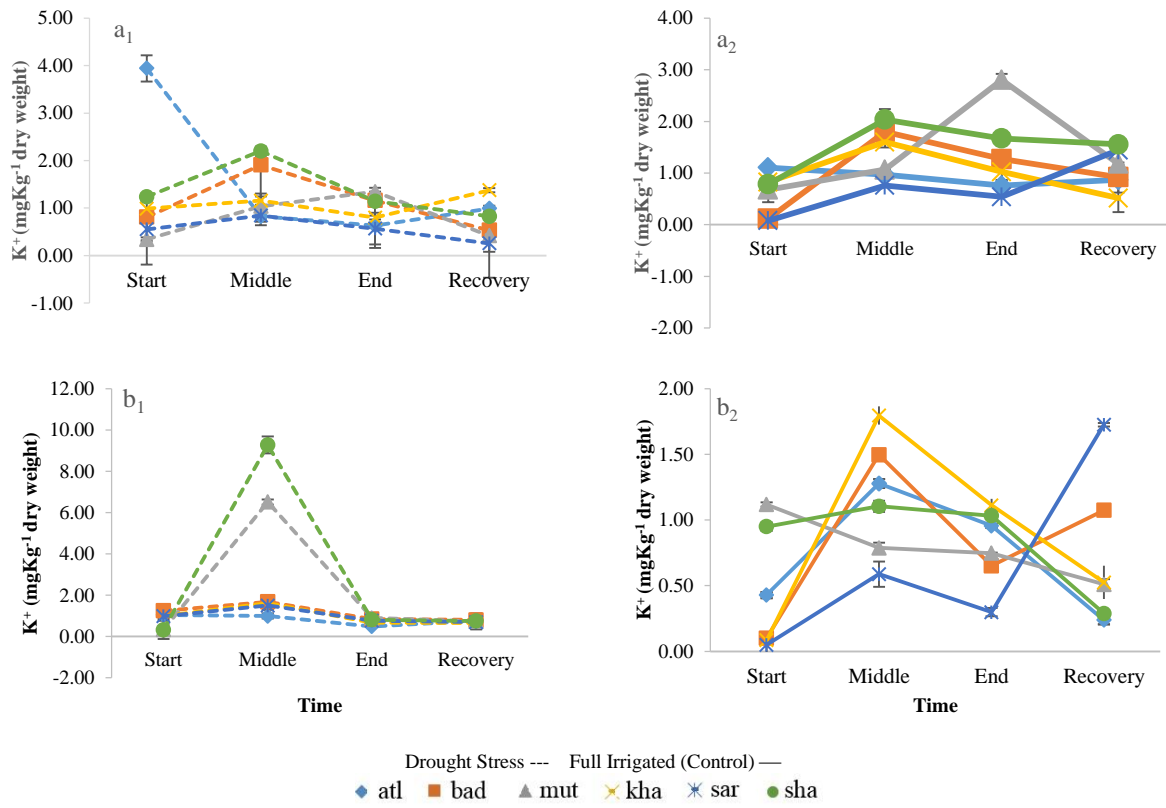


Fig.9. Changes of potassium (K⁺) ions concentration in drought stressed (---) and full irrigated (—) at different times for 12 pistachio rootstock (a: hybrid and b: non-hybrid).

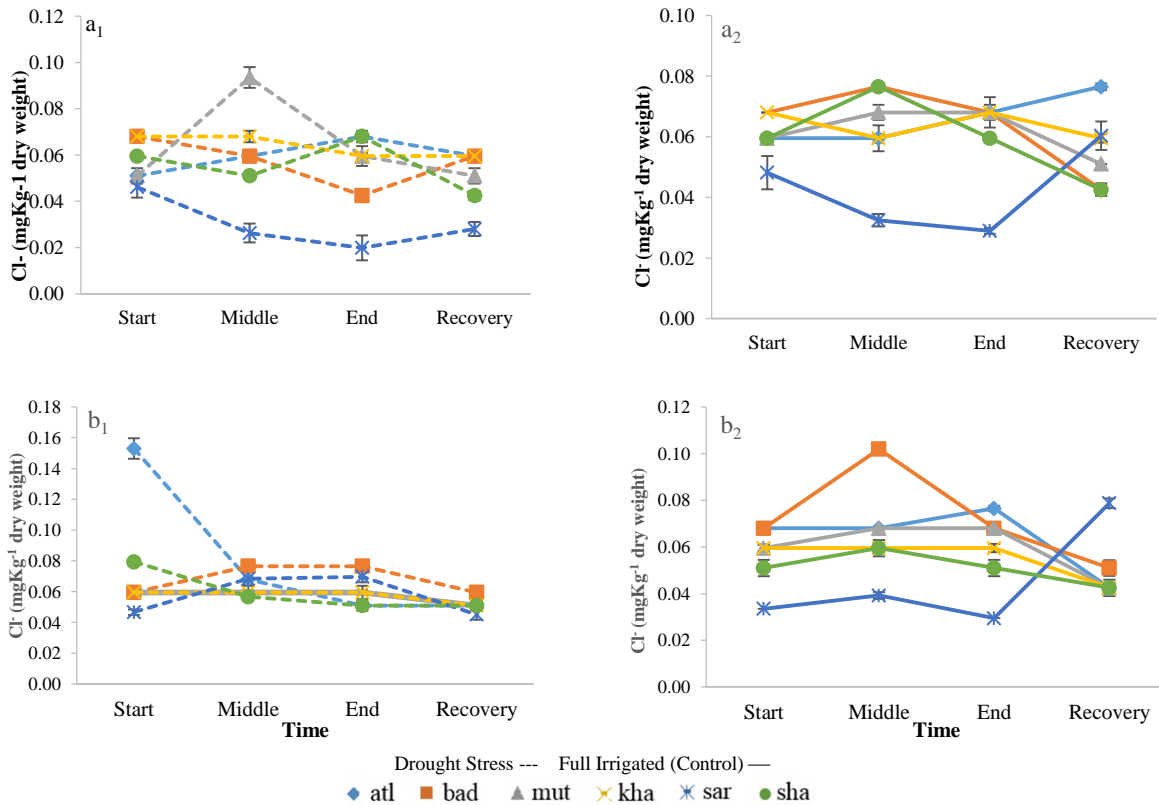


Fig.10. Changes of chloride (Cl⁻) ions concentration in drought stressed (---) and full irrigated (—) at different times for 12 pistachio rootstock (a: hybrid and b: non-hybrid).

Carotenoids content

The results showed that there were no significant interaction effects of rootstocks and stress treatment but triple effects with the time were significant. Based on the results, the highest levels of carotenoids (cars) at the

beginning, end of stress treatment and recovery were observed in *shaO*, *khaO* and *khaH* respectively. At the end of drought stress and rehydration, *sarH* and *mutH* were the lowest respectively (Fig.11).

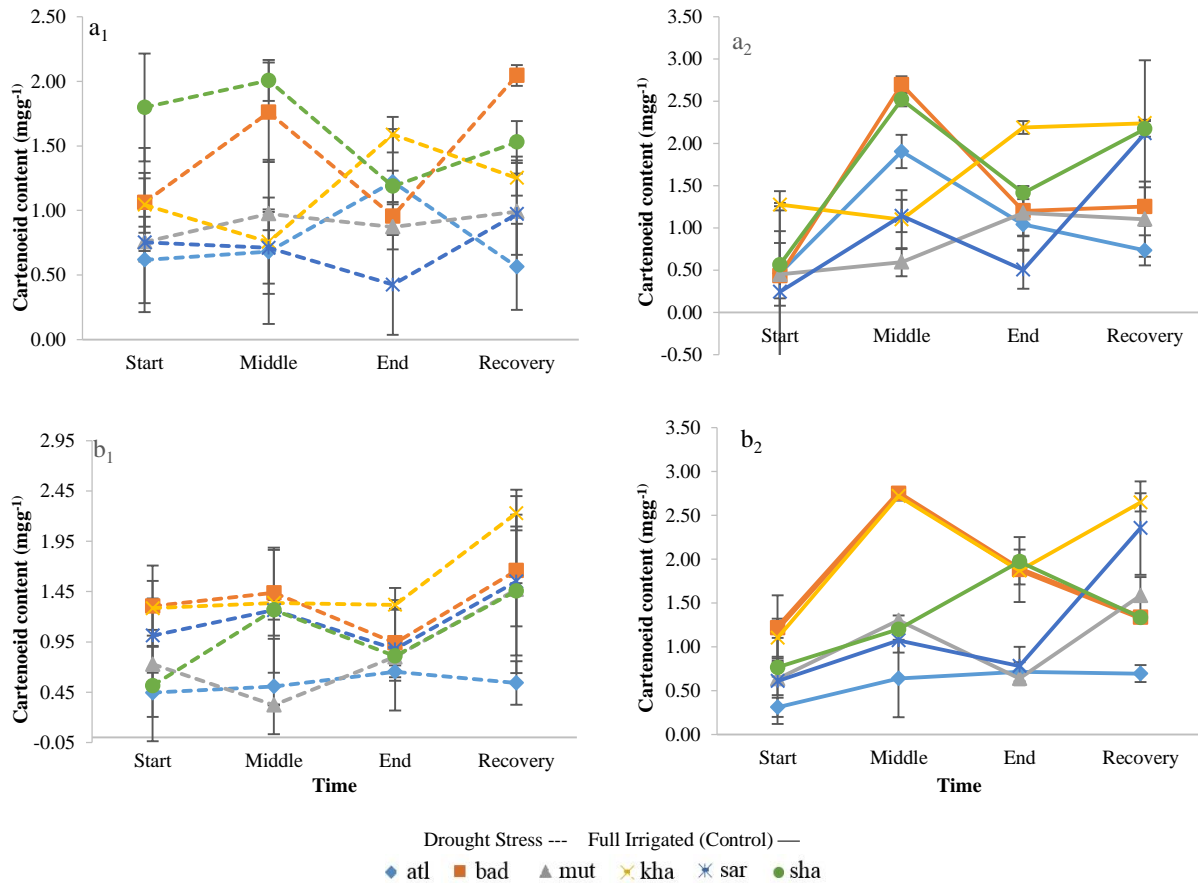


Fig. 11. Effect of water stress and rehydration on Carotenoids content (cars) of irrigated (—) and drought stressed (---) for 40 days period on hybrid (a₁ and 2) and non-hybrid (b₁ and 2) pistachio Seedling.

Anthocyanins content

There is a significant interaction among drought treatment, rootstocks and time of stress period (P < 0.01) for amount of the anthocyanins (anths) levels. In some of hybrids, anths levels decreased in fully-expanded leaves of stressed plants at the middle and end

of drought stress compared to full irrigated plants at all times. In the present study, the highest and lowest amount of anths at the end of stress was *atlO* and *sarO*, while 15 days after rehydration were *khaO* and *atlH* (Fig.12).

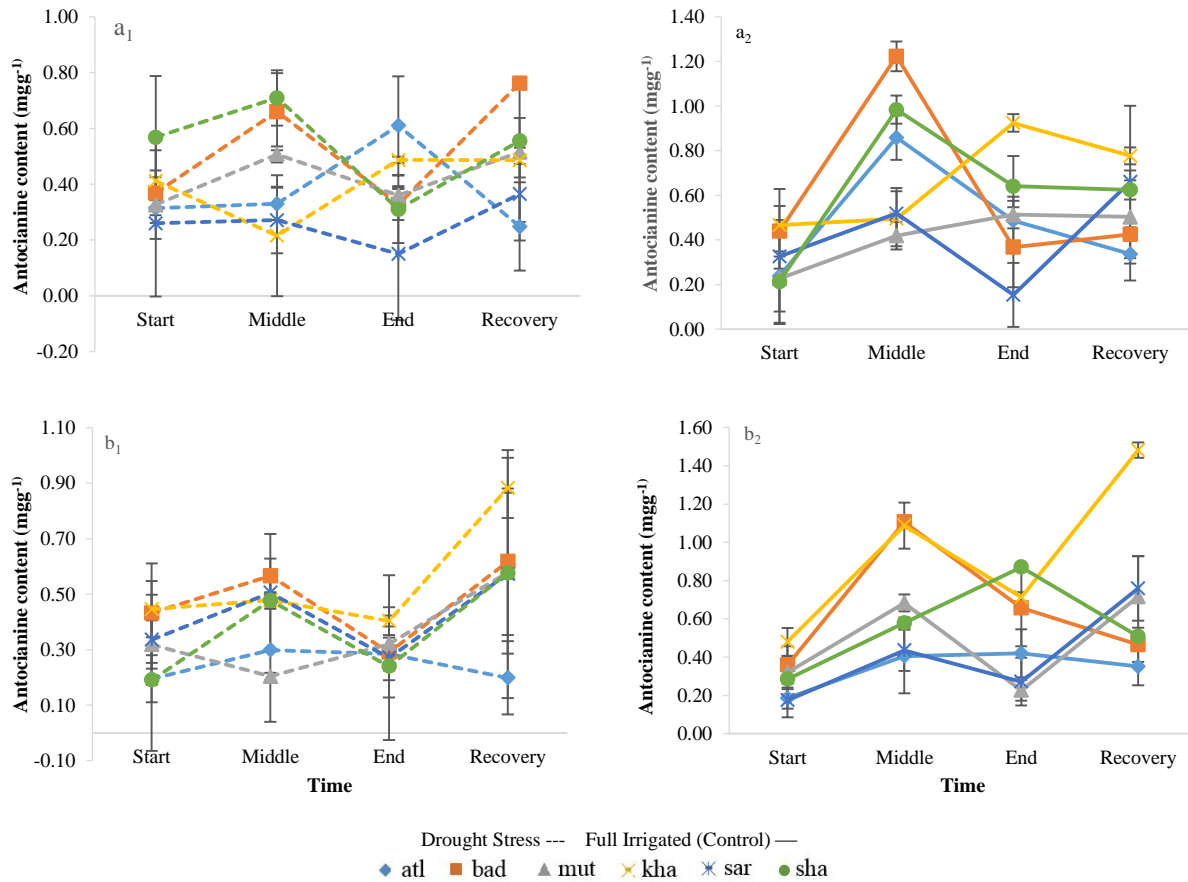


Fig.12. Effect of water stress and rehydration on anthocyanins content (anths) of irrigated (—) and drought stressed (---) for 40 days period on hybrid (a₁ and 2) and non-hybrid (b₁ and 2) pistachio Seedling.

Chlorophyll content

The total chlorophyll (TChl) content of pistachio rootstocks was significantly affected by water stress. Induced water deficient decreased TChl significantly in comparison with the control in most rootstocks. The maximum and minimum of TChl were observed in

khaH (2.05 mgg⁻¹) and *sarO* (0.79 mgg⁻¹) at end of drought stress respectively, but after rehydration the lowest amount (0.86 mgg⁻¹) of TChl was obtained in *atlH*, while *khaO* showed a slight decrease in TChl and it also recovered well (Figs.13- 15).

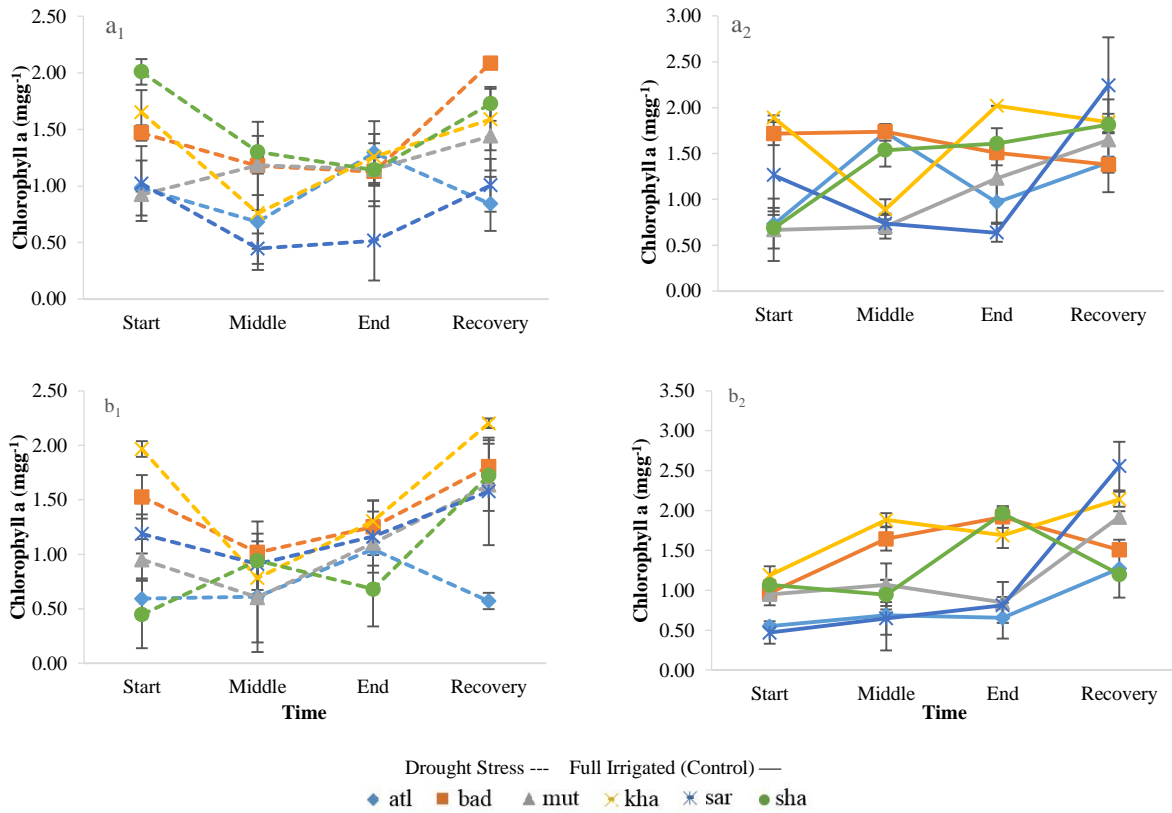


Fig.13. Effect of water stress and rehydration on Chlorophyll a content (Chl a) of irrigated (—) and drought stressed (---) for 40 days period on hybrid (a₁ and a₂) and non-hybrid (b₁ and b₂) pistachio Seedling.

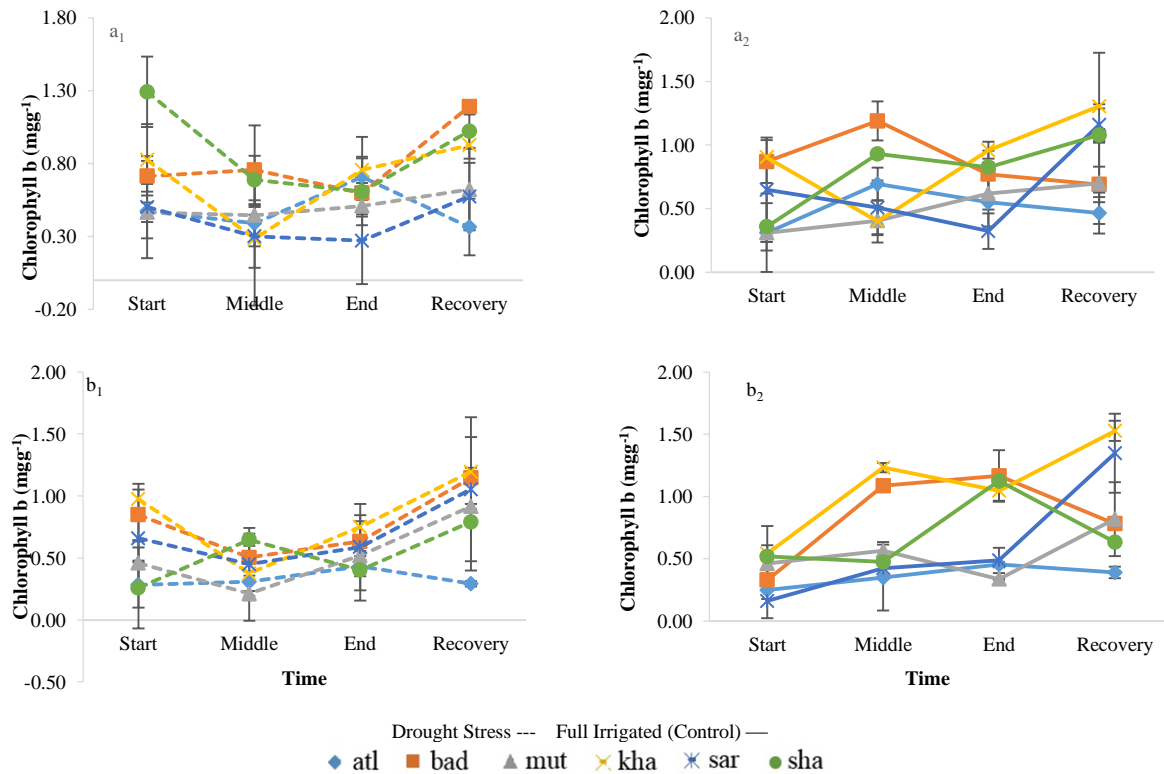


Fig.14. Effect of water stress and rehydration on Chlorophyll b content (Chl b) of irrigated (—) and drought stressed (---) for 40 days period on hybrid (a₁ and a₂) and non-hybrid (b₁ and b₂) pistachio seedling.

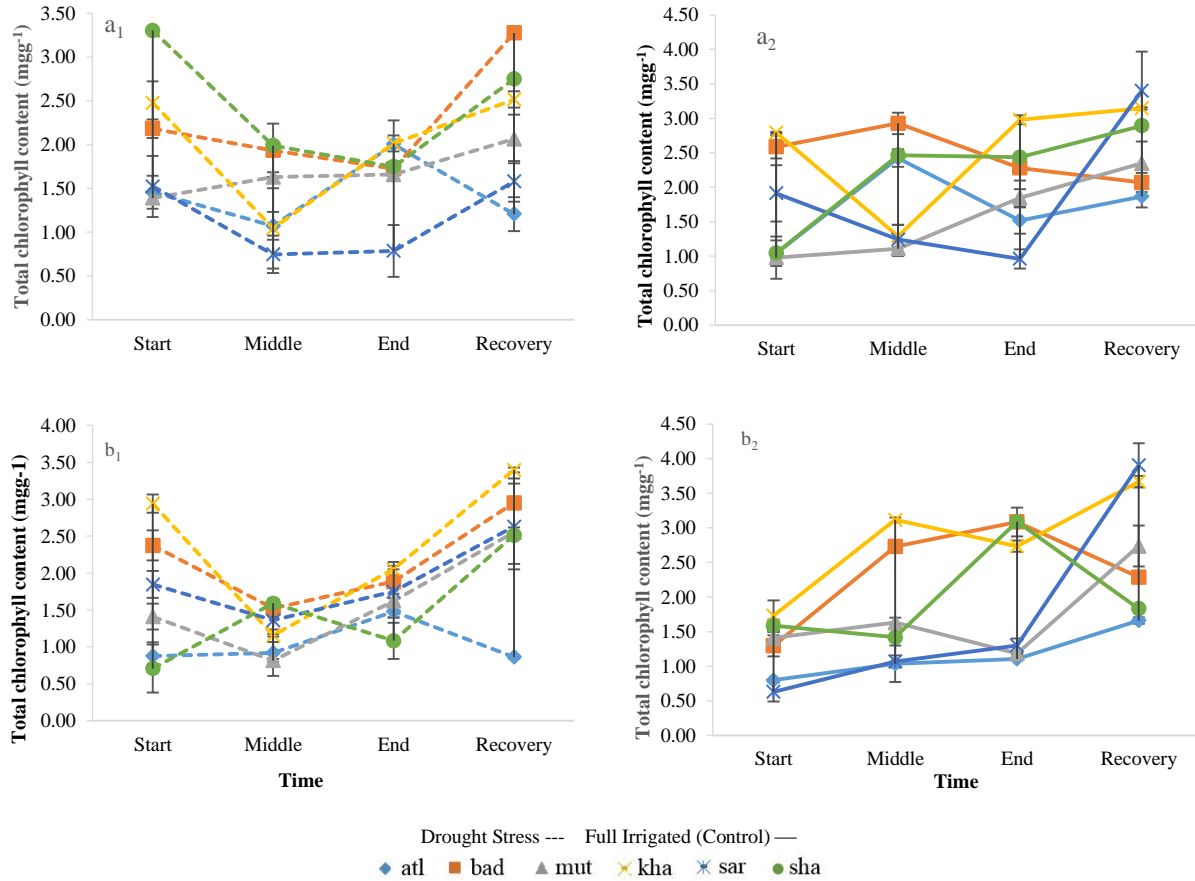


Fig. 15. Effect of water stress and rehydration on total chlorophyll a content (TChl) of irrigated (—) and drought stressed (---) for 40 days period on hybrid (a₁ and a₂) and non-hybrid (b₁ and b₂) pistachio seedling.

Multivariate analysis

PC analysis

In this study, PCA was performed to determine parameters quota in total variation at two separate times included the end of stress and after recovery. At the *End of stress*, the results showed that the first principal component could explain 35% of the variation. Furthermore, the first and second component explained generally 55% of the variation. The first component was influenced by TChl content, C_i, anths, carts, fluorescence parameter and Cl⁻, Mg²⁺ ions and Pn, E, mesophyll efficiency and Na⁺ ions were contributed to

the second principal component. *After Recovery*, all measured traits were classified under three major principal component axes (PC1, PC2 and PC3) with 69% of total variance being accounted. The PC1 accounted 31% of the total variation which is consists of Pigments, Pn, and WUE and mesophyll efficiency. Minerals, E, C_i and F₀F_m were the main component of PC2. The Fluorescence parameter traits such as F_v, F₀ and F_vF₀ were contributed to the third component (Fig.16).

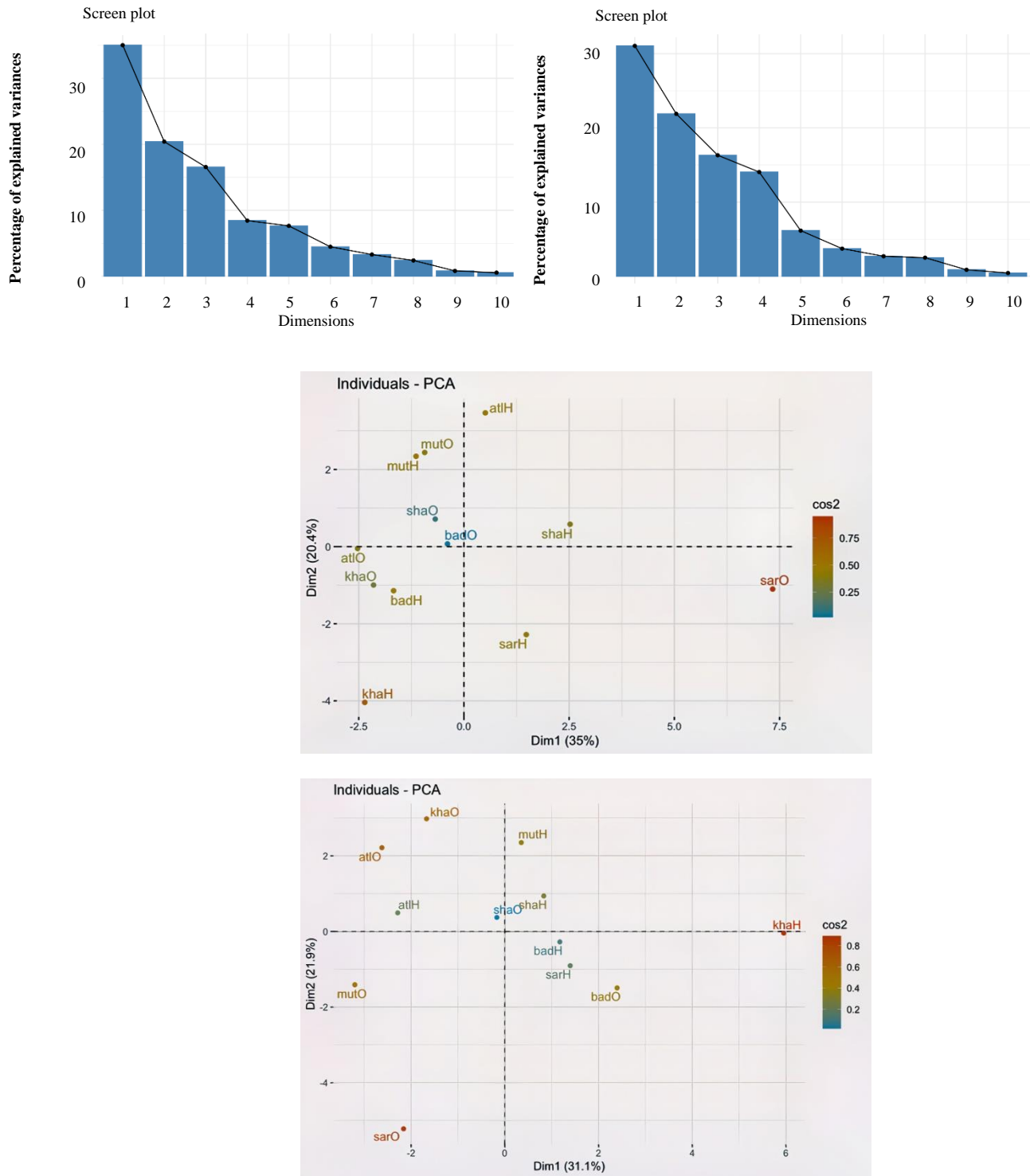


Fig.16. Principal component analysis (PCA) of the studied *Pistachio* rootstock under normal (full irrigated) and drought stress conditions at end of stress (left) and recovery (right) time. (A) Scree plot (percentage of explained variances), (B) PCA individual plot, (C) Principal component analysis biplot.

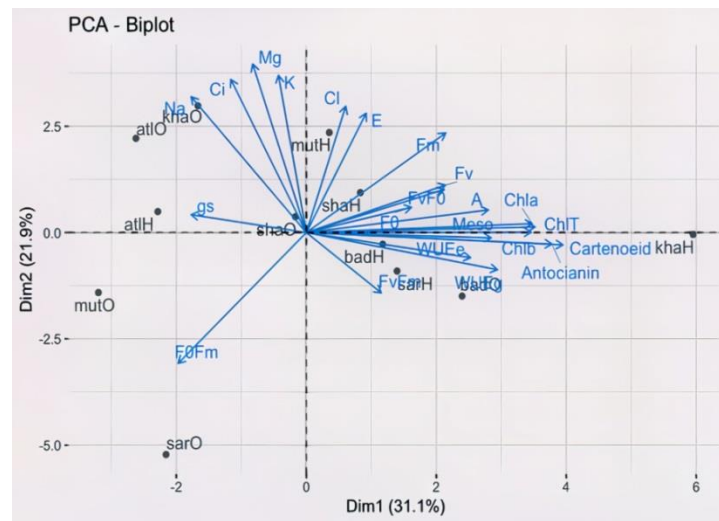
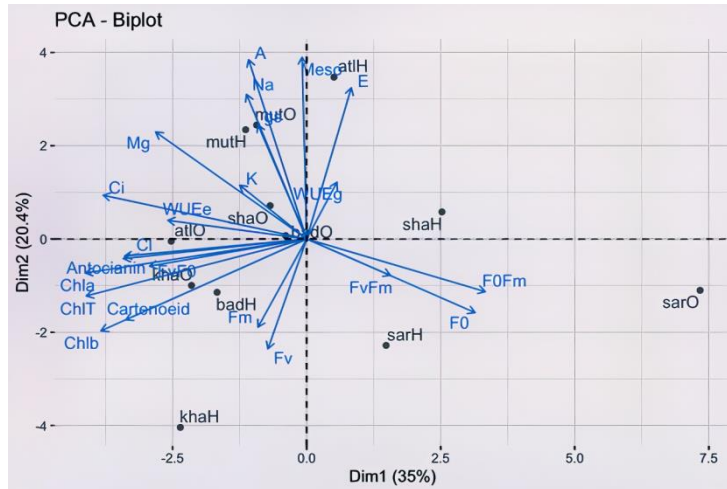


Fig.16. Continued.

Cluster analysis

As in the PCA analysis, clustering of rootstocks was performed at two different times. At the end of the Stress, 12 studied rootstocks were classified into four groups. Three out of 12 rootstocks (9, 12 and 2) were placed in the same group (group1) having the least pigments, Cl^- and K^+ ions and highest WUE_g . Rootstocks 10, 4 and 8 were clustered in group 2 having the highest Cl^- and mesophyll efficiency. 1 and 7 as highest ants and carts rootstocks were classified in cluster group 3 and the fourth cluster includes 11,3,5 and 6 had highest K^+ and gs (Fig.17).

Like the previous clustering, also after recovery, cluster analysis divided pistachio rootstocks into four mains clusters. Rootstocks 6, 10 and 12 were clustered in group 1 having the highest F_v/F_0 , F_v and F_0 . Cluster 2, the largest group, consisting of 4,11,3 and 8 rootstocks having the highest pigments, Pn and mesophyll index. 9 and 5 as Highest F_0/F_m rootstocks were classified in cluster group 3. Three out of 12 rootstocks (7, 1 and 2) were placed in group 4 having the highest mineral nutrient (Fig.17).

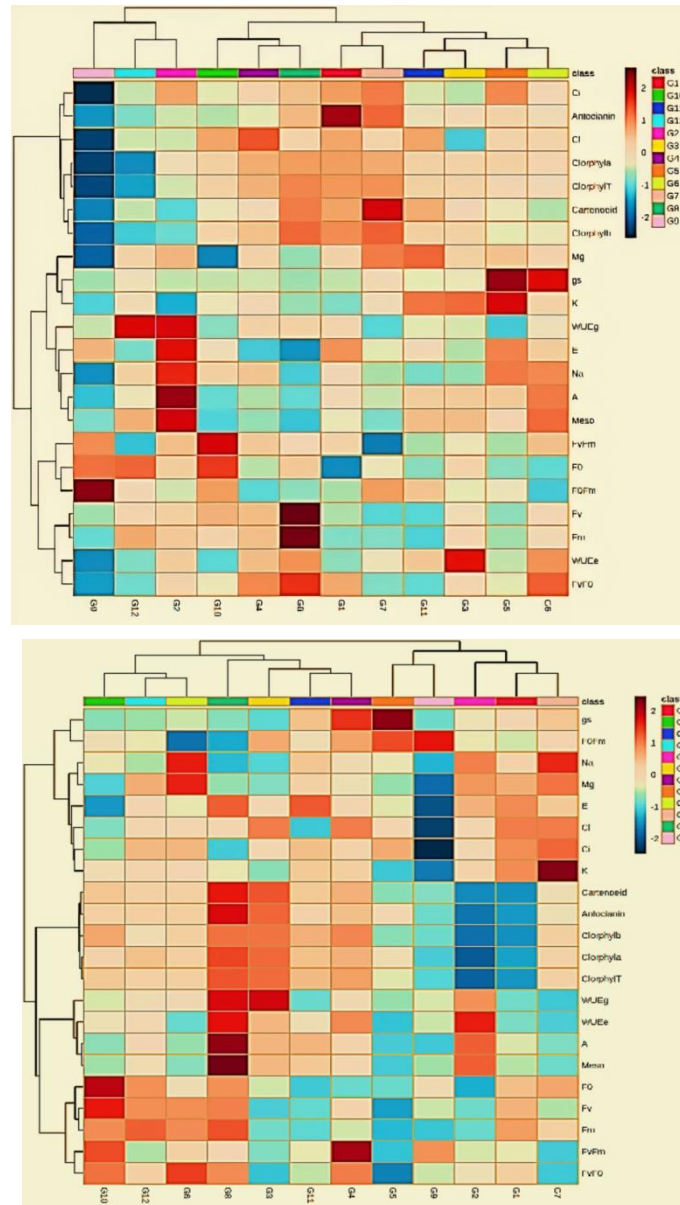


Fig.17. Cluster analysis of the studied pistachio rootstock at end of stress (left) and rehydration (right) time.

Correlations

A positive correlation was observed between the content of Pn with the E, mesophyll efficiency, WUE, Mg^{2+} , Cl^- and Pigments amounts; and a significant negative correlation between Pn rate and F_v/F_m . We also observed a negative correlation between the F_v/F_m and Pn, E, mesophyll efficiency, Mg^{2+} , Cl^- and Na^+ ions. As seen, Na^+ ions show a positive correlation with other mineral nutrients (K^+ , Mg^{2+} and Cl^- ions), there was also

a significant positive correlation between chlorine with WUE, Carts and anths content. The correlation between K^+ with E and gs was negative. We found significant positive correlations between pigments, also TChl with Pn, E and mesophyll efficiency. A positive correlation was observed between the content of Carts with the Pn, E, mesophyll efficiency and Mg^{2+} , Na^+ and Cl^- ions (Table 4).

Table 4. Correlation analysis of some physiological Characteristics in drought-exposed Pistachio rootstocks

	Pn	E	gs	Ci	mesophyll index	F _v /F _m	WUEg	WUEe	Na ⁺	K ⁺	Mg ²⁺	Cl ⁻	Carts	Anths	TChl
Pn	1														
E	0.35 **	1													
gs	0.18 **	0.42 **	1												
Ci	-0.13 *	0.21 **	0.02 ^{ns}	1											
mesophyll index	0.94 **	0.27 **	0.14 *	-0.40 **	1										
F _v /F _m	-0.19 **	-0.14 *	-0.06 ^{ns}	-0.13 *	-0.16 **	1									
WUEg	0.41 **	-0.23 **	-0.34 **	-0.27 **	0.48 **	-0.09 ^{ns}	1								
WUEe	0.41 **	-0.49 **	-0.14 *	-0.34 **	0.44 **	-0.04 ^{ns}	0.51 **	1							
Na ⁺	0.08 ^{ns}	0.05 ^{ns}	0.11 ^{ns}	0.03 ^{ns}	0.06 ^{ns}	-0.16 **	-0.01 ^{ns}	0.02 ^{ns}	1						
K ⁺	-0.06 ^{ns}	-0.12 *	0.19 **	0.03 ^{ns}	-0.06 ^{ns}	-0.03 ^{ns}	-0.06 ^{ns}	0.06 ^{ns}	0.12 *	1					
Mg ²⁺	0.13 *	-0.09 ^{ns}	-0.01 ^{ns}	-0.02 ^{ns}	0.13 *	-0.18 **	0.07 ^{ns}	0.20 **	0.50 **	0.16 **	1				
Cl ⁻	0.13 *	-0.10 ^{ns}	0.05 ^{ns}	0.05 ^{ns}	0.11 ^{ns}	-0.20 **	0.06 ^{ns}	0.18 **	0.29 **	0.23 **	0.31 **	1			
Carts	0.18 **	0.10 ^{ns}	-0.05 ^{ns}	-0.10 ^{ns}	0.20 **	-0.03 ^{ns}	0.06 ^{ns}	0.07 ^{ns}	-0.15 **	0.04 ^{ns}	0.18 **	0.12 *	1		
Anths	0.25 **	0.12 *	-0.03 ^{ns}	-0.10 ^{ns}	0.26 **	-0.02 ^{ns}	0.10 ^{ns}	0.07 ^{ns}	-0.11 ^{ns}	0.06 ^{ns}	0.23 **	0.13 *	0.90 **	1	
TChl	0.17 **	0.24 **	0.05 ^{ns}	0.02 ^{ns}	0.14 *	-0.09 ^{ns}	-0.02 ^{ns}	-0.07 ^{ns}	-0.10 ^{ns}	-0.01 ^{ns}	0.07 ^{ns}	0.09 ^{ns}	0.82 **	0.77 **	1

Discussion

Photosynthesis characteristics and water use efficiency

Decreasing the amount of photosynthesis in drought-stressed plants led to a decrease in stomatal and nonstomatal factors which was in line with the results of Chatzistathis *et al.*, (2020) and Alipour (2018). According to our results, the photosynthetic factors were affected by drought stress, that the hybrids induced the highest amount of stomatal regulator in answer to water deficient, preserving of highest g_s and maximum rate of P_n . Stress led to a 55% reduction in photosynthesis, this was in line with Gijón *et al.*, (2019) results, they reported the drought stress leads to significant reduction in photosynthetic indices. There are several functional apparatuses to survive in drought environments (Li *et al.*, 2019) which are exposed by different plants and depend on the drought stress severity. The stomatal control is generally related to an inception level of leaf water potential, that the leaf resistance increases gradually (Gijón *et al.*, 2011). The effect of drought stress on stomatal conductance and P_n amount has been reported in many species especially pistachios (Abbaspour *et al.*, 2012). Our study on seedling pistachio showed that stomatal closure led to restrictive CO_2 uptake in the trend of water stress which was previously reported by Flexas *et al.* (2002). In fact, the decreasing CO_2 assimilation rates in drought stress alleviate CO_2 diffusion through the mesophyll of leaf as showed by mesophyll efficiency (Flexas *et al.*, 2012). However, drought stress reduced photosynthetic indices but species that could definitely respond when re-watered could improve leaf water potential, and hence productivity. There are many studies that show that stressed and then re-irrigation plants exposed a well stomatal adjustment, and later better efficiency: Regrowth after rehydration often continued at a faster than in the continuously irrigated plants (2000; Xu *et*

al., 2010) that our results also confirmed previous finding.

Fluorescence parameters

F_v/F_m is not sensitive to moderate and early stresses and its value showed no significant difference upon water deficit management but as the tension continued, its value decreased. Our results on F_v/F_m are similar to the findings of Ranjbar Fordoei, 2018 who proposed that any decrease in F_v/F_m shows that PSII suffers from destruction and that the main photosynthetic responses are repressed. A similar result was also exposed Tezara *et al.*, 2021. In other words, the decline in F_v/F_m ratio was associated to decrease in F_m values under water stress showing the disruption of antenna complex of PSII and increased wasted energy, damage of reaction center at PSII, and impaired ribulose-1,5-bisphosphate (RuBP) regeneration ability (Vieira Santos *et al.* 2001), thus, electron transport ability of PSII was degraded. At the end of drought stress, the F_v/F_m ratio less decreased in *sarH*. It is suggested that these rootstocks maintained lesser disruption in antenna complex and RuBP regeneration ability under water stress which confirms Ranjbar Fordoei, (2018) findings.

Mineral nutrient

Water status impacts the potassium accumulation in plants and interacts with K^+ nutritious status in some species (Liang *et al.*, 2018). Our experimental results showed that under stress conditions, potassium leaf content increased in the middle of drought stress while its value decreased (22%) with continued stress. Bagheri *et al.*, (2012) has previously reported that drought stress leads to a 10% increase in potassium in pistachio stems. As discussed in Fernando *et al.* (1992), the primary reactions of plants to potassium deficit is the net

removal of K^+ from the vacuole to the cytosol and then to the shoot via the xylem. As potassium stress intensifies, there is a more reduction of the transfer of K^+ from roots to shoots. Chlorine is generally found in soils as a monovalent ion such as chloride and is a compound involved in the photolysis of water by photosystem II. It plays a key role in the stomatal movement, osmoregulation and the water relation. This ability is due to biochemical inertness, which allows Cl^- to show osmotic and cation neutralization function that may have the biophysical or biochemical results (Heckman 2007; Reddy 2006). Our funding showed that increasing water stress leads to an increase in the concentrations of chloride ions in the leaves. Aligned with our results Fattahi *et al.*, 2021 found an increase in chloride ions amount in both roots and leaves under water stress in pistachio. Hence, together with proline, sugar and other compounds (Lotfi *et al.*, 2010), these ions contribute to osmotic regulation in plants in order to tolerate drought.

The major function of magnesium is its role as the central atom of chlorophyll molecules. As magnesium is not obtainable under conditions of water deficit, the plant roots are not able of absorbing sufficient magnesium to maintain usual plant growth (Merhaut 2007). We observed that with the onset of drought stress, the concentration of magnesium decreased and reached its lowest level at the end of the stress, this finding is confirmed by many researchers in pistachio, apple, strawberry and etc (Tadayyon *et al.*, 2018; Liang *et al.*, 2018; Celiktopuz *et al.*, 2021). After re-irrigation, some of them were recovered and the amount of magnesium in the leaves increased.

Some Previous studies have shown a water stress mainly resulted in an over accumulation of Na^+ (Teakle and Tyerman, 2010; Behzadi Rad *et al.*, 2021; Pacheco *et al.*, 2021), and even imbalanced mineral components in plants (Silva *et al.*, 2015). Our study showed that Na^+ content of non-hybrid rootstocks increased under severe

drought stress but in hybrids had very mild leaf burn, which was maybe due to an ability to accumulate more Na in roots and transport less Na^+ to branches and leaves (Munns, 2002). Hence, we speculate that it is a mechanism for pistachio to modulate the damaging effects of water deficiency. Plant drought resistance has been linked to the retention of Na^+ ions in roots (Tattini *et al.*, 1993) and/or preventing the accumulation in shoots and leaves in other researches (Gucci and Tattini, 1997). This observation was in agreement with the results of other researches under salinity stress in pistachio (Akbari *et al.*, 2018; Behzadi Rad *et al.*, 2021).

Pigments

Our results showed that under drought stress, photosynthetic pigments (TChl, carts and anths) have significantly changed in all of the rootstocks. Under severe stress, the amount of chlorophyll decreased. Severe stress reduced chlorophyll content 9% compared to control. The decrease in the chlorophyll content under water stress is a commonly observed phenomenon (Hamdani *et al.*, 2021). Hamdani *et al.*, (2021) reported that drought stress reduced chlorophyll content by 10 to 35% in pistachio. Also, our results are in agreement with those of Ranjbar (2016). Previous studies have shown that reduction in chlorophylls under drought stress conditions may be due to perturbation in biosynthesis (Khoyerdi *et al.*, 2016) and or its degradation by chlorophylls (Woodward and Bennett, 2005).

Carotenoids and anthocyanin act as assisting pigments and it has antioxidant functions which protect and stabilize the photochemical proceedings under water stress condition. In the middle of drought stress, the content of carts increased but anthocyanins reduced, while in severe tension both of them declined in most rootstocks. Therefore, the rootstocks with more pigments content are more resistant to drought stress (Abbasi *et al.*, 2014).

Conclusions

In summary, our results showed that under water stress conditions, pistachio rootstock developed an active drought tolerance mechanism to cope with water deficiency. The present study has shown that drought stress treatments increased mesophyll efficiency, the maximum quantum yield of PSII, potassium concentration and water use efficiency and decreased the net photosynthesis rate, stomatal conductance and total chlorophyll content. According to the results of this research, diversity in response to drought stresses was established among all studied rootstocks and changed at different times (beginning, middle and end of stress and then recovery), and some rootstocks were more tolerant than others. The main mechanism to reduce the effect of drought stress was to maintain leaf chlorophyll content and *P. vera* "Khanjari" hybrid and *P. vera* "Khanjari" rootstock had the highest chlorophyll content at the end of stress and after recovery, respectively and recovered well.

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