Journal of Nuts

Journal homepage: sanad.iau.ir/journal/ijnrs

ORIGINAL ARTICLE

Physio-Biochemical Changes of Some Pistachio Rootstocks in Response to Drought and Recovery Periods

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K E Y W O R D S

Chlorophyll fluorescence; Chlorophyll pigments; Osmoregulation

A B S T R A C T

The reduction of water resources in pistachio production areas of Iran has led to an increase in the frequency of irrigation, so the pistachio trees are continuously exposed to periods of drought and recovery after irrigation during the growing season. Choosing rootstocks that have the highest resistance to drought stress and the highest recovery speed can be considered as one of the basic strategies for facing such conditions. This experiment was carried out as factorial and in the form of a completely randomized design with three replications. The experimental factors were the type of rootstock in five levels and the sampling stage in three levels for destructive biochemical parameters and six levels for non-destructive chlorophyll fluoresce parameters. Drought stress was achieved by withholding irrigation for 15 days, and in the recovery phase, the pistachio seedlings were irrigated daily up to field capacity for 10 days. At the end of the experiment, shoot and root dry weights were measured. Evaluation of pistachio seedlings biomass showed that the periods of drought and recovery did not affect the dry weight of shoot and root and rootstock type was the only influencing factor. The highest amount of shoot and root dry weight was observed in lentisk *(Pistacia lentiscus)* and Bane-Baghi respectively, and the lowest amount was recorded in Bane and Sarakhs. Chlorophyll fluorescence indices were completely sensitive to drought stress and recovery. Dry period caused the measured biochemical parameters known as osmolytes to increase and the changes in these parameters were different in different rootstocks.

Introduction

Drought stress is the main limiting factor affecting plant growth and development in arid and semiarid regions of the world where plants are often exposed to periods of water deficit. Responses of plants to drought stress are multiple and interconnected (Vahdati *et al.*, 2009). Plants use three different strategies to deal with drought stress: drought escape, avoidance, or tolerance (Basu *et al.*, 2016). 'Drought escape' has a proper timing of lifecycle, allowing plants to complete their sensitive developmental stages in water abundant period (Manavalan *et al.*, 2009), drought avoidance related to increase in water uptake and decrease in water loss (Luo *et al.*, 2010; Tardieu *et al.*, 2013), and drought tolerance by osmotic adjustment, antioxidant capacity, and desiccation tolerance (Luo *et al.*, 2010). It is well

[*]**Corresponding author**: Email address: shamshiri88@gmail.com
Received: 9 February 2023; Received in revised form: 22 June 2023; Accepted: 8 July 2023
DOI: 10.22034/jon.2023.1979552.1214

documented that water stress impairs numerous metabolic and physiological processes in plants. Reduction in photosynthesis and chlorophyll pigments and changes in fluorescence parameters due to water stress has been well documented (Mohsenzadeh *et al.*, 2006; Li-Ping *et al.*, 2006; Gharaghani *et al.*, 2018). Previous studies have shown the biochemical responses of water stressed plants including production and accumulation of some secondary metabolites such as soluble sugars, amino compounds (proline and glycine betaine), sugar alcohols (mannitol) and other low molecular weight metabolites classified as osmotic protectors or osmolyte compounds and oxidative enzymes (Hare *et al.*, 1998; Lotfi *et al.*, 2010a, b, 2019; Soroori and Danaee, 2023). As a consequence, increase in water absorption power, maintaining the available water and dealing with oxidative stress will happen (González-Villagra *el al.*, 2019). The activation speed of these mechanisms in a plant has a direct relationship with its resistance to drought (Hare *et al.*, 1998).

Recovery means a return to a normal state of health, which in the case of plants under stress, refers to a state in which, after removing the stress factor, the plant returns to its initial state of growth or to relatively favourable growth conditions. The ability to tolerate and recover from severe drought stress is becoming an important issue for tree species because more dry periods may occur in the coming decades (Gallé *et al.*, 2007). The response of plants to drought stress has been widely studied, while the recovery after stress, despite its importance, has attracted less attention (Ruehr *et al.*, 2019). In any case, it is necessary to understand the behaviour of tree species in facing water scarcity and what strategies they use to deal with the water shortage. However, when water shortage is relieved, the plant needs to restart growth as quickly as possible. Recovery after stress is a very complex process involving the rearrangement of many metabolic pathways to repair drought-induced damage and resume plant growth.

Pistachio (*Pistacia vera* L.) is the most important commercial fruit crop grown in arid and semi-arid regions of Iran especially in Kerman province where the most of pistachio crop is produced (Shamshiri and Hasani, 2015; Eslami *et al.*, 2019). In 2014, Iran was the first producer and exporter of pistachios with production of 440,000 tons of this product (Sharifkhah *et al.*, 2020; Nazoori et al., 2022).Increased establishment of irrigated pistachio orchards during the last three decades in this region has decreased the availability of underground water resources and prolonged drought periods is the major concern for the pistachio producers. Based on the predictions of future global environmental change, Iran will experience an increase of 2.6°C in mean temperature and a 35% decline in precipitation in the next decades (Mansouri, 2019). Increases in the both severity and frequency of drought in the near future is expectable. Pistachio is commercially propagated by grafting on rootstock and the responses of grafted plants to drought stress are mostly related with rootstock. Therefore, finding more efficient and better drought adapted rootstocks is becoming increasingly important. To achieve this goal, there is an urgent need to find affordable and trustworthy physiological or biochemical indicators that can help in the selection of drought-adaptive rootstocks. Moreover, while drought resistance of pistachio rootstocks has been a major concern in previous studies on plant drought adaptation, the role of drought recovery in pistachio rootstocks received much less attention over the last researches. According to the above, the current research aims to investigate the growth and biochemical responses of some pistachio rootstocks to the drought and recovery period at seedling stage. Moreover, the other goal was to find the best biochemical index that can be used as a sensitive marker to evaluate the drought tolerance of these rootstocks.

Materials and Methods

The experiment was started in April, 2018 in Vali-e-Asr University of Rafsanjan, Iran. It was carried out

as a factorial based on a completely randomized design with three replications. Experimental treatments were rootstock type including *P. vera* cv. Badami-riz-zarand (BRZ), *P. vera* cv. Sarakhs (SRKH), *P. mutica* (Bane, BN), *P. vera* × *P. mutica* (Bane-Baghi, BB) and *P. lentiscus* (LNT) and sampling times including three levels (5 days before the beginning of drought stress, end of drought stress and end of recovery stage) for destructive biochemical parameters including chlorophyll a and b, proline, sucrose, starch, total soluble proteins, total phenolic compounds and six levels (5 days before drought stress onset, 5 days, 10 days and 15 days after the start of drought stress and finally 5 and 10 days after the start of recovery) for non-destructive chlorophyll fluorescence parameters. At the end of the experiment, the dry weight of shoots and roots was measured.

Seed preparation and cultivation

The seeds of BN, BB, BRZ and SARKH were provided by Pistachio Research Institute, Rafsanjan, Iran and LNT seeds were donated by Professor Barbara Rufoni (Consiglio per la Ricerca e la Sperimentazione in Agricoltura, CRA-FSO, Sanremo, Italy). The seeds of BN and BB were soaked in water for 24 hours and after removing their hard shell, the kernels were disinfected with 5% sodium hypochlorite for 3 minutes and washed 3 times with distilled water. After that, they were treated with Mancozeb fungicide (0.1%) for 5 minutes and mixed with wetted perlite and stored in a refrigerator at 4ºC for 60 days to remove physiological dormancy (Cherighi *et al.*, 2015). When the first sign of radicle was appeared, BRZ, SARKH and LNT seeds were surface sterilized as described above and placed in a wet cloth under laboratory conditions for germination.

Germinated seeds of all rootstocks were transferred to 5 kg pots and five seeds were planted in each pot and immediately irrigated with distilled water. The soil used was a sandy loam with the following characteristics: sand 74.1%, silt 14.1%, clay 11.8%, pH 7.9, P 6.15 mg kg^{-1} soil, K 21.2 mg kg^{-1} soil, Mg0.8 meq L^{-1}, Ca 7meq L^{-1}, Fe 0.09µg g^{-1}, Zn 1.27µg g^{-1}, Mn 1.2µg g^{-1}, Cu 1.35µg g^{-1} and cation exchange capacity 1.5Ds m^{-1}. The pots were kept for 12 months in greenhouse conditions and irrigated every two days up to field capacity. During this period, Hoagland's nutrient solution was given to the pistachio seedlings every 10 days and 15-20% more than field capacity to cover seedlings requirements.

Treatments

After 12 months of seed germination, drought and recovery treatments were implemented in such a way that irrigation was stopped entirely for 15 days and then the pots were irrigated daily up to field capacity for 10 days (by weighting method). During the experiment period, the mean temperature and relative humidity was about 35±2ºC and 55±5% respectively.

Measurements

Root and shoot dry weight

After the periods of drought and recovery, plants were divided to shoot and root and placed in an oven at 72°C for 48 hours. The dry weight of the shoot and root was measured individually by a digital balance.

Chlorophyll content

Chlorophyll a and b contents were determined by the method of Porra (2002). Fresh leaves (0.25 g) were triturated in 80% acetone. The absorbance of the extracts was measured at 645 and 663 nm using a spectrophotometer (T80 UV/VIS, PG Instruments Ltd, UK).

Chlorophyll fluorescence parameters

Three fully expanded leaves were selected from each plant for measurements. They were measured with a portable Photosynthetic Efficiency Analyzer (PEA) model (Hansatech Inc. Co., UK). The leaves were dark adapted for 30 min by fixing leaf clips on

each upper leaf blade before measurements were taken. After 30 min of dark adaptation, the sensor head was fitted on the leaf for measurement (Strasser *et al.*, 2000).

Proline content

Proline colourimetric determination proceeded based on the reaction with ninhydrin (Bates *et al.*, 1973). A 1:1:1 solution of proline, ninhydrin acid and glacial acetic acid was incubated at 100ºC for 1 hour. The reaction was arrested in an iced bath and the chromophore was extracted with 4 ml toluene and its absorbance at 520 nm was determined. Proline concentration was calculated with a standard curve and expressed as µg g^{-1} fresh mass.

Sucrose and starch

Fresh leaf tissue (0.5g) was ground with 5 ml of 80% ethanol and then extracted three times with 5 ml of 70% ethanol. After centrifugation (3500 ×g for 10 min.), 0.2 ml of supernatants were mixed with 0.1 ml of 30% KOH and heated at 100 °C for 10 min. After cooling (at room temperature) 3 ml anthrone (150 mg anthrone and 100 ml of 70% sulfuric acid) was added. After 10 min, the samples were cooled and their absorbance was read at 620 nm. Sucrose concentration was calculated with a standard curve and expressed as mg g^{-1} fresh weight (Van handel's, 1968). The ethanol-insoluble residue was used for starch extraction following the protocol of Rose (1991). After removing ethanol by evaporation, 2 mL of distilled water was added to the samples, and then the samples were incubated at 100°C for 15 min. Starch was hydrolyzed by adding 9.2 M and 4.6 M $HClO_4$ to the samples, separately. The content of starch was determined spectrophotometrically with anthrone reagent at A620 nm.

Total soluble proteins

0.5 g of leaf sample was weighed and ground with 5 ml potassium phosphate buffer (50mM, pH 7.2) and centrifuged at 15000 rpm for 10 min. 1 ml of supernatant was taken and 5 ml of Bradford's reagent was added to it and vortexed immediately. After 25 minutes, the light absorbance of the solution was recorded at 595 nm. The protein concentration was expressed in mg g^{-1} fresh weight of the leaf. Bovine serum albumin was used to prepare the standard curve (Bradford, 1976).

Total phenolic compounds

0.1 g of fresh leaf sample was ground in 5 ml of 95% ethanol and kept in the dark for 48 hours. After this period, 1 ml of the supernatant solution was taken and 1 ml of 95% ethanol was added to it and the volume was brought to 5 ml by distilled water. The extraction was done four times. Total phenolics were determined colourimetrically using a Folin-Ciocalteu reagent as described by Pinelo *et al.* (2004). Tenfold diluted reagent (2.5 ml) and 5% sodium carbonate (1ml) and ethanolic extract (0.5 ml) were mixed and kept in the dark for 1 hour and then the absorbance of the samples was measured at 725 nm against blank. The phenolic content was reported as mg tannic acid equivalent per gram fresh weight of the sample.

Data analysis

Data processing was performed by SPSS 20 software. Duncan's test was used for comparing the mean values. Graphs were drawn with Microsoft Excel.

Results

Root and shoot dry weight

According to the analysis of variance results, root and shoot dry weight was influenced by rootstock type, while the effect of drought and recovery periods and their interaction had no significant impact on dry biomass production (Table 1). Among the investigated rootstocks, the highest and lowest shoot dry weight was produced by LNT and SARKH, respectively (Fig. 1-a). The highest amount of root dry weight was

recorded by BB in comparison to other rootstocks (Fig. 1-b).

Table 1. Analysis of variance results concerning the effect of rootstock, drought-recovery and their interaction on shoot and root dry weight

Source of variations	df	Mean squares	
		Shoot dry weight	Root dry weight
(R) Rootstock	4	28.28**	12.08**
(SS) Sampling stage	1	0.20ns	0.11ns
R×SS	4	1.17ns	0.20ns
Error	14	1.39	0.64
cv	-	18.1	29.6

* and ** significant at probability of 1% and 5% respectively, ns: non-significant

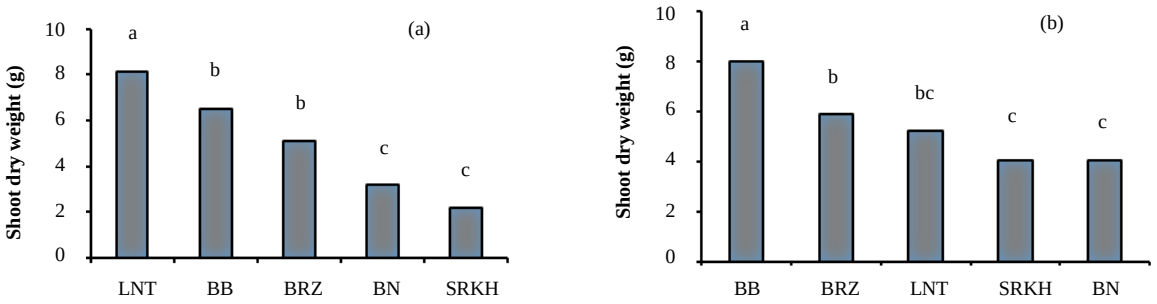

Fig. 1. Effect of rootstock on shoot (a) and root dry weight (b) of pistachio seedlings
BN: Bane, LNT: *Pistacia lentiscus*, BB: Bane-Baghi, SRKH: Sarakhs, BRZ: Badami-Riz-Zarand
Columns with at least one common word have no significant difference at 5% probability based on Duncan test.

Chlorophyll fluorescence and pigments

The effects of experimental treatments on chlorophyll fluorescence and chlorophyll pigments are presented in Table 2. As it is evident from the results, the main effects of treatments and their interactions caused a significant difference in measured parameters.

Table 2. Analysis of variance results concerning the effect of rootstock, drought-recovery and their interaction on chlorophyll fluorescence parameters of pistachio seedlings

Source of variations	df	Mean squares			
		Fv/Fm	PI	Chl a	Chl b
Rootstock (R)	4	0.23**	125.21**	0.46**	0.02ns
Sampling stage (SS)	5	0.17**	65.54**	0.87**	0.58**
R×SS	20	0.06**	33.26**	0.12**	0.16**
Error	48	0.003	3.20	0.02	0.01
cv	-	4.5	5.8	16.38	25.13

* and ** significant at probability of 1% and 5% respectively, ns: non-significant

The interaction of drought-recovery treatments and rootstock showed that no significant change in maximum efficiency of PSII (Fv/Fm) occurred until 10 days after the onset of drought stress, but at the last stage of sampling in drought period (15th day), except for BN, a decrease was observed in other rootstocks (Table 3). The highest reduction in this stage was related to LNT by 60% in comparison with the previous stage (10th day). Fv/Fm was increased during the recovery period in all rootstocks and reached to

pre-drought stage except with LNT seedlings which dried up in the recovery stage (Table 3). The interaction results of drought and recovery treatments and type of rootstock showed that no significant change in performance index (PI) occurred until 10 days after the onset of drought stress, but at the end of the drought stress period (15^{th} day), a significant decrease was observed in all rootstocks ($p \leq 0.01$). The highest decrease was related to LNT by 98% and the lowest was related to BN by 34% in comparison with the previous stage. After 10 days of recovery, PI returned to the pre-drought stage. The same increase was observed in SARKH and BN while all LNT seedlings were dried. In BRZ seedlings, PI remained unchanged until 10 days after drought stress commencement and decreased sharply up to the end of the drought period and the recovery treatment had no significant effect on this parameter (Table 3).

Biochemical parameters

ANOVA results concerning the effects of rootstock and drought-recovery treatments on biochemical parameters are presented in Table 4. From the results, it is apparent that almost of the measured parameters were influenced by the main and interaction effects of experimental factors ($p \leq 0.01$).

Chlorophyll pigments

Comparison of means showed that at the pre-drought stage, the highest amount of chlorophyll a was related to BN and SRKH and the lowest to LNT and BRZ. When the seedlings entered to drought stress stage, a significant increase in the concentration of chlorophyll a was observed in LNT and BRZ by 60% and 43% respectively in comparison with the pre-drought stage. However, at the recovery stage the amount of chlorophyll a was decreased in BRZ, SRKH and BB by about 32%, 35% and 27% respectively in comparison to the pre-drought stage. Chlorophyll a remained unchanged in BN throughout the experiment. Changes in leaf Chlorophyll b at different sampling stages and between the rootstocks were negligible (Table 5).

Proline

Response of rootstocks to drought-recovery period in terms of leaf proline accumulation was completely different. In BN, it started to increase at drought stage and reached its maximum value at the end of the recovery period (Table 5). SRKH and BRZ produced the highest amount of proline under drought stress conditions and returned to pre-drought stage values after 10 days of recovery (Table 5). Proline concentration in the leaf of BB seedlings was not affected by drought and recovery periods (Table 5).

Sucrose

Changes in sucrose concentration in the leaves of different rootstocks during drought and recovery periods had a trend similar to that of proline. The concentration of sucrose in the leaves of BB seedlings increased during the drought and recovery periods, so that at the end of the recovery phase, it was about 3.5 times more than the pre-drought stage (Table 5). In SRKH and BRZ, the amount of sucrose increased at the end of the dry phase and decreased at the end of the recovery stage, although these changes were not statistically significant. In BB seedlings, sucrose concentration was not influenced by drought and recovery periods (Table 5).

Table 3. Interaction of rootstock and sampling stage on chlorophyll fluorescence parameters of pistachio seedlings

Sampling time	BRZ	SRKH	BB	LNT	BN	BRZ	SRKH	BB	LNT	BN
	PI					Fv/Fm				
5DBD	7.77bc	5.31c-f	6.14c-e	7.36b-d	12.47a	0.78a	0.72ab	0.75a	0.78a	0.78a
5DAD	4.81c-g	6.78b-d	7.02b-d	7.25b-d	12.02a	0.74a	0.75a	0.76a	0.77a	0.79a
10DAD	4.56c-g	5.32c-f	7.24b-d	4.62c-g	10.2ab	0.74a	0.74a	0.77a	0.74a	0.79a
15DAD	0.47hi	1.19g-i	1.49g-i	0.11i	8.14bc	0.4e	0.6cd	0.52d	0.29f	0.76a
5DAR	1.94f-i	2.3e-i	1.48g-i	0.00i	10.02ab	0.62b-d	0.68a-c	0.62b-d	0.00g	0.79a
10DAR	2.84e-i	4.6c-g	3.87d-h	0.00i	12.74a	0.71a-c	0.73ab	0.71a-c	0.00g	0.80a

Columns with at least one common word have no significant difference at 5% probability based on Duncan test. 5DBD: 5 days before drought, 5DAD: 5 days after drought, 10DAD: 10 Days after drought, 15DAD: 15 days after drought, 5DAR: 5 days after recovery, 10DAR: 10 Days after recovery

Table 4. Analysis of variance results concerning the effect of rootstock, drought-recovery (sampling stage) and their interaction on some biochemical parameters of pistachio seedlings

Source of variations	df	Mean squares				
		TPC	TSP	Starch	Sucrose	Proline
Rootstock (R)	4	0.29**	10311**	76.2**	3740**	313.7**
Sampling stage (SS)	2	0.26**	3757**	28.7**	976ns	83.6**
R×SS	8	0.07**	4258**	13.4**	1138**	47.9**
Error	30	0.01	16.30	3.17	325	14.7
cv	-	25.76	2.59	30.82	51.04	35.66

* and ** significant at probability of 1% and 5% respectively, ns: non-significant;TSP: total soluble proteions, TPC: total phenolic compounds

Chlorophyll pigments

Comparison of means showed that at the pre-drought stage, the highest amount of chlorophyll a was related to BN and SRKH and the lowest to LNT and BRZ. When the seedlings entered to drought stress stage, a significant increase in the concentration of chlorophyll a was observed in LNT and BRZ by 60% and 43% respectively in comparison with the pre-drought stage. However, at the recovery stage the amount of chlorophyll a was decreased in BRZ, SRKH and BB by about 32%, 35% and 27% respectively in comparison to the pre-drought stage. Chlorophyll a remained unchanged in BN throughout the experiment. Changes in leaf Chlorophyll b at different sampling stages and between the rootstocks were negligible (Table 5).

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Changes in sucrose concentration in the leaves of different rootstocks during drought and recovery periods had a trend similar to that of proline. The concentration of sucrose in the leaves of BB seedlings increased during the drought and recovery periods, so that at the end of the recovery phase, it was about 3.5 times more than the pre-drought stage (Table 5). In SRKH and BRZ, the amount of sucrose increased at the end of the dry phase and decreased at the end of the recovery stage, although these changes were not statistically significant. In BB seedlings, sucrose concentration was not influenced by drought and recovery periods (Table 5).

Starch

Except with LNT, starch accumulation was increased in other rootstocks at the end of the drought period. However, the differences were significant just in the case of BN and SRKH in comparison with the pre-drought stage. During recovery, starch accumulation was continued to increase in BN and BB and reached its maximum value although it was not different statistically in comparison with the previous stage. In SAR, it was reduced to the pre-drought stage. Starch accumulation in the leaves of BRZ seedlings remained unchanged throughout the experiment. The maximum mean starch accumulation was observed in BN and SAR while the minimum was recorded in LNT (Table 5).

Total soluble proteins (TSP)

Results showed that the effects of rootstock and period of drought and recovery, as well as their interaction, had a significant impact on leaf TSP (Table 4). The highest amount of TSP at the pre-drought stage was found in BN and the lowest in LNT. When the seedlings entered the drought stress stage, the amount of TSP in SRKH increased by about 4% and no significant difference was observed in other rootstocks. When the seedlings started the recovery stage by re-watering, the TSP in leaves of all rootstocks had no significant change in comparison with the previous stage (Table 5).

Table 5. Interaction effects of sampling stages and rootstock on some biochemical parameters of pistachio seedling

Sampling stages	Rootstock	TPC (mg g^{-1}fw)	TSP (mg g^{-1}fw)	Starch (mg g^{-1}fw)	Sucrose (mg g^{-1}fw)	Proline (µmol g^{-1}fw)	Chlorophyll a (mg g^{-1}fw)	Chlorophyll b (mg g^{-1}fw)
Before drought stress	BN	0.59 b	177.9 a	6.80 b	27.06 c-e	15.85 bc	1.12 a-c	0.48 bc
	LNT	0.36 cd	140.7 f	3.85 b	19.32 de	6.00 d-g	0.70 d	0.33 cd
	BB	0.28 cd	164.8 de	3.99 b	32.82 c-e	7.28 d-f	0.92 b-d	0.46 bc
	SRKH	0.39 b-d	165.3 de	4.59 b	30.46 c-e	8.88 c-f	1.10 a-c	0.47 bc
	BRZ	0.23 d	169.6 c-e	3.69 b	30.85 c-e	4.56 fg	0.76 d	0.33 cd
End of drought stress	BN	0.95 a	174.9 a-c	10.60 a	75.10 ab	17.72 b	1.34 a	0.59 b
	LNT	0.28 cd	146.8 f	3.36 b	14.66 de	7.84 d-f	1.12 a-c	1.18 a
	BB	0.41 b-d	163.0 e	4.95 b	30.01 c-e	8.98 c-f	1.19 ab	0.51 bc
	SRKH	0.83 a	173.1 a-c	11.44 a	59.26 bc	18.38 b	1.24 a	0.51 bc
	BRZ	0.48 bc	174.4 a-c	6.15 b	40.60 cd	13.19 b-d	1.09 a-c	0.49 bc
End of recovery	BN	0.38 b-d	177.4 ab	12.28a	97.44 a	25.91 a	1.17 ab	0.39 b-d
	LNT	0.00 e	0.00 g	0.00 c	0.00 e	0.00 g	0.00 e	0.00 e
	BB	0.45 bc	170.0 b-e	5.16 b	58.59 c-e	9.07 c-f	0.86 cd	0.34 cd
	SRKH	0.60 b	170.10 b-e	6.26 b	31.40 c-e	12.04 b-e	0.80 d	0.32 cd
	BRZ	0.33 cd	171.30 a-d	3.58 b	13.84 de	5.69 e-g	0.74 d	0.25 d

Columns with at least one common word have no significant difference at 5% probability based on Duncan test.
TSP: total soluble proteins, TPC: total phenolic compounds

Total soluble proteins (TSP)

Results showed that the effects of rootstock and period of drought and recovery, as well as their interaction, had a significant impact on leaf TSP (Table 4). The highest amount of TSP at the pre-drought stage was found in BN and the lowest in LNT. When the seedlings entered the drought stress stage, the amount of TSP in SRKH increased by about 4% and no significant difference was observed in other rootstocks. When the seedlings started the recovery stage by re-watering, the TSP in leaves of all rootstocks had no significant change in comparison with the previous stage (Table 5).

Total phenolic compounds (TPC)

Drought stress increased TPC in leaves of BN, SRKH and BRZ seedlings. The amount of this parameter in these rootstocks decreased in response to the recovery period and returned to the initial values of the pre-drought stage. In BB and LNT rootstocks, its changes were not significant statistically (Table 5).

Discussion

The present study was planned to identify the response of some pistachio rootstocks to drought and recovery periods at the seedling stage and to find a sensitive index that can be used to evaluate the tolerance of pistachio rootstocks to drought and their recovery ability. Therefore, the effect of drought and recovery periods on PSII photochemistry and some biochemical parameters associated with better drought tolerance among pistachio rootstocks were investigated.

According to the results of this research, the period of drought and recovery caused the cessation of vegetative growth, which means that the period of recovery could not force the resumption of growth (based on dry matter production in shoots and roots) in seedlings regardless of rootstock type. However, the development of new leaf buds was observed in BN and BB at the end of the recovery stage (visual observations). The results showed that only the LNT seedlings were affected by drought stress and completely dried which indicates their lower resistance to drought stress. Changes in the shoot and root dry weight was under the effect of rootstock type (Fig. 1). The results obtained in this study are consistent with the results of previous studies (del Carmen Gijón *et al.*, 2010; Esmaeilpour *et al.*, 2015; Fahimi *et al.*, 2016; Moriana *et al.*, 2018). The insignificant effect of drought and recovery period on growth parameters of pistachio rootstocks in this study (except with LNT) can be attributed to the short period of drought and recovery. Most pistachio cultivars and species are genetically resistant to drought due to some anatomical and physiological characteristics such as deep root system, thick cuticle layer and low stomatal conductivity (Arzani *et al.*, 2013). It seems that the drought stress and recovery period used in this experiment was not enough to completely restore the negative effects of drought stress.

Results of chlorophyll fluorescence parameters (Fv/Fm and PI) showed that they are completely sensitive to drought stress and recovery. Except for BB, Fv/Fm and PI indices in all rootstocks were decreased under the influence of drought stress and increased at the end of the recovery period, although this increase was not significant in the case of PI. These results are consistent with the results of previous studies (Esmaeilpour *et al.*, 2015). The results showed that no decrease in chlorophyll fluorescence indices was observed in any of the rootstocks until 10 days after the stopping of irrigation indicating the tolerance threshold of pistachio seedlings to drought stress. Based on these results, chlorophyll fluorescence parameters can be used as an effective tool in identifying drought-resistant and sensitive rootstocks (Fahimi *et al.*, 2016).

Plants face a variety of abiotic stresses that led to oxidative stress and obstruct normal growth and development of plants. To prevent cellular damage

caused by oxidative stress, plants accumulate specific compatible solutes known as osmolytes to protect the cellular structures (Behzadi Rad *et al.*, 2021). The most common osmolytes that play a crucial role in osmoregulation are proline, glycine-betaine, polyamines, phenolic compounds and soluble carbohydrates. These compounds stabilize the osmotic differences between the surroundings of the cell and the cytosol (Azevedo Neto *et al.*, 2010). Plants are more resistant to drought stress when they have a higher accumulation rate of osmolyte compounds. It is why the LNT seedlings could not withstand the drought, and as a result, they all dried up during the recovery period. A look at the results of Table 5 shows that none of the osmotic regulating compounds increased during drought stress in LNT seedlings. Accumulation of proline, sucrose, starch and TPC in BN, SAKH and BRZ were according to drought and recovery stages showing the activity of osmoregulation mechanism in these rootstocks (Table 5). These results are in agreement with previous studies on pistachio (Esmaeilpour *et al.*, 2016; Fahimi *et al.*, 2016; Shamshiri and Fattahi, 2014). There are some reports showing the accumulation of TSP under drought stress condition (Vaidya *et al.*, 2015). Usually under drought stress, an increase in total soluble proteins is a result of high amino acids content as plants accumulate small molecular mass proteins as there may be an increase de novo synthesis or inhibition of amino acids degradation (Azevedo Neto *et al.*, 2010) and higher protein content might impart better drought tolerance (Yadav *et al.*, 2013) as it helps in osmotic balance. However, our results showed no changes in TSP under drought and recovery periods in different rootstocks (Table 5). Seedlings of BB showed no significant difference in terms of osmolyte compounds under drought and recovery periods. On the other hand, they had the most vigorous root system among the other rootstocks (Fig. 1-b). It can be assumed that with a more efficient root system, it was less affected by drought stress and therefore did not enter the osmotic regulation stage. Confirming this hypothesis requires more experiments with a more extended period of drought stress.

Conclusions

Based on the results of this research, except for LNT which was the most sensitive species to drought stress, the others had almost the same response to the period of drought and recovery. However, BB seedlings were superior in terms of growth. According to the aim of this experiment, the most sensitive indicators to drought stress and recovery were chlorophyll fluorescence parameters, which due to the ease of measurement, can be used to evaluate the response of different varieties and species of pistachio to drought stress if these results are confirmed by field experiments.

Acknowledgements

This research is a part of M.Sc. thesis of the second author. Financial support by Vali-e-Asr University of Rafsanjan is greatly acknowledged.

Conflict of interests

The authors declare that there is no conflict of interest.

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