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Effect of Water Stress on the Growth, Nutritional and Biochemical Status of Two Varieties of Pomegranate Seedlings

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ABSTRACT

A pot experiment was carried out during successive seasons of 2015 and 2016 to investigate the effect of water stress on growth, nutritional and biochemical status of two pomegranate seedlings (Early 116 and wonderful) as in Egypt. The experimented seedlings were obtained from cuttings of two pomegranate varieties, namely Early 116 as well as wonderful. One year old pomegranate seedlings were grown in pots filled with sandy soil under greenhouse conditions, where they subjected to four water levels, 100% of field capacity (control), 80%, 60% and 40% of available water. The results indicated that the maximum values of vegetative growth parameters (shoot length, number of sprouted shoots as well as leaf surface area) were obtained when the plants were irrigated with 100 % of field capacity, while the minimum values were recorded at 40 % of available water. Wonderful seedlings gave the highest values of tested growth parameters at all water levels as compared with Early 116 variety. The decrease in irrigation water levels led to increase the hard leaf character, proline content, while leaf succulence grade and relative water content were increased by increasing the given amount of irrigation water in both pomegranate varieties. Although the two tested varieties accumulated high values of proline in their leaves when subjected to water stress (80%, 60% and 40% of available water), but the Wonderful variety accumulated high values of proline content under water stress treatments as compared with Early 166 variety . Therefore, Wonderful variety might be more tolerant to drought than Early 116 variety. The content of some macronutrients (N, P and K %) and micronutrients (Fe, Zn, Mn and Cu mg/kg) in leaves of both varieties were decreased by decreasing irrigation water levels. The highest significant values of some macro and micronutrients were found with control treatment (100 % field capacity), while the lowest values of these nutrients were found at sever water stress (40 % available water).On the other hand, Wonderful seedlings significantly showed a higher content of N, P, K, Fe, Zn, Mn and Cu under all water irrigation treatments than those of Early 116.

Keywords: *Punica granatum* L., Wonderful, Early 116, irrigation, stress, growth, proline content, nutrients.

INTRODUCTION

Pomegranate (*Punica granatum* L.),belongs to the family *Punicaceae* and is a very interesting fruit plant species from a commercial point of view due to its adaptation to a wide range of climates and soil conditions. Commercial orchards of pomegranate trees are now grown in many regions of the world, including the Mediterranean Basin countries (Holland *et al.*, 2009).There are many local varieties in Egypt such as Manfalouty, Wardy, Nab El-Gamal, El-Araby and recently foreign (Spain) varieties like Early 116 and wonderful, which introduced to Egypt. Wonderful variety is known for its unusual sweet flavor, outstanding red color and exceedingly long shelf-life (up to three months in cold storage). Early 116 pomegranate variety have a citrus like taste and is very juicy, high rate of acid and earlier existing than wonderful. Pomegranates are fairly drought resistant but require normal watering to produce good fruit crops (El-Kassas *et al.*, 1992).Pomegranate trees are a rustic crop, with low water and nutrient demands. Water restrictions limit the growth of new buds, shoots and trunks, in young and adult plants (Moriana and Fereres, 2002). Generally, nutrients uptake by crop plants grown in soil is greatly influenced by several factors including climate and water stress, where water stress affects nutrient transportation to the root and root growth. However, crop species and genotypes within a species are known to differ in their ability to take up nutrients under drought stress conditions (Garg, 2003). Drought stress depresses plant growth and development, which results in the production of smaller organs, and hampered flower production and grain filling. Following drought, stomata close progressively with a parallel decline in net photosynthesis and water use efficiency (Farooq *et al*,. 2009).There is extensive literature indicating that drought stress induces an accumulation of proline (Bradford and Hsiao, 1982), suggesting that the amino acid may be a useful early indicator of drought stress effects, although there is evidence in the literature that proline accumulation seems to occur only when plant growth is already retarded by drought stress (Aspinall, 1986). There were wide climatic differences between years and 2008 were found to be more dry and hot compared to 2007. The proline content varied among pomegranate varieties and particularly years. In year 2007, average proline content of three pomegranate varieties were 30 mg/l while it was 93 mg/l in year 2008, indicating that climatic change affects proline accumulation in pomegranate fruits and in hot and dry years, proline accumulation in fruits increases. (Hanim and Nesrin 2009). Under deficient irrigation, pomegranate plants develop mechanisms to avoid and tolerate stress (Rodríguez *et al.*, 2012).One of hypothesis is that drought resistant genotypes will maintain high nutrient uptake under drought conditions and this ability will be a part of the reason for their drought tolerance and improved yield under drought conditions (Junjittakarn *et al.*, 2013).Under the conditions of water scarcity, the cultivation of crops more tolerant to drought and use less water, especially in the newly reclaimed soil must be taken into consideration. Therefore, the main goal of this experiment is to study the effect of water stress at different irrigation levels on some vegetative growth parameters, proline content, some macro and micronutrients content (N, P, K, Fe, Zn, Mn and Cu) and some water relations in leaves of pomegranate seedlings (Early 116 and wonderful).

MATERIALS AND METHODS

The current investigation was carried out during seasons of 2015 and 2016 in the green house of the research farm of Horticulture Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, to study the effect of different irrigation levels on vegetative growth, proline, N, P, K, Fe, Zn, Mn, Cu and some water relations in leaves of Early 116 and wonderful pomegranate seedlings as newly varieties in Egypt. The plants were obtained as cuttings and were planted in the field for one year. The uniform and healthy of one year old seedlings of both pomegranate varieties were chosen as plant material for this study. The irrigation treatment consisted of three replicates, each replicate consisted of three plants. The pomegranate plants were planted on 15th February 2015 and 2016, in pots capacity of 7 kg and 30 cm in diameter, which filled with a mixture of 6.8 Kg sandy soil and 0.2 kg compost per pot before planting. The pomegranate plants were irrigated with tab water for 15 days before starting the main treatments of irrigation water. The cultivated pomegranate plants were fertilized according to the general recommendation by Egyptian Ministry of Agriculture after calculation of the added amounts of NPK from compost [250 g/plant of ammonium sulfate (20.6%), 190 g/plant of calcium super phosphate (15.5% P_2O_5) and 125g/plant of potassium sulfate (48% K_2O)]. Calcium super phosphate was mixed with compost before planting, while ammonium sulfate and potassium sulfate were added as soil application in three equal doses at March, April and May. On 1st March, the irrigation treatments started as follows:

Treatment 1: Each pot received 100% of field capacity (control).
Treatment 2: Each pot received 80% of available water.
Treatment 3: Each pot received 60% of available water.
Treatment 4: Each pot received 40% of available water.

The irrigation treatments were carried out by weighing the pots every three days and adding the depleted amount of water during the whole period of experiment to attain the percentage of moisture in the treatment. The analysis of soil, water and compost used were done according methods described by Jackson (1973) and presented in Table1and 2(a, b).

Table 1. Some physical and chemical properties of the used soil.

Particle size distribution			Texture	Field capacity %	Permanent wilting point %	Available water %	pH (1:2.5)	EC (dSm^{-1})	OM %	CEC meq/100 g soil	Soluble ions meq L^{-1} (1:2.5)							
											Cations				Cations			
Sand (%)	Silt (%)	Clay (%)									Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	SO_4^{--}	Cl^{-}
88.50	4.90	6.60	sandy	10.00	5.10	4.90	7.30	1.65	0.31	2.51	1.95	3.00	10.53	0.35	0.00	2.12	4.15	9.21

Table 2a. Some chemical properties of the used compost.

pH	EC dS m^{-1}	OC %	MO%	C/N ratio	Total macronutrients %			Total micronutrients mg kg^{-1}			
					N	P	K	Fe	Mn	Zn	Cu
6.87	2.51	17.50	30.10	12.96	1.35	0.53	1.00	120	85	70	12

Table 2b. Some chemical properties of the used tab water.

Soluble ions meq L^{-1}								EC dS m^{-1}	pH
Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO^{--}_3	HCO_3^{--}	Cl^{-}	SO_4^{--}		
1.25	1.02	1.10	0.45	-	2.34	1.04	0.45	0.45	7.2

Measurements:

1. Vegetative growth parameters:

Shoot length: Shoot length (cm) of pomegranate (Early 116 and wonderful) was measured through calculating the difference between main shoot length per seedling at the beginning of the spring flush and at the end of growth season.

Number of new sprouted shoots: Number of the growing shoots/ plant was recorded at end of growth season.

Leaf area: The fourth distal leaf was used to determine leaf area according to Ahmed and Morsy (1999) as follows:

Leaf area = 0.41 (Leaf length (cm) × width (cm) +1.83 = -- cm²

Water relations of leaves: Some water relations of leaves were measured as follows:

Relative water content (R.W.C): Leaf disks (1 cm2) were taken from adult leaves (the fourth distal adult leaf) at cessation of growth and weighed, then put in distilled water for 45 minutes and weighed. Leaf disks, thereafter were dried at 70oC for 24 hrs. The saturated disks were used to calculated the relative water content (R.W.C) using the following equation according to Nomier (1994).

$$\text{R.W.C.} = \frac{\text{Original fresh weight} - \text{dry weight}}{\text{Saturated weight} - \text{dry weight}} \times 100$$

Hard leaf character (H. L. C.): Samples of 9 fully developed leaves (3 replicate × 3 plants) located at the third or fourth apical node were taken on Sept. 29th of each seasons and devoted for measuring the water relation parameter according to Youssef (1990).

The hard leaf character was measured according to the following equation as described by Abdel-Razik and Abd-Raboh (2007).

$$\text{H. L. C} = \frac{\text{Dry weight of leaf (mg)}}{\text{Leaf area (cm}^2)} = (\text{mg. dry weight / cm}^2 \text{ of leaf area).}$$

Succulence grade (S.G): The succulence grade was determined according to the following equation as described by Nomier (1994).

$$\text{S.G} = \frac{\text{Leaf water content (mg)}}{\text{Leaf area (cm}^2)} = (\text{mg water content / cm}^2 \text{ of leaf area).}$$

2. Proline content: Proline content (%) as a biochemical leaf character was colorimetrically measured according to method described by Bates *et al.*, (1973) as follows: Mature fresh leaf sample (0.5 gm) was homogenized by grinding in 10 ml of 3% (w/v) of sulphosalicylic acid. The mixture was then filtered through Whatman No.1 filter paper. The filtrate (2 ml) was added to 2 ml ninhydrin reagent and 2 ml glacial acetic acid and then the mixture boiled on water bath at 90 ◦C for 30 min. The boiled mixture was allowed to cool at room temperature. The mixture was extracted with toluene, and then colourimetrically estimated at 520 nm using spectrophotometer. The proline concentration in the sample was determined from a standard curve using analytical grade proline.

3. Some macro and micronutrients in leaves:

The content of some macronutrients (N, P, K %) and micronutrients (Fe, Mn, Zn Cu mg/kg) were estimated in leaves of pomegranate. leaves plant samples were washed with distilled water and dried at 70oC in an air forced oven for constant weight. Dried plant samples were digested in mixture of HClO4and H2SO4 acids according to the method described by Allen (1974).The contents of N, P, K, Fe, Mn, Zn and Cu were estimated in the acid digested solution. Total nitrogen was determined by Kjeldahl method and phosphorus content was determined by colorimetric method (ascorbic acid) using spectrophotometer according to Jackson (1973). Potassium content was determined photo-metrically using Flame photometer as described by Chapman and Pratt (1961). The micronutrients (Fe, Mn, Zn and Cu) were determined by Inductively Coupled Plasma Spectrometer (ICP) plasma 400.

Statistical Analysis: A complete randomized block design was followed and the analysis of variance (ANOVA) was performed using ANOVA Co-stat software according to Stern (1991).

RESULTS AND DISCUSSION

Effect of different water stress on vegetative growth parameters:

Shoot length:

Data in Table 3 show a decrease in shoot length (cm) during the growth period under different water stress (80%, 60% and 40% of available water) compared with those of control treatment (100% of field capacity) in the two studied pomegranate varieties. The results showed that shoot length parameter significantly declined by decreasing the irrigation water levels, where planted irrigated with 100% of field capacity gained the maximum of shoot length followed in descending order by 80%, 60% and in last 40% of available water, which possessed of the lowest values. Under water stress treatments or control treatment (100 % of field capacity), Wonderful seedlings possessed the highest values of shoot length compared with Early 116 variety. Therefore, Wonderful seedlings were superior to Early 116 at different irrigation levels in the two studied seasons. These results could be enhanced by Mezghani *et al.* (2012), they concluded that under deficit irrigation regimes, where the requested amounts of water are not applied, many problems can arise affected such as shoot growth, root development, water and mineral uptake by young and old olive transplants. The difference in plant growth between the two varieties as affected by water stress may be due to the hormonal balance as explained according to Webster *et al.*, 2000 and Liu *et al.*, 2005.They explained that water stress decreased the cytokinin transport from root to shoots and increased the amount of leaf abscisic acid. These changes in hormone balance caused a reduction in shoot growth and leaf enlargement and expansion. On the other hand, accumulation of abscisic acid in roots induced by soil drying might play the main hormonal role regarding drought tolerance. Where, increase concentrations of abscisic acid in the root may maintain root growth and lead to an increase in water uptake by increasing the permeability of root tissues to water and thereby postpone the development of water deficit in the shoot. Also, root abscisic acid is also transported in the xylem to the shoots, where it causes stomatal closure and reduced leaf expansion, thereby preventing dehydration of leaf tissues and enhancing the chance for survival under prolonged drought.

Table 3. Effect of water stress on some of vegetative growth characteristics of Early 116 and Wonderful pomegranate seedlings in2015 and 2016seasons.

Varieties	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean
Character	Shoot length (cm)			Number of new sprouted shoots			Leaf area (cm^2)		
Season / Treatments	2015								
100% of field capacity (control)	22.00b	28.00a	25.00A	9.17b	10.17a	9.66A	8.79b	10.11a	9.45A
80% of available water	15.50c	23.25b	19.38B	6.83cd	7.67c	7.25B	6.73cd	7.74bc	7.24B
60% of available water	12.25d	16.00c	14.13C	6.33d	7.00cd	6.66C	5.41d	6.48cd	5.95C
40% of available water	8.00e	12.00d	10.00D	5.47e	6.33d	5.90D	5.37d	5.13d	5.25C
Mean	14.44B	19.81A		6.95B	7.79A		6.58B	7.36A	
	2016								
100% of field capacity (control)	21.00b	25.33a	23.17A	11.33b	13.00a	12.16A	7.91b	9.77a	8.84A
80% of available water	14.33c	21.00b	17.67B	7.67de	10.00bc	8.83B	6.44cd	6.85c	6.64B
60% of available water	11.00d	14.52c	12.76C	7.33de	9.67bc	8.50B	5.41d	6.38cd	5.73C
40% of available water	7.45e	11.11d	9.28D	6.17e	8.58cd	7.37C	5.32d	5.59cd	5.46C
Mean	13.45B	17.99A		8.12B	10.31A		6.27B	7.06A	

Means followed by the same letter (s) are not significantly different at 5%.

Number of sprouted shoots:

Data in Table 3 indicated that plants that received 100% of field capacity possessed the highest values of sprouted shoots compared with other treatments, where these values were decreased by decreasing the available water up to 40%, which possessed the least values. Also, Wonderful variety was superior to Early 116 at all different irrigation levels (stress or non-stress treatments) in the two studied seasons. Similar results was found by Bugueño *et al.*, (2016), they reported that canopy growth of pomegranate was at a maximum when soil watering was equivalent to 100% or 130% of evapotranspiration (Etc.). On the other hand, plants subjected to water deficits have been known to modify their patterns of water absorption, transpiration, enzyme activity and photosynthesis, interfering directly with the development of new shoots and canopy expression (Nortes *et al*., 2005).Reducing the number of new shoots could be a phenomenon by the plants to minimize the transpiration surface hence the water lost to the atmosphere, where water deficits decrease leaf growth by slowing rates of cell division and expansion due to turgor loss and increased synthesis of abscisic acid (Tezara *et al*., 2002).

Leaf area:

Data in Table 3 showed that leaf area (cm^2) of both tested pomegranate varieties was decreased by decreasing the available water levels. Under different irrigation levels the highest values of leaf area can be arranged in the descending order, 100% of field capacity> 80% > 60% > 40% of available water, respectively. Plants received 100% of field capacity possessed the highest values of leaf area, while the plants that irrigated with 40% of available water gave the lowest values. In this regard, Wonderful pomegranate variety was superior to Early 116, since it possessed the highest values of leaf area compared with those of Early 116, which gained the least values in the two studied seasons. These results are in agreement with those obtained by Egea *et al*., (2013) who found that reduction of the leaf area has been already reported for almonds and peach seedlings under water stress. Our results also are in harmony with the conclusion given by Abou El-Wafa (2002) who noticed that, pomegranate transplants leaf area possessed the highest values of growing under lower water stress as compared with higher water stress. The ability of Wonderful seedling on possessed higher leaf surface area at all water levels than those of Early 116 variety may be explained according to Webster *et al*., (2000), they concluded that water stress decreased the cytokinin transport from root to shoots and increased the amount of leaf abscisic acid. These changes in hormone balance caused a reduction in shoot growth and leaf enlargement and expansion.

In conclusion, all tested vegetative growth parameters (shoot length, shoot number as well as leaf area) of both varieties were negatively affected by reducing water levels since plant that received 100 % of field capacity possessed the highest values of these parameters followed in descending order by 80%, 60% and finally 40% of available water, which possessed the least values of the above-mentioned parameters.

Effect of water stress on some water relations of leaves

Succulence grade (SG).

Data in Table 4 indicated that the leaf succulence grade (leaf moisture mg /1 cm^2 of leaf blade) of Early 116 and wonderful pomegranate plants was significantly affected by different irrigation treatments. Leaf succulence grad increased by increasing the given amount of irrigation water in both pomegranate plants, where succulence grade is attaining the maximum value under the effect of 100 % of field capacity, which indicated that the leaf cells showed to be completely turgid, while the lowest values were obtained at 40 % of available water. On the other hand, leaves of Wonderful variety gave the highest values of succulence grade than Early 116 variety under all irrigation water levels. Also, Wonderful pomegranate variety showed higher succulence grade of leaves under the three irrigation regimes condition (80%, 60% and 40% of available water) than Early 116 in the two seasons. Therefore, leaves of Wonderful variety might be more tolerance to drought than Early 116 variety . The results are in harmony with those obtained by Abdel-Razik (2011), who found that succulence grade was increased as the available water increased. Those obtained by Abo - Taleb *et al*., (1998) on some pomegranate varieties and Hassan (1998) on olive and pomegranate transplants.

Table 4. Effect of water stress on succulence grade of both Early 116 and Wonderful pomegranate seedlings in 2015 and 2016 seasons.

Varieties	**Early 116**	**Wonderful**	**Mean**	**Early 116**	**Wonderful**	**Mean**
Character	**Succulence grade(mg. water content / cm^2 of leaf area)**					
Season / Treatments	**2015**			**2016**		
100% of field capacity (control)	7.71d	10.19b	8.94A	9.99ab	11.20a	10.59A
80% of available water	6.13f	10.90a	8.51B	9.11b	10.81a	9.96A
60% of available water	7.03e	9.38c	8.20C	7.09c	10.34ab	8.71B
40% of available water	7.03e	7.35e	7.18D	6.44c	7.82c	6.96C
Mean	7.05B	9.37A		8.15B	10.04A	

Means followed by the same letter (s) are not significantly different at 5%.

Hard leaf character (HLC).

Data illustrated by Fig. 1 show that hard leaf character (HLC) of pomegranate varieties (Early 116 and wonderful) was significantly affected by different irrigation treatments. Hard leaf character increased gradually by reducing the amount of given irrigation water. In other words, hard leaf character attained the lowest value by 100 %of field capacity and increased gradually by decreasing the amount of irrigation water to 80%, 60 % and 40% of available water treatments. The increase in HLC values due to reduction in given amount of irrigation water means that dry substances of leaf increased, hence protect the leaves against drought (Al-Khateeb, 1996).The present results are in agreement with those obtained by Tahir *et al*., (2003) on mango, who found that the hard leaf character increased gradually by reducing the amount of irrigation water. Hard leaf index was positively affected by lowering the irrigation regimes. It could be concluded that when the given irrigation water decreased, hard leaf character (dry weight /cm^2 of leaf) increased.

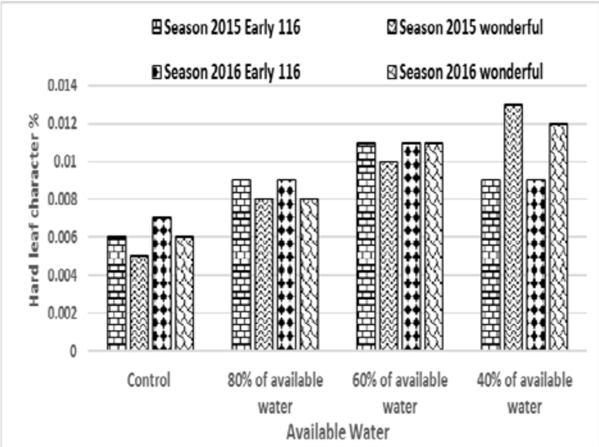

Fig. 1. Effect of water stress on hard leaf character (HLC) of both Early 116 and Wonderful pomegranate seedlings in 2015 and 2016 seasons.

Relative water content of leaf

The increase in values of the relative water content (RWC %) indicates that the plant has got its need from water to accomplish the different plant physiological functions. Relative leaf water content (RWC) is an integrative index of plant water status, which is used to evaluate the tolerance to water stress. Reduction in RWC under drought stress leads to stomatal closure, which further resulting in decreased CO_2 assimilation (Gindaba *et al*., 2004). Data in Table 5 showed that relative water content (%) of pomegranate tested varieties leaves was increased as amount of added irrigation water increased. This was true in the two pomegranate Early 116 and wonderful plants. Data showed that RWC values were significantly reduced by decreasing the amount of added irrigation water. Therefore, the treatment at 100 % of field capacity showed the maximum significant RWC values compared with that were got at 80 %, and 60 % or 40% of available water. Hence, the leaves of studied pomegranate varieties might suffer from water deficit stress when treated with 80 % of available water or with that less than 60 % or 40% of available water. These results are in agreement with those obtained by Tahir *et al*., (2003) on mango, who found that water saturation deficit was decreased by increasing soil moisture. Similar results were also reported by Abdel-Razik (2011) and Khalifa (2013), they found that mango plants produced low values of relative water content as a result of high water deficit.

Based on the above results we concluded that, the highest values of tested growth parameters (shoot length, number of new sprouted shoots and leaf area) of two pomegranate varieties under the effect of different irrigation levels were increased as a result of increasing available water and can be arranged in the descending order, 100% of field capacity> 80% > 60% > 40% of available water, respectively. In contrast, the hard leaf character was arranged in the descending order40% > 60%>80%of available water> 100% of field capacity, respectively. While succulence grade and relative water content in leaves take the same trend as mentioned with growth parameters. Also, wonderful seedlings were superior on Early 116 at all irrigation water levels.

Table 5. Effect of water stress on relative water content of leaf (RWC %) of both Early 116 and Wonderful pomegranate seedlings in 2015 and 2016 seasons.

Varieties	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean
Character	Relative water content of leaf (R.W.C. %)					
Season / Treatments	2015			2016		
100% of field capacity (control)	24.05c	35.48a	29.76A	25.05c	36.15a	30.60A
80% of available water	17.65d	30.56b	24.10B	16.98d	30.22b	23.6B
60% of available water	15.49e	23.08c	19.28C	14.83e	22.74c	18.78C
40% of available water	13.61f	18.64d	16.12D	12.27f	17.98d	15.12D
Mean	17.69B	26.93A		17.28B	26.77A	

Means followed by the same letter (s) are not significantly different at 5%.

Proline content (%):

Data in Fig.2 indicate that plants of the two tested varieties accumulated high values of proline when subjected to water stress (80%, 60% and 40% of available water) as compared with control treatment (100% of field capacity). Therefore, the lowest value of proline accumulation was obtained at 100% of field capacity, while the highest value was recorded at 40% of available water. Differences between two varieties concerning proline content (%) in pomegranate leaves were insignificant in the two seasons. Wonderful seedlings produced the higher proline level in their leaves at all levels of irrigation water in the two seasons than those obtained with Early 116 variety. In this concern, production of high proline content in the leaves indicates that such plant is more tolerant to drought than that of low proline content. It could be concluded that Wonderful variety more tolerant to drought than Early 116variety .These results were in agreement with those obtained by Abdel-Razik and Abd-Raboh (2007) and Abdel-Razik (2011), who found that mango plant produced a highest values of proline as a result of high water stress.

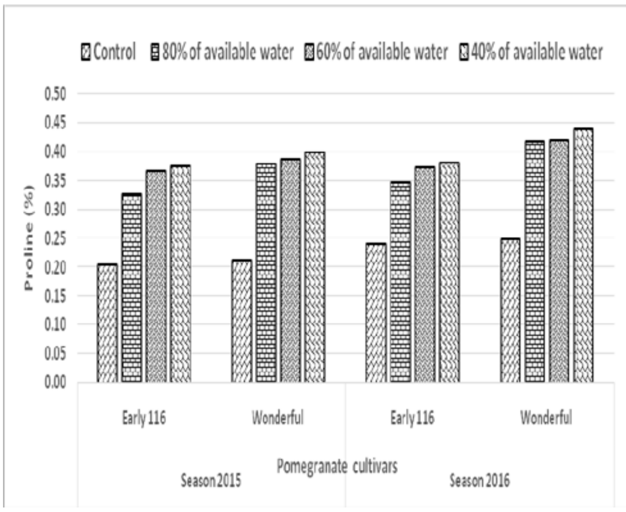

Fig. 2. Effect of water stress on proline content (%) of both varieties Early 116 and Wonderful pomegranate seedlings in 2015 and 2016 seasons.

Leaf macronutrients (N, P and K) content:

Nitrogen content (%)

The results in Table 6 show that the highest significant values of leaf nitrogen content were obtained at irrigation level of 100% of field capacity, while the lowest significant nitrogen values were obtained at irrigation level of 40% of available water. This was true for the two pomegranate plants and two seasons. Moreover, Wonderful seedlings possessed higher significant values of leaf nitrogen content than Early 116 at all water stress (80%, 60% and 40% of available water) or at control treatment (100%field capacity).These results are in harmony with those obtained by Tahir *et al*., (2003), they found that leaf nitrogen content was decreased by decreasing the available water percentage. They added that, under soil water stress, nitrogen solubility was decreased and the plant did not receive the necessary nitrogen amount. Similar results were also reported by Khattab *et al*., (2011), they found that leaf nitrogen percentage of pomegranate increased by increasing irrigation water level. It is interesting to say that under drought stress conditions, the available soil N ($NO_3^-$and NH_4^+), N_2 fixation, nitrogen uptake and nitrogen use efficiency will be greatly decline and such reduction leads to low N accumulation and consequently low dry matter production and low crop yield as recorded by many investigators (Pimratch *et al*., 2008; Hoseinlou, 2013and Pimratch *et al*., 2013). 3.4.2. Phosphorus content (%)

Table 6 showed that the highest values of leaf phosphorus content were obtained when the plants of the two varieties were irrigated with 100% of field capacity, while the lowest values were noticed at irrigation level of 40 % of available water. Differences between the two studied varieties in two seasons were significant at all irrigation levels. Similar findings were observed by Luvaha (2005) and Abdel-Razik (2011) on mango rootstocks and Junjittakarn *et al*., (2013) on peanut genotypes, they found that, phosphorus content in leaves of mango and shoot of peanut genotypes were significantly reduced by decreasing irrigation level. They stated that water deficit negatively affected on nutrient uptake and transport. As mentioned before with nitrogen, also Wonderful seedlings were superior to Early 116 on phosphorus content at all irrigation levels, whether at water stress levels (80%, 60%, 40% of available water) or at control treatment (100% of field capacity). These results were agreement with Junjittakarn *et al*., (2013) they found that, under different water regimes (field capacity, 2/3 and 1/3 available water), peanut genotypes that showed the high uptake of nitrogen also showed increases in the uptake of other nutrients including phosphorus.

Potassium content (%)

Data in Table 6 also show that leaf potassium content in the two studied pomegranate varieties were increased by increasing water availability. The highest values of potassium content were found at irrigation level of 100% of field capacity followed in descending order by 80%, 60% and 40% of available water for the two studied pomegranate varieties and two seasons. Also, Wonderful seedling leaves significantly showed higher potassium content under all water stress treatments than those of Early 116 seedlings. The present results are agreement with those obtained by Abdel-Razik and Abd-Rabboh (2007) on mango rootstocks and Khattab *et al*., (2011) on pomegranate, they found that potassium content in leaves was significantly reduced by decreasing irrigation rate. It is worth to be mentioned that higher soil moisture usually means greater availability of K, where increasing soil moisture increases movement of K to plant roots and enhances availability of potassium. Also, under such kind of drought conditions, it can be clarify that enhancing the concentration of K in plants body parts could be of significant importance for obtaining high sustainable yield under drought situation (Valadabadi and Farahani, 2010). In conclusion, the content of macronutrients (NPK) in leaves of two studied pomegranate (Early 116 and Wonderful) were significantly affected by available water levels, where the highest values of these nutrients were found at high irrigation level, while the lowest values were recorded at the low available water. Wonderful variety was superior to Early 116 at all studied irrigation water levels. The high content of these nutrients in leaves of Wonderful variety was found at the highest available water (100 % of field capacity). In contrast, the low content of NPK was recorded in leaves of Early 166 variety at the lowest available water (40% of available water). There is a general significant positive effect on the percent of N, P and K in leaves due to increasing available soil water. These results may be led to the conclusion that nutrient content was retarded under water stress condition, where a substantial decrease in transpiration rates and impaired active transport and membrane permeability and resulting in a reduced root absorbing power of plant. So depletion of soil moisture level caused a reduction in leaf mineral content. These results are confirmed by many investigators such as Kirnak *et al*., (2001) on eggplants*;* Mikhael (2007) on apple; Ibrahim and Abd El-Samad (2009) on pomegranate and Junjittakarn *et al*., (2013) on peanut genotypes, they mentioned that, there is a general significant positive effect on the percent of N, P and K in leaves of these plants due to increasing available soil water and vice versa.

Table 6. Effect of water stress on nitrogen, phosphorus and potassium content (%) in leaves of both varieties Early 116 and Wonderful pomegranate seedlings in 2015 and 2016 seasons.

Varieties	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean
Character	**N %**			**P%**			**K%**		
Season / Treatments	**2015**								
100% of field capacity (control)	2.39b	2.62a	2.50A	0.25a	0.26a	0.26A	1.59c	1.94a	1.76A
80% of available water	1.80cd	1.85c	1.82B	0.18c	0.22b	0.20B	1.49c	1.82b	1.65B
60% of available water	1.72d	1.74d	1.73C	0.12e	0.15d	0.14C	1.31d	1.73b	1.51C
40% of available water	1.41e	1.47e	1.44D	0.06g	0.09f	0.08D	1.24d	1.49c	1.36D
Mean	1.83B	1.92A		0.15B	0.18A		1.41B	1.74A	
	2016								
100% of field capacity (control)	2.54b	2.67a	2.60A	0.24a	0.25a	0.25A	1.58ab	1.82a	1.70A
80% of available water	1.94c	1.96c	1.95B	0.19c	0.21b	0.20B	1.37bc	1.70a	1.53B
60% of available water	1.60e	1.71d	1.65C	0.14e	0.16d	0.15C	1.21c	1.59ab	1.40B
40% of available water	1.39f	1.56e	1.47D	0.07g	0.10f	0.09D	1.17c	1.30bc	1.23C
Mean	1.86B	1.97A		0.16B	0.18A		1.33B	1.60A	

Means followed by the same letter (s) are not significantly different at 5%.

Leaf micronutrients (Fe, Mn, Zn and Cu mg kg^{-1}) content:

Table 7 showed the effect of different available water levels on some micronutrients (Fe, Mn, Zn and Cu) content in levees of pomegranate varieties (Early 116 and Wonderful) in two seasons of 2015 and 2016. The results showed that plants that irrigated with 100% of field capacity (control) gave the highest significant values of Fe, Mn, Zn and Cu followed by 80 and 60 % and finally 40% of available water, which possessed the lowest significant values. In other words, decreasing available water levels caused a decreasing in the content of Fe, Mn, Zn and Cu in leaves of the two studied pomegranate varieties. The low content of micronutrients (Fe, Mn, Zn and Cu) in leaves of two pomegranate varieties under water stress can be explained on the negative effects of water stress on mineral nutrition (uptake and transport of nutrients), where water stress decreased the availability of moisture in the soil and this consequently decreased the availability of nutrients and intern affected the absorption of nutrients (Mengel and Kirkby, 2001; Hu and Schmidhalter, 2005; Amtmann and Blatt, 2009).The results also showed that under all irrigation levels (100% of field capacity, 80%, 60% and 40% of available water), leaves of Wonderful variety contains higher values of these nutrients than Early 116 in the two seasons. On the other hand, the high content of micronutrients in leaves of Wonderful seedlings as compared with Early 116 under water stress treatments (80%, 60% and 40% of available water) may be due to its ability on adaptation and tolerant of water stress conditions by accumulation of abscisic acid in roots, where increased concentrations of this hormone in the root induced by soil drying may maintain root growth and lead to an increase in water uptake by increasing the permeability of root tissues to water and this consequently increased the uptake of nutrients (Liu *et al.*, 2005). Based on the above results of nutrients (macro and micro), we concluded that nutrients uptake by roots and their translocation to shoots were retarded under water stress condition, where a substantial decrease in transpiration rates and impaired active transport and membrane permeability and resulting in a reduced root absorbing power of plant as mentioned by Marschner (1995), Alam (1999), Baligar *et al.*, (2001) and Khattab *et al.*, (2011). So depletion of soil moisture level caused a reduction in leaf mineral content.

Table 7. Effect of water stress on the content of Fe, Mn, Zn and Cu in leaves of both Early 116 and Wonderful pomegranate seedlings in 2015 and 2016 seasons.

Varieties	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean	Early 116	Wonderful	Mean
Character	**Fe mg kg^{-1}**			**Mn mg kg^{-1}**			**Zn mg kg^{-1}**			**Cu mg kg^{-1}**		
Season / Treatments	**2015**											
100% of field capacity (control)	80.0b	96.0a	88.0A	58.0b	70.0a	64.0A	56.0b	66.0a	61.0A	40.0a	44.0a	42.0A
80% of available water	70.0c	91.0a	80.5B	49.0c	60.0b	54.5B	43.0c	54.0b	48.5B	28.0b	32.0b	30.0B
60% of available water	54.0d	71.0c	62.5C	30.0d	48.0c	39.0C	30.0d	45.0c	37.5C	19.0c	22.0c	20.5C
40% of available water	39.0e	55.0d	47.0D	20.0e	32.0d	26.0D	20.0e	30.0d	25.0D	8.0d	19.0c	13.5D
Mean	60.8B	78.3A		39.3B	52.5A		37.3B	48.8A		23.8B	29.3A	
	2016											
100% of field capacity (control)	84.0b	99.0a	91.5A	57.0b	72.0a	64.5A	50.0c	68.0a	59.0A	42.0a	45.0a	43.5A
80% of available water	72.0c	95.0a	83.5B	46.0c	60.0b	53.0B	44.0d	58.0b	51.0B	29.0b	30.0b	29.5B
60% of available water	58.0d	74.0c	66.0C	32.0d	46.0c	39.0C	34.0e	42.0d	38.0C	21.0c	24.0c	22.5C
40% of available water	43.0e	56.0d	49.5D	21.0e	29.0d	25.0D	22.0f	32.0e	27.0D	8.0d	23.0c	15.5D
Mean	64.3B	81.0A		39.0B	51.8A		37.5B	50.0A		25.0B	30.5A	

Means followed by the same letter (s) are not significantly different at 5%.

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تأثير الإجهاد المائى على النمو والحالة الغذائية والبيوكيميائية لصنفين من شتلات الرمان

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أجريت تجربة اصص خلال موسمى ٢٠١٥ و ٢٠١٦ بهدف دراسة تأثير الإجهاد المائى على النمو والحالة الغذائية والبيوكيميائية لشتلات الرمان صنفى Wonderful وEarly 116. تم تنمية شتلات الرمان تحت ظروف صوبة قسم البساتين – كلية الزراعة – جامعة الازهر بالقاهرة – مصر وذلك فى اصص سعتها ٧ كجم تربة رملية ، حيث تم تعريض شتلات الرمان الى اربع مستويات رى مختلفة هى: ١٠٠% من السعة الحقلية (معاملة الكنترول) و٨٠% ، ٦٠%،٤٠% من الماء الميسر والتى تمثل ظروف الاجهاد المائى. أشارت النتائج ان اعلى قيم النمو الخضرى (طول النبات، عدد النموات الحديثة ،مساحة الورقة) لكلا الصنفين (Wonderful وEarly 116) تم الحصول عليها عند رى شتلات الرمان بـ ١٠٠ % من السعة الحقلية (معاملة الكنترول)، بينما اقل القيم للنمو الخضرى تم الحصول عليها عند رى النباتات بـ ٤٠ % من الماء الميسر. أظهرت شتلات الرمان صنفWonderful تفوقاً معنويا فى قيم النمو الخضرى تحت معاملات الرى المختلفة بالمقارنة بالصنف Early 116 . أدى الانخفاض فى نسب الماء الميسر فى التربة الى ارتفاع محتوى الاوراق من البرولين و خصائص الصلادة للورقة فى كلا الصنفين ، بينما ادت زيادة نسب الماء الميسر فى التربة الى زيادة عصارية الاوراق وكذلك محتوى الاوراق من الرطوبة. أظهر صنف Wonderful تفوقاً فى محتوى الاوراق من البرولين تحت معاملات الاجهاد المائى (٨٠% ،٦٠%، ٤٠% من الماء الميسر) مقارنة بالصنف Early 116 وبالتالى فان صنف Wonderfulيعتبراكثر تحملا للجفاف من الصنف Early 116. أدى ارتفاع نسب الماء الميسر فى التربة الى زيادة محتوى الاوراق من العناصر الكبرى (النيتروجين والفوسفور والبوتاسيوم) وكذلك العناصر الصغرى (الحديد والمنجنيز والزنك والنحاس) حيث كانت اعلى القيم لهذه العناصر تحت تأثير معاملة الكنترول (١٠٠% من السعة الحقلية) ، بينما كانت اقل القيم عند معاملة ٤٠ % من الماء الميسر وذلك لكلا الصنفين (Early 116 و Wonderful) فى موسمى الدراسة. أظهرت النتائج تفوق صنف Wonderful فى محتوى اوراقه من العناصر الكبرى والصغرى مقارنة بالصنف Early 116 تحت معاملات الرى المختلفة.