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ORIGINAL PAPER

EVALUATION OF ALMOND CULTIVARS FOR THE MORPHOLOGICAL, PHYSIOLOGICAL, AND NUTRITIONAL TRAITS UNDER WATER DEFICIT CONDITIONS[1]

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Abstract

Drought stress is one of the most critical environmental stresses. Water deficit is a multidimensional stress and affects physiological, morphological, biochemical and molecular traits in plants. Many plants could be improving their tolerance mechanisms over drought stress, although the mechanisms vary and depend on a plant species. Almond species are mainly known as drought-tolerant trees and shrubs and are adapted to arid and semi-arid environments. This study aimed to evaluate morphological, nutritional, and physiological responses of 4 almond cultivars (Talkh, Nonpareil, Azar, and Sanghi) under drought stress. During 90 days of the growing season, drought treatment was applied at 4 levels of field capacity (control, 75%, 50% and 25%). To assess the response of the almond varieties to drought stress, the plant height, root length, internode length, fresh (FW) and dry weight (DW) of shoot and root, leaf area, proline, malondialdehyde (MDA) and elements content such as N, P, K, Ca, Mg and Fe were evaluated. The present study results showed that drought stress decreased morphological traits and increased proline and MDA accumulation in all the evaluated almond cultivars. Also, increasing drought stress was associated with reducing the content of the element in all the cultivars. According to our findings, it can be concluded that Sanghi and Azar cultivars are more tolerant to drought stress than Talkh and Nonpareil.

Keywords: almond, antioxidant enzyme, macronutrient, drought.

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INTRODUCTION

Almond is one of the oldest nut crops as well as one of the most commercially produced nuts around the world nowadays. The almond germplasm is a valuable source of important physiological characteristics such as drought tolerance that can be identified and used for breeding plans (Akbarpour et al. 2017). Environmental stress is one of the severe threats to agricultural crops. Drought stress is one of the most important abiotic stresses causing significant changes in physiological and biochemical activities, including photosynthesis, respiration, metabolism, hormones, and enzymatic activities in most plants (Okunlola et al. 2017). Under drought stress, the plant cell membrane is more sensitive to rapid damage and electrolyte leakage. This membrane leakage is caused by uncontrolled free radicals, which trigger lipid peroxidation. Degradation of cell membrane fatty acids can produce small hydrocarbons such as malondialdehyde (MDA). In other words, MDA is the final product of lipid peroxidation and is a sign of membrane cell damage (Cheng et al. 2018).

Isaak et al. (2013) reported that environmental factors could strongly affect plants' oxygen radicals and MDA accumulation. Drought stress modifies MDA, H_2O_2, and proline accumulation in plants. Reactive oxygen species (ROS), including O_2, H_2O_2, O^{2-}, and OH^-, are produced by plants during cellular metabolism under stress conditions (Jyoti, Sudesh 2012). Excessive ROS results in oxidative damage to cellular structures, cell metabolism and macromolecules, such as membrane lipids, nucleic acids, and proteins (Chakhchar et al. 2016). The plants ability to use antioxidant enzyme activity to reduce drought stress varies in cultivars, plant growth stages and even in different drought stress conditions (Zhang et al. 2019). Along with soluble osmolytes, proline plays a significant role in free radical scavenger, cell regenerative balancer, cytosolic pH buffer, and stabilizer of the intracellular structure during osmotic stress. Accumulation of proline under drought stress reduces atomic oxygen production and thus decreases the likelihood of damage to the thylakoid membrane (Lawlor, Cornic 2002, Zhang et al. 2019).

A reduction in the uptake and transfer of macroelements has been reported in various plant species under drought stress, due to reduced root volume and inaccessibility of the nutrients in dry soils (Noman et al. 2018). Drought stress also reduces the active transport and membrane permeability of cations (K^+, Ca^{2+}, and Mg^{2+}), thus reducing the uptake of these cations through the root (Farooq et al. 2012). A similar reduction of the Ca, K, and P concentrations has been reported in wheat roots and shoot under water stress (Noman et al. 2018). Drought can also cause the deficiency of trace elements, such as Fe, Mg and Mo (Hu, Schmidhalter 2005). Sohag et al. (2020) reported that drought stress reduced the content of Ca, K and Mg in rice compared to the control.

Gholamhoseini et al. (2013) stated that drought stress significantly reduced the aerial part of sunflowers. In a study carried out by Sohag et al. (2020), it was reported that drought stress reduced shoot and root fresh weight (FW) and dry weight (DW) in rice seedlings. This study aimed to assess the effect of drought stress on morphological, physiological and nutritional response in different almond cultivars to identify a cultivar suitable for arid and semi-arid areas and to identify marker parameters for rapid screening almond seedlings.

MATERIALS AND METHODS

The experiment was carried out in the horticultural research greenhouse of Maragheh University in 2019-2020. Greenhouse environmental conditions included a temp. of 25-30°C in daylight and 15-20°C in nighttime, relative humidity of 60-70%, and natural light of 70-80%. The drought treatments corresponded to 4 levels: control, 75%, 50%, and 25% of field capacity (FC), applied to 4 cultivars: Talkh, Nonpareil, Azar, and Sanghi. Three seedlings were planted in each pot with a capacity of 7 liters, leaving 2 plants per pot after germination. The soil of the pots was sandy loam with pH=7 and EC=0.36. Half Hoagland solution was used to meet the nutritional requirements. After the required growth of seedlings, drought stress was applied based on field capacity for 90 days.

After 90 days, morphological characteristics, such as shoot length, shoot diameter, internode length and root length, were measured. Also, shoot and root DW were measured after drying in an oven at 72°C for 48 h after their FW measurement. Leaf area (LA) was recorded using a Leaf Area Meter (CI 304 CID model). The content of Ca, Fe, P, K and Mg of plants was measured by the fresh digestion method. Ninety days after the drought treatment, fully developed leaves were sampled and washed with distilled water. Then, they were dried in an oven at 55-65°C and digested using nitric acid-hydrochloric acid (4:1 v/v) and K was measured in a flame photometer (Jenway, PFP7 Germany) while Ca, Mg, P and Fe were measured using the atomic absorption (Shimadzu, AA6300, Japan) method. After grinding, to measure nitrogen of plant tissues, 2 g of each sample was separated. The samples are digested in special tubes with sulfuric acid, salicylic acid, hydrogen peroxide and selenium. The nitrogen concentration was measured in plant tissues by the titration method after distillation using Kjeldahl Auto 1030 Analyser Techator equipment (Emmami 1996).

The proline content was determined according to Bates et al. (1973). First, 0.5 g of plant leaf tissue was digested with 10 ml of 3% sulfosalicylic acid. After centrifugation, 2 ml of the extract and 2 ml of ninhydrin acid were mixed with 2 ml of glacial acetic acid and placed in a water bath. Then, 4 ml of toluene were added and results were read at 520 nm.

The MDA content was measured according to Heath and Packer (1968). Then, 0.5 g of fresh leaf sample was digested with 1.5 ml of 0.1% trichloroacetic acid. After centrifugation, 0.5 ml of 0.1% thiobarbituric acid containing 20% trichloroacetic acid was added. Then, it was heated at 95°C for 30 min and instantly placed on ice to stop the reaction, and results were read at 532 and 600 nm.

The study was conducted as a factorial experiment based on a completely randomized design (CRD) with four replications. ANOVA was performed using MSTATC software (ver. 2.1 Michigan University), and comparison of the means of data was accomplished with the Duncan's multiple range test at a probability level of 5%.

RESULTS AND DISCUSSION

The results revealed that plant height decreased with the application of drought stress in different almond cultivars. The lowest reduction was observed in Nonpareil cultivar (46%) and the highest reduction was observed in Azar and Sanghi cultivars (52%). The effects of different drought treatments on almond cultivars indicate a reduction in internode length during the applied stress. According to the results, it can be stated that the highest and the lowest decrease occurred in Talkh cultivar (17%) and Azar cultivar (14%), respectively (Figure 1*a*,*b*). The results suggested that the effect of cultivars and treatment was significant in shoot diameter, so that the highest shoot diameter was observed in Sanghi cultivar, decreasing with the increasing drought stress: the highest diameter was observed in control (0.2813 mm) and the lowest – in the 25% FC (0.2694 cm) – Figure 1*c*,*d*. Based on the results, the highest root length was observed at 50% and 75% FC in Talkh cultivar (46.15 cm and 45.19 cm, respectively), and the lowest was observed at 25% FC in Azar cultivar (34.81 cm). Application of drought stress on different almond cultivars significantly reduced leaf area. According to the findings, the highest reduction in leaf area up to 80% was observed in the Azar cultivar. The lowest reduction up to 60% was observed in the Sanghi cultivar (Figure 1*e*,*f*). Our studies suggest that the shoots FW in different almond cultivars was significantly reduced with increasing drought stress, so that the highest shoot FW was observed in control and in Sanghi cultivar (4.832 g), and the lowest was observed in the 25% FC in Talkh cultivar (1.938 g). The results of shoot DW showed a reduction against drought stress in different cultivars so that the highest shoot DW was observed in the 75% FC and control (2.415 g and 2.365 g, respectively) in Sanghi cultivar, and the lowest was observed in the 25% FC (0.777 g) in Talkh cultivar (Figure 2*a*,*b*). The highest and lowest root FW were observed in Sanghi cultivar (5.765 g) and Talkh cultivar (2.128 g), respectively. The reduction of root DW in all the almond cultivars under water deficit was the same. The highest and low-

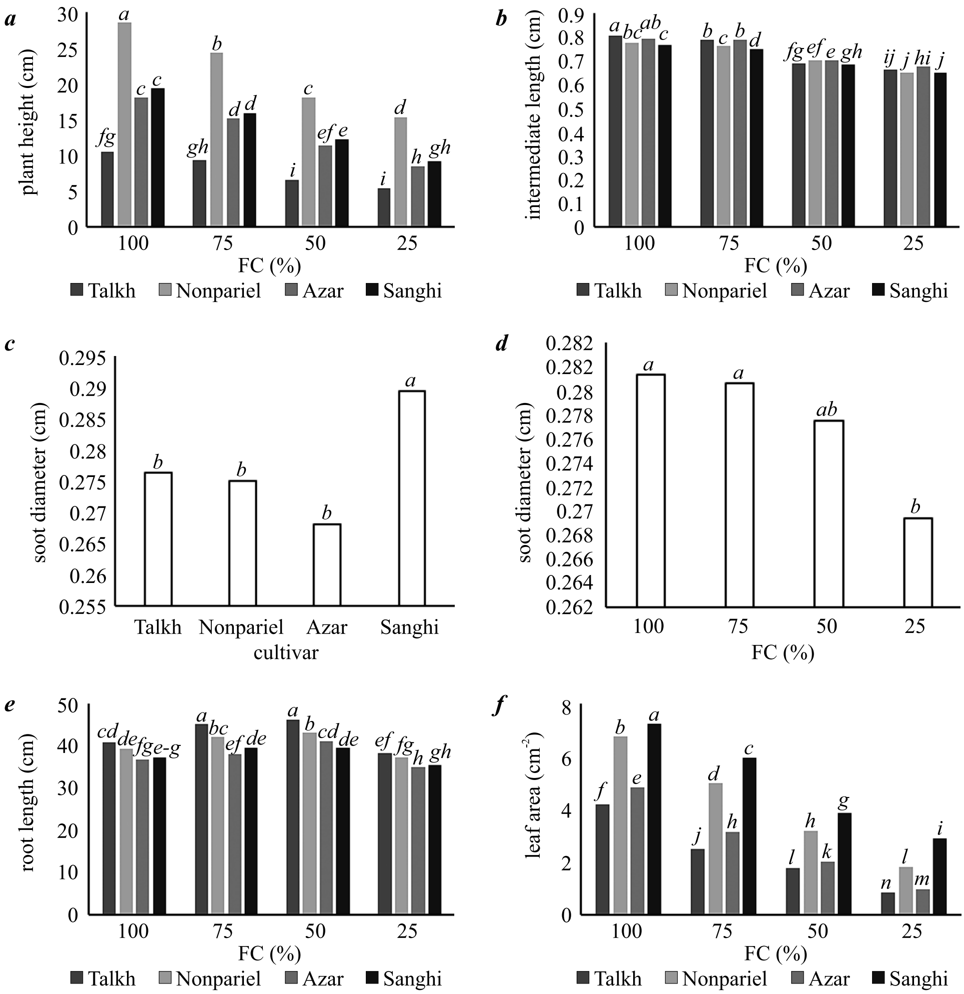

Fig. 1. The effect of drought stress and almond genotypes on shoot length (*a*), intermediate length (*b*), root length (*e*) and leaf area (*f*), the effect of genotypes on shoot diameter (*c*) and drought stress on shoot diameter (*d*). FC refers to field capacity. Means with the same letter in each figure are not significantly different by the Duncan multiple ranges at $P<0.05$

est dry weight reduction was observed in Nonpareil (26%) and Azar (18%) cultivars, respectively (Figure 2*c*,*d*). According to the results of this study, drought stress has a significant effect on morphological traits. With the increasing drought stress, plant growth is inhibited more severely. The plant growth is probably inhibited due to the reduced turgor pressure and low cell metabolism (Singh et al. 2018). The reduction may also be due to decreased net uptake caused by the diminution in leaf water potential. The effect of water stress on growth parameters may be highlighted, since the photosynthesis rate may be faster than respiration under water deficit stress (Rao

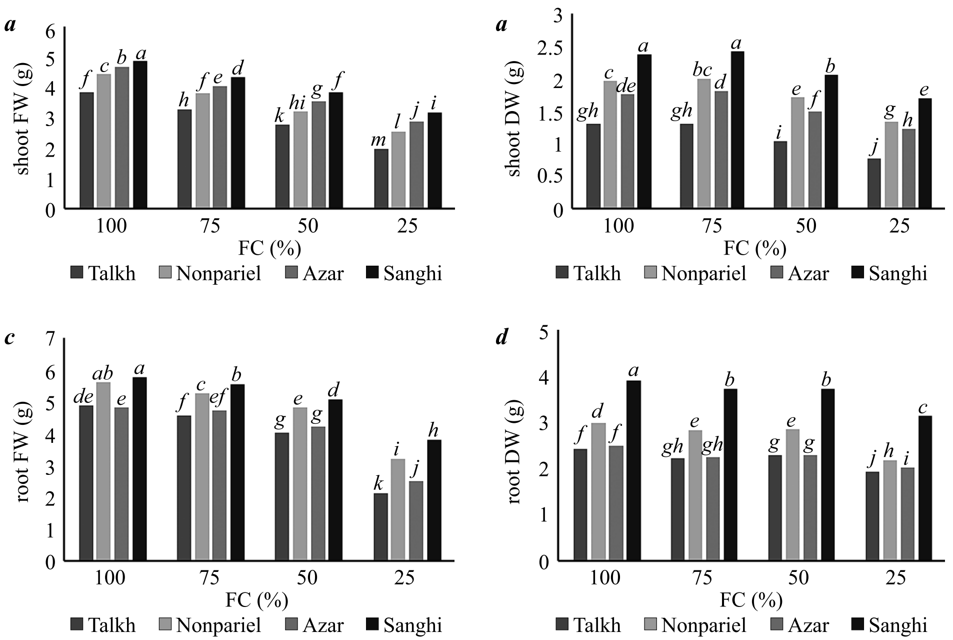

Fig. 2. The effect of drought stress and almond genotypes on shoot FW (*a*), shoot DW (*b*) root FW (*c*), root DW (*d*). FW, DW and FC refer to fresh weight, dry weight, and field capacity. Means with the same letter in each figure are not significantly different by the Duncan multiple ranges at $P<0.05$

et al. 2008). Also, water stress reduces plant growth by reducing cell division and cell enlargement, which leads to a severe reduction in plant growth (Osakabe et al. 2014). Morphological response to drought stress is an avoidance mechanism revealed in our experiment by regulating the plant growth rate, resulting in the reduction of plant height as well as dry weight of shoots and roots in different almond cultivars. Our results align with the studies reported by Ranjbar et al. (2019) and Sohag et al. (2020*a*).

In our experiment, the leaf N content was investigated at different drought levels, demonstrating that the N content in all the cultivars decreased significantly with increasing drought stress. The highest N content was observed in Nonpareil cultivar (2.327%), and the lowest was determined in Talkh cultivar (0.915%) under 100% and 25% FC, respectively – Figure 3*a*. The results showed that the application of drought stress reduced the Ca content significantly in all the almond cultivars, so that the highest Ca content was observed at 100% FC of Sanghi cultivar (5.628%) and the lowest at 25% FC of Talkh cultivar (3.063%) – Figure 3*b*. Drought stress caused a significant reduction in the Mg content in all the cultivars. The most tolerant and sensitive cultivars to drought stress in the Mg content were Azar and Talkh, with 29% and 38% reduction, respectively (Figure 3*c*). The results revealed that the highest P content was observed in the Nonpareil cultivar

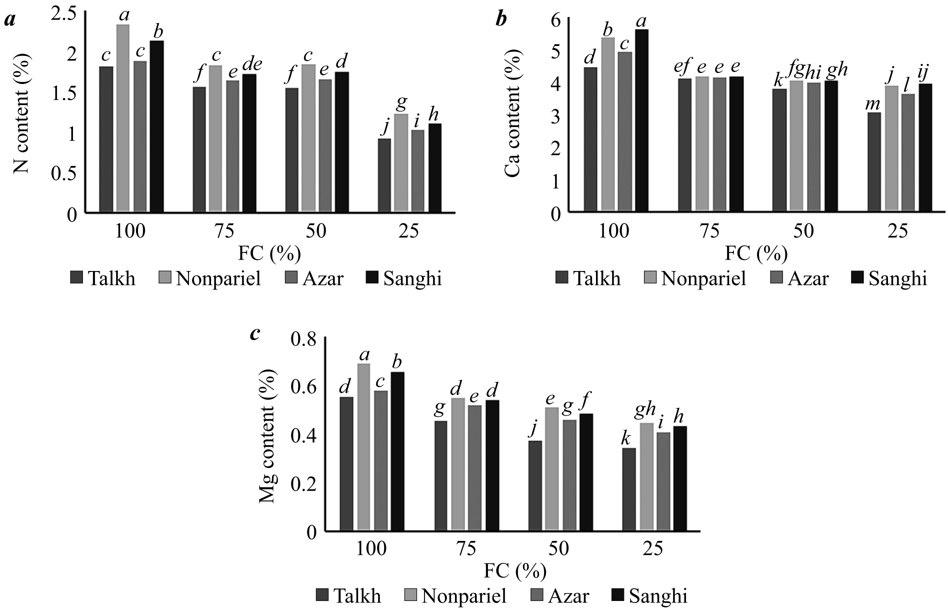

Fig. 3. The effect of drought stress and almond genotypes on N (*a*), Ca (*b*), and Mg concentration (*c*). N, Ca, Mg, and FC refer to nitrogen, calcium, magnesium, and field capacity. Means with the same letter in each figure are not significantly different by the Duncan multiple range at $P<0.05$

and the lowest in the Talkh cultivar. Application of water deficit caused a significant reduction of 25% in the P content at the highest level of stress (Figure 4*a*,*b*). Based on the findings, the effects of cultivar were significant on the K concentration, so the highest K content was observed in Nonpareil cultivar (Figure 4*c*). Furthermore, drought stress had a significant effect on the K content, and the highest was observed in control, and the lowest was observed in 25% FC (Figure 4*d*). The results showed that the application of drought stress caused a 36% reduction in the Fe content at 25% FC compared to control. Also, the Fe content was significantly different among the evaluated cultivars. The highest Fe concentration was observed in the Nonpareil cultivar and the lowest in Talkh (Figure 4*e*,*f*). Minerals play a major role in improving plant tolerance to stress conditions (Marschner 2011, Aazami et al. 2021). Drought stress reduces nutrient availability, uptake, transport, and metabolism (Farooq et al. 2009). K is an essential element in most plants, being an important osmolyte mineral in regulating osmotic and turgor pressure and plays a key role in plant growth and leaf stomatal conductance (Marschner 2011). Lower K reduces the osmotic pressure of protective cells, thus reducing the ability of cells to swell and perform partial closure of stomata (Battie-Laclau et al. 2013). Urbina et al. (2015) showed that K decreased under extreme stress in a single and mixed planting of plants. The lower content of K observed in the present study leads to stomatal dys-

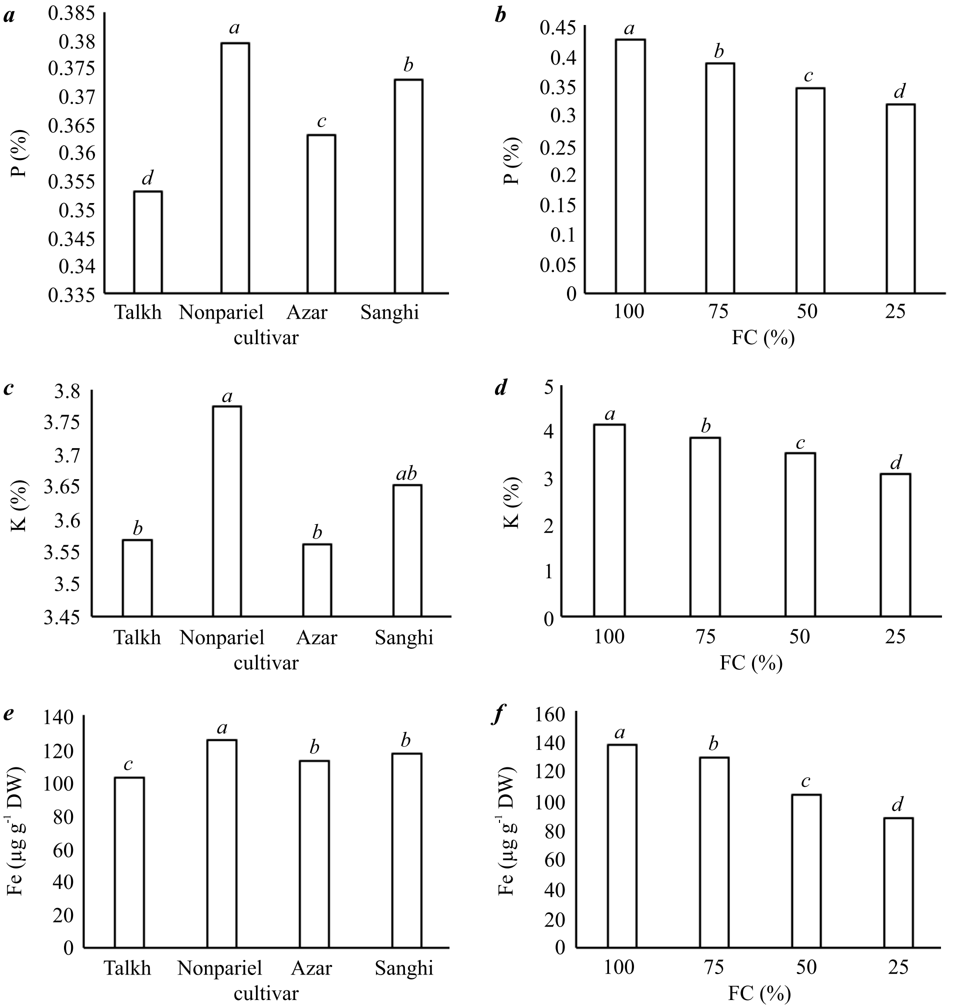

Fig. 4. The effect of genotypes on P (*a*), K (*c*), and Fe (*e*) concentration and the effect of drought stress on P (*b*), K (*d*), and Fe (*f*) concentration. P, K, Fe, and FC refer to phosphorus, potassium, iron and field capacity, respectively Means with the same letter in each figure are not significantly different by the Duncan multiple ranges at $P<0.05$

function, water loss due to poor stomatal regulation and reduced photosynthesis, causing poor almond growth in drought stress conditions. Mg uptake is influenced by soil conditions, such as drought or lack of access to water, which aggravates the symptoms of Mg deficiency. Since Mg is physically and physiologically unavailable under water deficiency conditions, plant roots cannot uptake enough Mg to maintain normal plant growth (Merhaut 2007). Brown et al. (2006) reported that Mg uptake was reduced in both roots and branches of *Spartina alterniflora* under drought conditions. The Mg concentration in tomato plants was reduced under drought stress conditions

(Nahar, Gretzmacher 2002). Ca ions are major secondary message transmitters that stimulate the basic physiological functions of cells in response to drought stress, and are required at almost all stages of plant growth. Ca also plays a major role in regulating the growth of polar cells and tissues, and in the adaptation of plants to stress. This element affects the closure of stomata due to abscisic acid and the adaptation of plants to drought stress. Drought stress tends to reduce the concentration of biomass Ca in the aerial parts, and this effect is attributed to the reduction in persistent transpiration changes (Sardans et al. 2008). Phosphorus is an essential mineral that is required in a relatively large amount to maintain plant growth. It plays a major role in maintaining and transferring energy to cell metabolism (Jin et al. 2006, Aazami et al. 2021). Under drought stress, P helps plants maintain leaf water potential, which increases stomatal conductance and photosynthesis rate (Waraich et al. 2011). Drought reduces P uptake and transport in plants (Sardans et al. 2008). Previous research suggests that drought stress regulates the balance of nitrogen metabolism through changes in nitrogen metabolic enzyme activities (Zhong et al. 2018, Zhang et al. 2019). Changes in enzyme activities can directly affect uptake efficiency and nitrogen use. Water deficiency and low nitrogen are the main limitations in wheat yield that cause the retarded water potential, chlorophyll fluorescence and photosynthesis processes as well as restricted plant growth rate, premature aging, reduced grain filling time, limited grain weight and poor yield (Mobasser et al. 2014). In general, water deficiency reduces the uptake of nutrients by the roots and their transfer to branches. Reduced mineral uptake can disrupt the nutrient uptake and depletion mechanisms, thereby reducing transpiration. Plant species and genotypes might show different reactions to the uptake of minerals under water stress (Hu, Schmidhalter 2005).

Based on the results, the effects of drought stress on the proline content showed that it had changed significantly under drought stress. Accordingly, the highest increase was observed in Sanghi cultivar (215%), and the lowest was observed in Talkh cultivar (101%). In addition, the highest content of proline was observed in the Nonpareil cultivar (715.3 μg g^{-1} FW), and the lowest was observed in the control of cultivar Azar (196 μg g^{-1} FW) – Figure 5*a*. The results revealed drought in all cultivars led to an enhancement of MDA content so that the highest level of MDA was observed in the highest stress level of Talkh cultivar (260 μmol g^{-1} FW) and the lowest was observed in the control of Sanghi cultivar (113 μmol g^{-1} FW) and Nonpareil cultivar (114 μmol g^{-1} FW). Also, the highest and lowest rate of increase in MDA content was observed in Talkh (119%) and Nonpareil (35%) cultivars, respectively (Figure 5*b*).

Proline is an essential organic material that maintains water content under stress through an osmotic protector and causes membrane stability (Liu et al. 2011). Proline accumulation is generally considered a drought tolerance mechanism in plants. Plants react to biotic stress by increasing

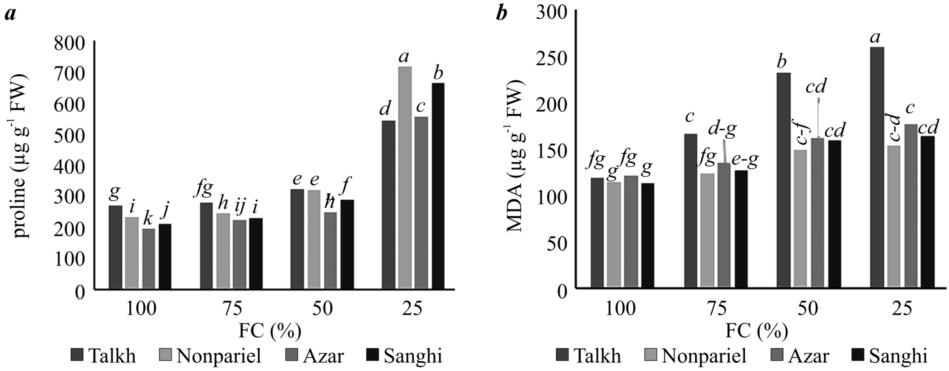

Fig. 5. Drought stress and almond genotypes affect proline (*a*) and MDA (*b*) content. FC refers to field capacity. Means with the same letter in each figure are not significantly different by the Duncan multiple range at $P<0.05$

proline concentrations, thus helping to maintain cell membrane integrity and protecting cell structure against damage to plants under drought stress (Penella et al. 2017). In a study conducted by Lawlor and Cornic (2002) on rice genotype, proline was significantly increased in genotypes under stress conditions. Oxidative stress in cells and tissues increases with increasing drought stress, so lipid peroxidation occurs with increasing water stress. The final lipid peroxidation product is MDA, which is used as an oxidative membrane damage indicator (Jaafar et al. 2012).

Furthermore, plant resistance can be assessed by measuring the MDA content. In the present study, the MDA content increased dramatically in the almond cultivars during exposure to drought compared to the control. Similar results were also reported in *A. tricolor* (Sarker, Oba 2018) and *Cucumis sativus* L. (Nie et al. 2018), which is in agreement with our findings.

The correlation of physiological attributes and nutritional characteristics is presented in Figure 6. A positive correlation was observed among nutritional traits, in addition to which a positive correlation was shown between morphological and nutritional attributes. On the other hand, a negative correlation was observed between proline and MDA with elements and morphological traits. The heat map (Figure 7) based on the response of almond cultivars' morphological, biochemical, and nutritional traits under drought stress revealed differentiations in all the cultivars. In plants under 100% FC, the N, P, K, Ca, Mg, and Fe concentrations were higher than under drought stress, and the accumulation of these elements decreased with increasing drought stress. Also, the other traits, such as shoot and root fresh weight, shoot and root dry weight, shoot length, shoot diameter and internode length, decreased under different drought stress. The results showed that root length increased in 50% FC.

On the other hand, MDA and proline were increased under drought stress compared to without water-deficit treatments. Cluster analysis and

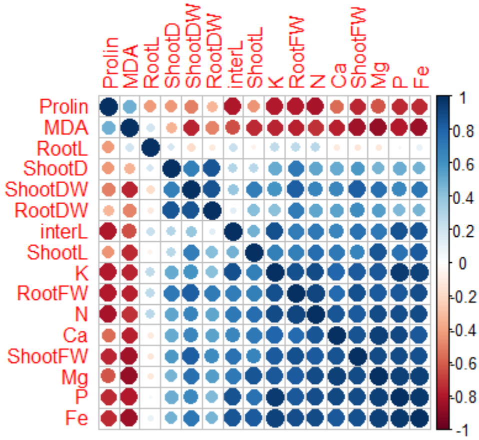

Fig. 6. Heat map of the Pearson correlation analysis. The studied attributes included: proline; MDA – malondialdehyde), RootL – root length), ShootD – shoot diameter, ShootDW – shoot dry weight, RootDW – root dry weight), interL – internode length, ShootL – shoot length, RootFW – root fresh weight, ShootFW – shoot fresh weight, N – nitrogen, K – potassium), P – phosphorus, Mg – magnesium, Ca – calcium and Fe – iron

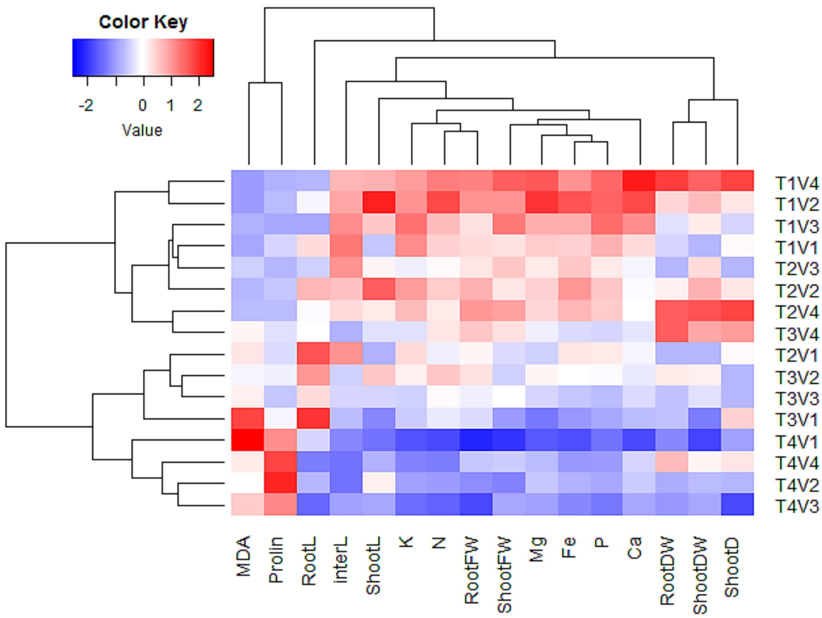

Fig. 7. Morphological, physiological, and nutritional changes in cultivars of V1 Talkh, V2 Nonpariel, V3 Azar and V4 Sanghi of almond. Heat map representing of proline, MDA – malondialdehyde), RootL – root length, ShootD – shoot diameter, ShootDW – shoot dry weight, RootDW – root dry weight, interL – internode length, ShootL – shoot length, RootFW – root fresh weight, ShootFW – shoot fresh weight, N – nitrogen, K – potassium, P – phosphorus, Mg – magnesium, Ca – calcium and Fe – iron responses in the cultivars under T1 100% FC, T2 75% FC, T3 50% FC and T4 25%FC

dendrograms in the heat map (Figure 7) showed two main groups in evaluated traits of the cultivars under different drought stress. Group 1 contained proline and MDA, and group 2 contained other traits, including shoot and root fresh weight, shoot and root dry weight, shoot length, shoot diameter and internode length. In general, cluster analysis of the heat map for cultivars under drought stress showed two main groups. Group 1 contained the cultivars under 100% and 75% FC, and group 2 comprised cultivars under 50% and 25% FC.

CONCLUSION

Morphological characteristics in response to drought stress in four almond cultivars were significantly affected. The almond cultivars' growth was more inhibited if less water was available. Drought stress led to decreased morphological traits and increased proline and MDA accumulation in all the four almond cultivars. Increased drought stress reduced the concentration of elements and changed their balance, which was observed in all the cultivars. Talkh cultivar was more sensitive than the other cultivars. According to these findings, it can be concluded that Sanghi and Azar cultivars are more tolerant to drought stress than Talkh and Nonpareil and could therefore be introduced as promising culticars, tolerant to drought stress, for use in breeding programs.

Conflicts of interest

The authors declare no conflict of interest.

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