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Influences of severe water stress on photosynthesis, water use efficiency and proline content of almond cultivars

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Abstract

Using drought tolerant almond cultivars under arid and semiarid regions such as Iran is important factor affecting production yield, especially in rainfed orchards. To evaluate responses of almond cultivars to drought stress under field condition, the experiment was carried out on six commercial cultivars namely 'Azar', 'Marcona', 'Mission', 'Nonpareil', 'Sahand', and 'Supernova'. Net photosynthesis rate (Pn) and water use efficiency (WUE) data during three stress periods indicated that Pn decreased in stress treatments, but WUE increased under stress treatments. The highest Pn occured in 'Azar' in July and August, and the highest WUE was recorded in 'Sahand' and 'Supernova'. Leaf abscission in 'Sahand' was very high and Supernova had no significant abscission. Leaf relative water content (RWC) showed a downward trend from June to August. In 'Azar', 'Nonpareil' and 'Supernova'cultivars, RWC resulted from severe stress treatment had close relationship with RWC in well-watered treatment. This result may be due to osmoregulation in leaves of stressed plants. So these cultivars could keep high water content in their leaves and tolerate severe drought stress conditions than other investigated cultivars. The highest and lowest proline accumulation was observed in the leaves of 'Marcona' and 'Sahand', respectively; both 'Marcona' and 'Sahand' were sensitive to drought stress than 'Supernova' which showed medium proline accumulation. In almond, accumulation of proline in response to longer interval between irrigation is a general trait and cannot be used as indicator for defining the tolerant trees. In general, 'Supernova' and 'Azar' showed best response under drought stress.

Key words: Drought tolerance, Pn, WUE, RWC, Proline, *Prunus dulcis* Mill.

Introduction

Water plays essential role in many physiological processes during plant life. Water absorbed by the roots is translocated to aerial portions of the plant and lost to the atmosphere via pores of the stomatal apparatus. Transpiration ratio is used to assess the plant effectiveness in regulating water loss which is essential for CO_2 uptake consumed in photosynthesis. An unbalance in water flow can result in water deficits and faulty functioning of numerous cellular processes (Taiz and Zeiger, 2006).

Photosynthesis and subsequently cell growth and productivity are severely affected by water potential and its components. Clearly, drought is one of the most common environmental factors affecting plant growth and productivity in arid and semiarid zones. WUE increases under reduced-water supply and this response to drought has an adaptive significance (Raviv and Blom, 2001).

Lower gas exchange reduces carbon assimilation in leaves under drought stress condition. Limitation of photoassimilates reduces vegetative growth and severely retard the development of plant reproductive organs (Boyer, 1970; Gehrmann, 1985; Singer *et al.*, 2003). Genotypic differences in drought tolerance have been observed for various crops (Bota *et al.*, 2001). Drought tolerant species have a capacity to maintain relatively high rate of photosynthesis under drought stress (Gu *et al.*, 1999). Romero *et al.* (2004) observed that during pre-harvest period, photosynthesis rate of drought-stressed almonds was lower than that of unstressed plants under control conditions, whereas during subsequent recovery their photosynthesis rate was the same as or even better than that of control plants.

Leaves of drought tolerant plants such as almond, olive, and some of forest trees can reach extremely low values of leaf RWC: 75-80%, before losing turgidity (Hinckley *et al.*, 1980; Lo Gullo and Salleo, 1988; Larsen *et al.*, 1989). One of the most widely distributed compatible osmolytes in higher plants is the amino acid proline. Accumulation of proline in plant leaves is related to irrigation intervals, as proline content in leaves increased sharply under drought stress and remained at higher levels during the stress condition (Al-Karaki *et al.*, 1996). The aim of this study was to characterize response of six almond cultivars to drought stress based on their photosynthesis activity, water use efficiency and proline accumulation in leaves.

Material and methods

Plant material and field condition: The experiments were carried out from May to September 2010 on six almond (*Prunus dulcis* Mill) cultivars *viz.*, 'Azar', 'Marcona', 'Mission', 'Nonpariel', 'Sahand', and 'Supernovoa'. These cultivars were raised in the open filed almond collection at Karaj-Iran (35 °55' N, 50 °54' E, 1312.5 m a.s.l.), under semi dry climate on calcareous soils (Table 1 and 2).

Experimental design: Almond cultivars 'Azar', 'Marcona', 'Mission', 'Nonpariel', 'Sahand', and 'Supernovoa' grafted on

Table 1. Soil properties at experimental site

Soil characteristics*	Depth (cm)		
	0-20	20-45	45-120
Soil texture	Sandy clay loam	Sandy clay loam	Sandy
Clay (%)	20-36	22-30	2-16
Silt (%)	26-36	22-38	4-18
Sand (%)	30-60	32-60	62-98
pH	7.4-7.8	7.6-7.9	7.5-7.8
EC	2.4	0.7	0.8
$CaCO_3$ (%)	10.4	14.5	9.8
Organic C (%)	0.6	0.27	0.1
Available P (mg kg^{-1})	11	2.6	2.3
Available K (mg kg^{-1})	225	86	54.5
Humidity percent in W.P	18.5	17	10
Humidity percent in F.C	9.5	9	5.3
Saturation humidity (%)	35.3	35.5	24.7

*Soil and Water Research Institute, Karaj, 2010

Table 2. Climatic conditions at experimental site, Karaj, 2010

Month	Apr	May	Jun	Jul	Aug	Sept	Oct
Average temperature (ºC)	17.2	20.3	26.6	32.4	28.4	25.6	19.6
Rainfall (mm)	35.4	28.8	0.2	0	1.2	0	2.0
Relative humidity percent	58	68	58	57	66	63	62

bitter almond seedling were planted at 5×5 m space in 2007. This experiment was arranged in a complete randomized design with three replications. Trees were subject to two irrigation regimes. In optimal irrigation regime (control), trees were irrigated to maintain 90–100% of soil water capacity (SWC); while in reduced irrigation regime (stress treatment), soil moisture was maintained at a level of about 10% of SWC. Both irrigation regimes were applied to the plants throughout their growing season. Rainfall was stopped at May 10th at the experimental site. The period of drought stress started since May 31th. Trees under stress and normal conditions were irrigated monthly and 7 days intervals, respectively.

Measurements: Net photosynthetic rate (Pn), photosynthetically active radiation (PAR) and transpiration rate (E) were measured by a LCA4 (ADC BioScientific Ltd., England) in the field at 10-12 AM at three stages during the stress period (25 June, 25 July, and 25 August). Water use efficiency was evaluated using following formula (Molden 1997):

$$WUE = Pn\ (\mu mol\ CO_2\ m^{-2}s^{-1}) / E\ (mmol\ H_2O\ m^{-2}s^{-1}).$$

Relative Water Content (RWC): Midday relative water content (RWC) was measured on June 25, July 25 and August 25 (beginning, middle and the end of drought stress) at the same time of day. To determine leaf fresh weight (FW), 5 leaves of each treatment/genotype were detached and weighed by electronic balance, Then leaves were hydrated until saturation (constant weight gained) for 48 h at 4 ^oC in darkness (turgidity weight: TW). Leaves were then dried in an oven at 105 ^oC for 24 h for determining dry weight (DW). RWC was calculated according to Filella *et al.* (1998):

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100.$$

Proline: Proline was measured according to the method described by Bates *et al.* (1973). In order to measure proline content changes within 30-day period of severe stress conditions, samples of leaves were collected from new shoots before irrigation on June 27th, July 27th and August 27th. Leaf sample of 0.5 g was used for extraction. A Shimadzu spectrophotometer (UV- 160A) was used for reading absorption at 520 nm.

Statistical analysis: The statistical analysis was performed using Microsoft Excel (Microsoft office version 2007 package) and SAS software (SAS Institute Inc, 1990) and means were compared using Duncan's Multiple Range Test at $P \leq 0.01$ (DMRT).

Results

Photosynthesis rate (Pn): As depicted in Fig. 1, at first stage, 'Nonpareil' with 3.93 µmol CO_2 $m^{-2}s^{-1}$ and 'Mission' with 3.93 µmol CO_2 $m^{-2}s^{-1}$ showed highest and lowest net photosynthetic rate, respectively. But in July and August, 'Azar' (5.2 and 2.9 µmol CO_2 $m^{-2}s^{-1}$) exhibited high Pn than other investigated cultivars. Pn of 'Sahand' and 'Nonpareil' (1.4 and 0.67 µmol CO_2 $m^{-2}s^{-1}$) were lowest at July and August, respectively.

Fig. 2 display Pn in studied cultivars in the well-watered treatments. The highest Pn was recorded on 25th June for all cultivars. At this stage Pn of 'Marcona' (12.54 µmol CO_2 $m^{-2}s^{-1}$) was the highest and 'Nonpareil' (7.12 µmol CO_2 $m^{-2}s^{-1}$) was the lowest. In July and August, 'Azar' with 8.83 and 6.45 µmol CO_2 $m^{-2}s^{-1}$ showed highest rate of photosynthesis, while the lowest rate was recorded in 'Mission' cultivar. In general, Pn decreased from June to August in each cultivars..

Water use efficiency (WUE): There was significant difference between cultivars in measured WUE. As shown in Fig. 3, 'Sahand' at first and the third stage showed the highest WUE (3.74 and 4.14 µmol CO_2 mmol H_2O^{-1}, respectively), while WUE of 'Supernova' was high (5.54 µmol CO_2 mmol H_2O^{-1}) at second stage (25th July). During that period, 'Mission' had the lowest WUE (1.05, 1 and 1.56 µmol CO_2 mmol H_2O^{-1}). In the three stages and also between cultivars WUE was different. But in the well-watered treatments, WUE at the first recording stage was several time more than that of later stages as well as stressed conditions (Fig. 4). Highest WUE was recorded in 'Azar' at the 1st stage, in 'Supernova' at the 2nd and 3rd stage whereas lowest WUE was in 'Nonpareil', 'Azar' and 'Mission' at 1st, 2nd and 3rd stage, respectively.

Relative water content (RWC): The data showed that the leaf relative water content ranged between 65 to 85% in all cultivars. RWC in the well-watered treatments was higher than RWC resulted in stress treatments. Although in the second stage of the experiment, except for 'Marcona', there weren't significant differences in RWC between well-watered and stressed plants. But at 1st and 3rd stage, measured RWC of 'Sahand', 'Marcona' and 'Mission' had significant differences between treatments. Overall 'Marcona' showed significant differences and 'Azar', 'Nonpareil' and 'Supernova' had no differences between treatments (Fig. 5).

Proline: Irrigation intervals had a significant effect on proline content of the leaves. Although proline content in the leaves at the beginning of the experiment was similar, it increased sharply in

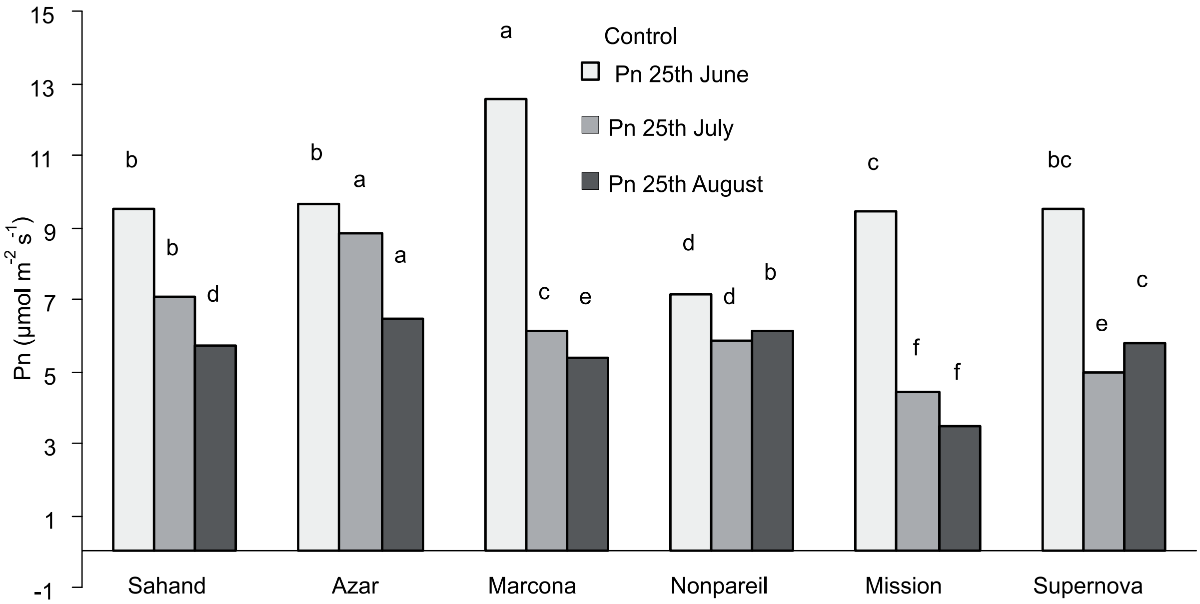

Fig.1. Pn of severe stressed treatments on 25th June, 25th July and 25th August

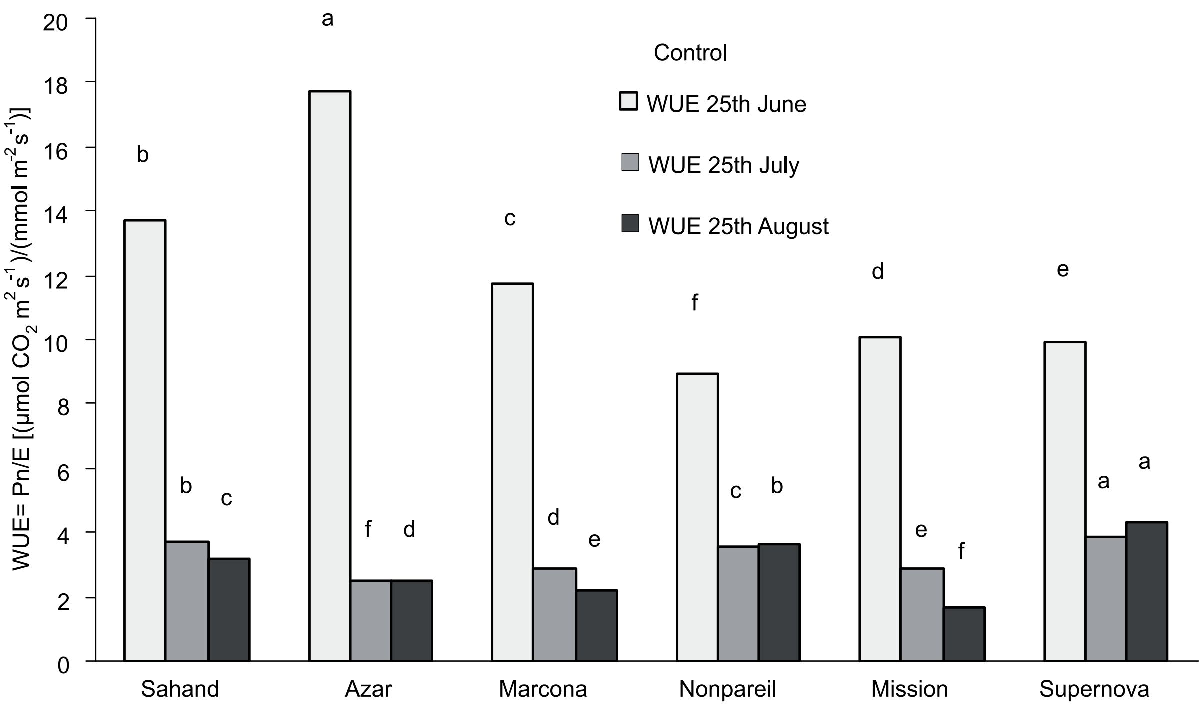

Fig. 2. Pn of normal irrigated treatments on 25th June, 25th July and 25th August

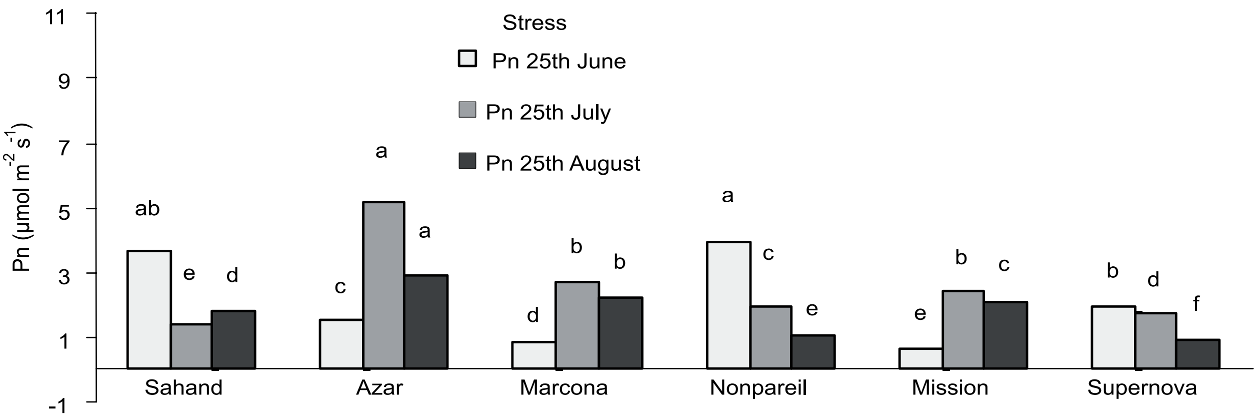

Fig. 3. WUE at three stages (25th June, 25th July and 25th August, named 1, 2 and 3) in the severe stressed plants.

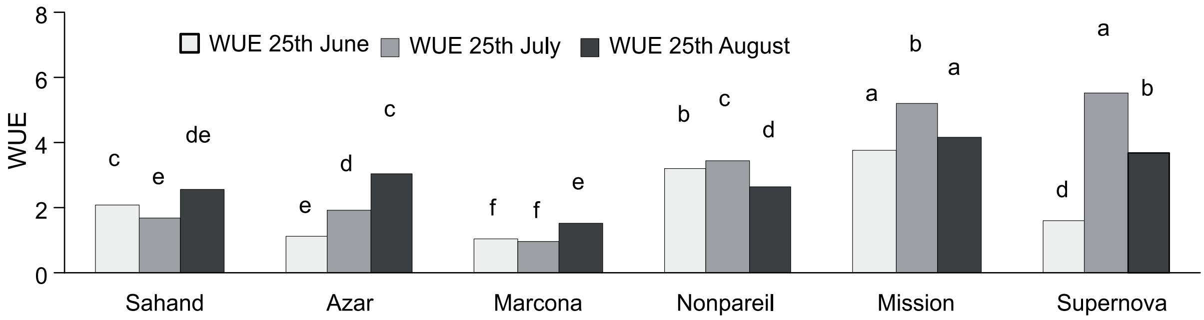

Fig. 4. WUE at three stages (25th June, 25th July and 25th August named 1, 2 and 3) in the well-watered plants

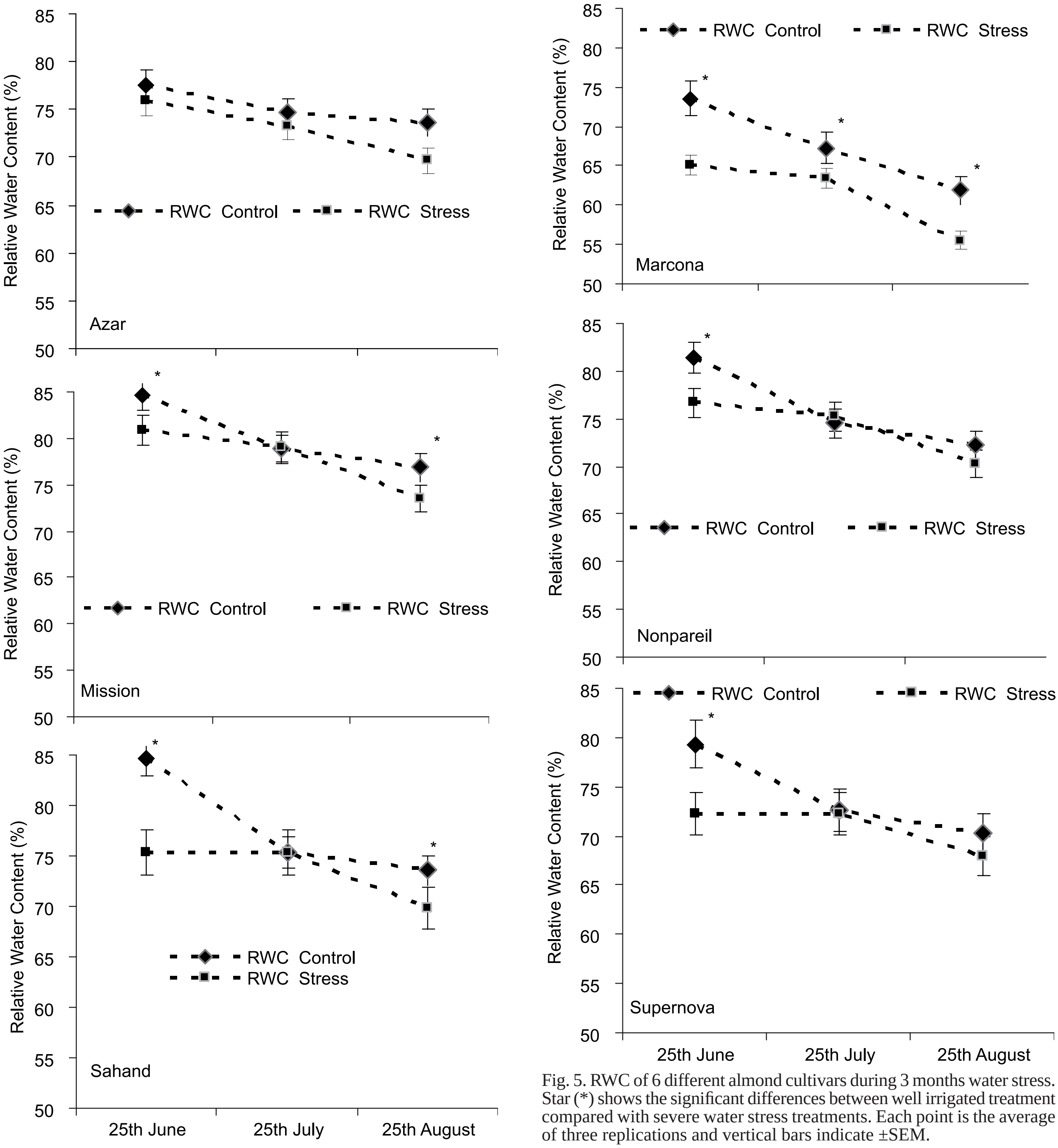

Fig. 5. RWC of 6 different almond cultivars during 3 months water stress. Star (*) shows the significant differences between well irrigated treatment compared with severe water stress treatments. Each point is the average of three replications and vertical bars indicate ±SEM.

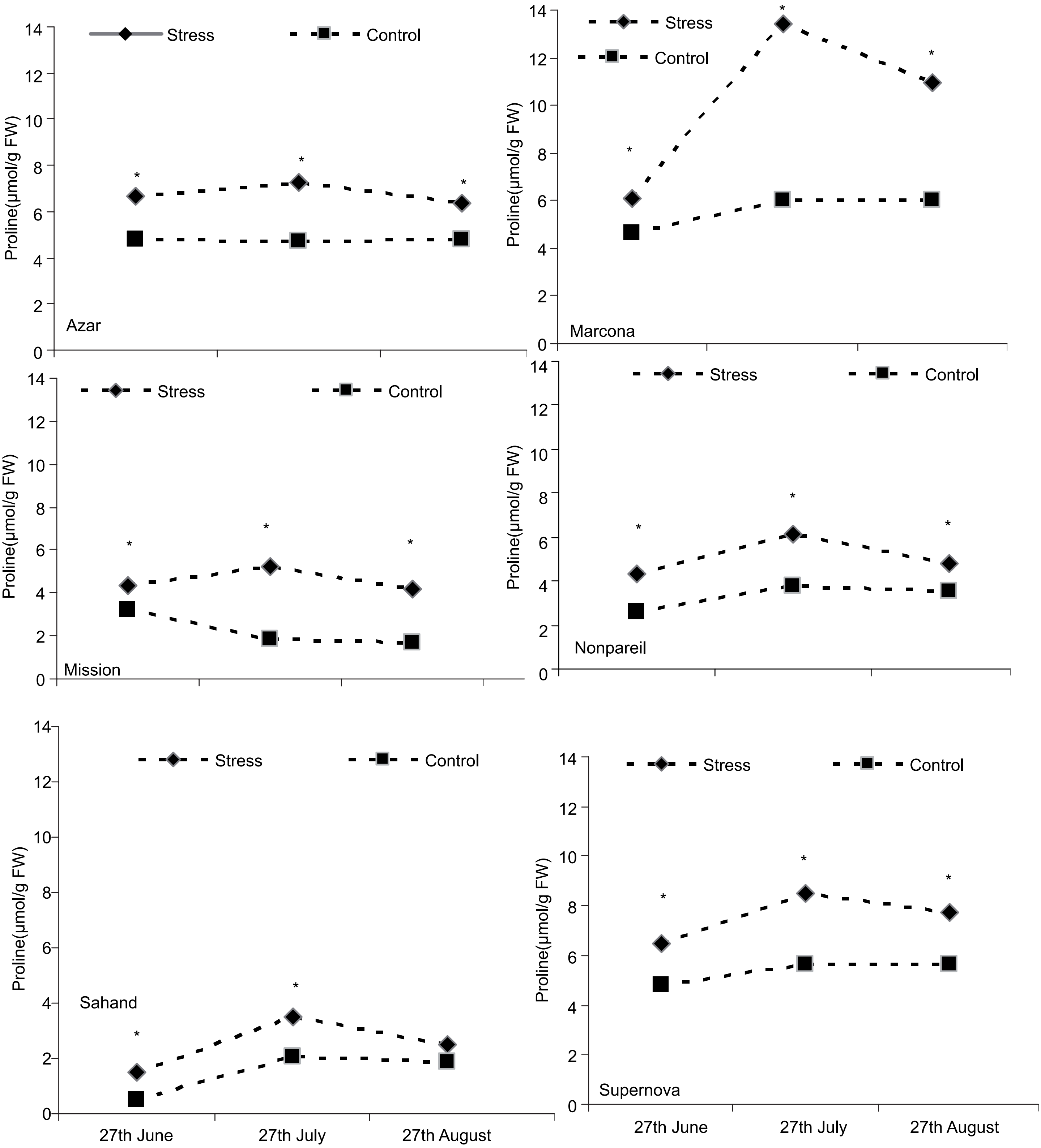

Fig. 6. Leaf proline content of 6 different almond cultivars during 3 months water stress. Star (*) shows the significant differences between well irrigated treatment compared with severe water stress treatments. Each point is the average of three replications and vertical bars indicate ±SEM.

the longer (30 days) irrigation treatment and remained at higher levels during the experiment. There were significant differences between severe stress and well-watered treatments during 3 stages (Fig. 6). The proline content of samples showed an increase in stress treatment during the first 30 day of the experiment. At the second stage of sampling proline content showed very high value in severe stress than well-watered treatments (Fig. 6).

Discussion

Drought can seriously reduce Pn and productivity of crops. Plant physiological responses such as photosynthesis and transpiration depend on the rapidity, severity and duration of drought (Lawlor and Cornic, 2002; Ramachandra Reddy *et al.*, 2004). The decline in photosynthesis during the early stages of water stress

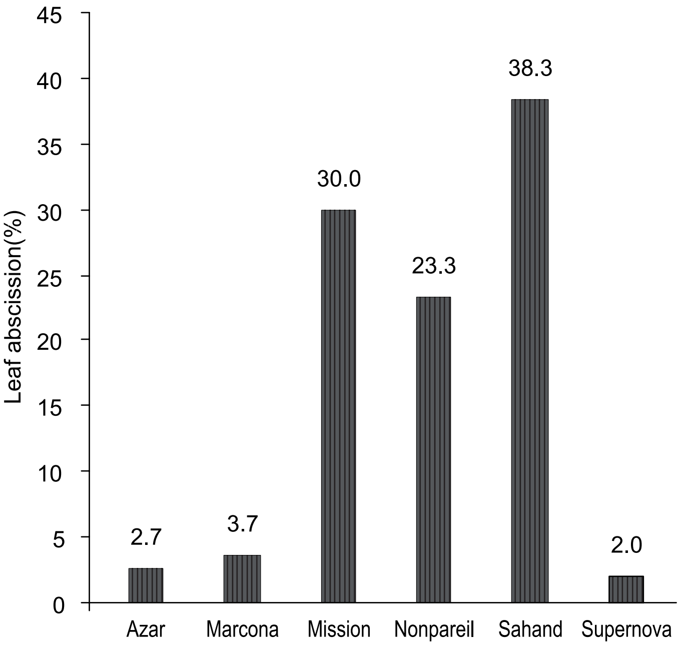

Fig. 7. Percentage of leaf abscission of cultivars under severe drought stress condition at the end of experiment.

was attributed to a combination of stomatal and non-stomatal factors, whereas a further decline in photosynthetic activity under more severe water stress conditions could be due to non-stomatal factors such as biochemical changes induced under severe stress. According to Williams *et al.* (1999), the decrease in photosynthetic rate at low water availability was primarily caused by stomata closure. Our results showed that decreased photosynthesis efficiency could be related to interference in stomatal conductance.

After 20 days of drought stress, 'Nonpareil' and 'Sahand' showed a higher rate of photosynthesis than other cultivars. At second stage, Pn of 'Azar', 'Marcona' and 'Mission' increased, but Pn of 'Sahand', 'Nonpareil' and 'Supernova' decreased. At 25th August, Pn decreased slightly in all stressed treatments. This change indicate adaptation to severe drought stress.

WUE increased under reduced-water supply. Since WUE increases as the soil water supply decreases, this response to drought has an adaptive significance (Raviv and Blom, 2001). Comparison of WUE data of well-watered and stressed treatments at three stages of growing season has shown that WUE increased after drought stress. Because of low dehydration and high photosynthesis during early season, WUE in control plants was very high. But simultaneous increase in temperature and transpiration influenced water loss. This condition leads to drastic reduction in WUE during season in well-watered plants. But in the plants under severe stress conditions, WUE was higher than control plants except at first stage. 'Sahand' and 'Supernova' had low and high WUE, respectively. Considerable leaf abscission in 'Sahand' and negligible abscission in 'Azar' and 'Supernova' was observed (Fig .7). Overall 'Supernova' and 'Azar' were superior than the other stressed cultivars.

Klein *et al.* (2001) suggested an overriding control on stomatal aperture exerted by low relative humidity, high vapor pressure deficit and temperature in almond leaves, although adjustments made in response to soil water deficit and low leaf water potentials were also observed (Torrecillas *et al.*, 1988; Klein *et al.*, 2001). The study of the patterns of leaf water relations and gas exchange activity is a good physiological approach for analyzing the optimum water use by plants (Hsiao, 1993) and can provide fundamental information on plant responses to irrigation treatments. This imbalance is more intense under high evaporative demand and/or severe soil water deficit (Yadollahi *et al.*, 2011). Changes in water content under severe drought stress applied initially (from 1st June to 29th June, RWC once measured at 25 June) indicate significant difference between well-watered and severe stress treatments. So due to lack of sufficient available water in the soil, osmotic adjustment in leaves was low in stressed treatments. At the second stage of RWC monitoring (25th July) two experimental treatments showed similar results. There were no significant differences in RWC between stressed and well-watered treatments in the most of cultivars. This result can be due to osmotic adjusting in leaves of stressed plants. The leaf RWC showed a downward trend from June to August. RWC in the leaves of severe stress treatment had close relationship with well-watered treatment in 'Azar', 'Nonpareil' and 'Supernova'. So these cultivars were able to keep high water content in their leaves and tolerate severe drought stress conditions than other experimental cultivars.

Proline has been primarily known as a protective and osmoregulatory agent for plant cells under environmental stresses (Shevyakova *et al.*,1985). Its production rate in plants with different stress tolerance is not similar and even proline content could be lower in more tolerant plants (Shevyakova *et al.*, 1985). Although proline concentration of almond leaves increased with longer irrigation intervals, differences between varieties were not significant to characterize and screen almond plants for drought tolerance based on their proline production. In our experiment severe stress also caused increased proline amount in leaves of all of cultivars; although, it was different between cultivars (Fig. 6). The highest proline accumulation was observed in the leaves of stressed 'Marcona' but it was least in 'Sahand'. Both 'Marcona' and 'Sahand' were sensitive to drought stress than 'Supernova'. It would be concluded that accumulation of proline in response to long time interval between irrigation is a general trend and cannot be used for defining the more tolerant almond trees.

Our data showed that cultivars 'Azar' and 'Supernova' are resistant cultivars under severe drought stress. Based on their high WUE and Pn, these cultivars use osmotic adjustment to keep high amount water in their leaves during drought stress conditions. Proline cannot be used as an indicator in determining drought resistant cultivars. Applying 3 time irrigation in semiarid regions such as Karaj, Iran situations will be enough, without any significant effect on performance of mentioned cultivars.

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