دراسة استجابة غراس مختلفة من التفاح (*Malus domestica* Borkh) للإجهاد المائي

Study of Responses of Different Seedlings of Apple (*Malus domestica* Borkh.) to Water Stress

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المُلَخَّص

يهدف هذا البحث إلى دراسة الاستجابات المورفولوجية والفيزيولوجية لغراس أصناف مختلفة من التفاح لفترات قصيرة من الإجهاد المائي باستخدام الري واستخدام تراكيز مختلفة من الجهود الأسموزية لمحلول البولي ايتيلين غليكول 6000. أُجريت التجارب على غراس من أصناف تفاح بعمر ثلاثة أشهر وهي: (Rubintte, Golden Delicious, Elstar, Cox Orange)، تم تعريض الغراس للإجهاد المائي في ظروف البيت الزجاجي، حيث تم ري بعض الغراس ثلاث مرات أسبوعياً كشاهد، ومعاملة الري كل عشرة أيام مرة، وأخرى كل عشرين يوماً مرة. أما معاملتي الإجهاد المائي بوساطة محلول البولي إيتيلين غليكول، فاستخدم فيها مستويين من الجهود الحلولية وهي (- 0.7 و- Mpa 1.6). لدراسة تأثير الإجهاد المائي في النباتات، أخذت القراءات التالية: ارتفاع النبات، عدد الأوراق، مساحة المسطح الورقي، محتوى الماء، فلورة اليخضور، محتوى اليخضور ومحتوى الأوراق من البرولين.

أظهرت نتائج الإجهاد المائي بالتعطيش لمدة عشرين يوماً وباستخدام البولي إتيلين غليكول تأثيرات مختلفة في الصفات المورفولوجية والفيزيولوجية لغراس التفاح. حيث أدى الإجهاد المائي بوساطة البولي ايتيلين غليكول إلى انخفاض معنوي في عدد الأوراق، وفي مساحة المسطح الورقي وارتفاع النبات في كلا الصنفين Golden Delicious وElstar، وانخفاض غير معنوي في الصنفين Cox Orange و Rubinette وذلك مقارنةً مع الشاهد. في حين انخفض المحتوى المائي لأوراق الصنفين Rubinette و Golden Delicious عند تعطيش النباتات لمدة 20 يوماً أو بالري بمحلول PEG. في الوقت الذي كان عنده التأثير أقل في الصنفين Cox Orange و Elstar. ولم تظهر نتائج فلورة اليخضور ومعدل اليخضور في الأوراق أي فرق معنوي نتيجة الإجهاد المائي. كما تبين أن محتوى الأوراق من البرولين يتعلق بالصنف. وتم ترتيب الأصناف حسب مدى تحملها للإجهاد المائي كما يلي:

Rubinette > Elstar > Golden Delicious ثم Cox Orange الذي كان أكثر تأثراً بالإجهاد المائي.

الكلمات المفتاحية: غراس تفاح، يخضور، الفلورة، PEG، *Malus domestica*، نمو النبات، البرولين، الإجهاد المائي.

Abstract

The objective of this work was to study the morphological and physiological responses of apple seedlings of different cultivars to short-term water stress by irrigation with water and different osmotic potential of PEG 6000. Experiments were performed in a green house on three month old seedlings of different apple cultivars

(*Malus domestica* Borkh. Cv. Rubinette, Golden Delicious, Elstar and Cox Orange). Water stress was induced by water reduction and by polyethylene glycol (PEG)6000. Part of plants were irrigated three times a week, as control. Others were irrigated one time per ten days and one time per 20 days. Water stress with PEG solution was induced by two osmotic potentials of -0.7 and -1.6 MPa, plant length, number of leaves, leaf area, water content, fluorescence, chlorophyll value and proline accumulation were measured. The results suggest that water stress by water irrigation (20 days drought) or by PEG 6000 showed different effects on the morphological and physiological characteristics of apple seedlings. Water stress induced by PEG decreased plant leaves number, leaf area and plant length significantly in Golden Delicious and Elstar, but there was no significant difference in Rubinette and Cox Orange compared to the control. Water content in leaves of Rubinette and Golden Delicious were decreased by high water stress after 20 days drought and by PEG treatments, however Elstar and Cox Orange were less affected. Fluorescence and chlorophyll value were not significantly affected by short term water stress. The results indicated that Proline accumulation depends on apple cultivar. This work showed a gradient of drought stress effects in the order of different varieties Golden Delicious, Elstar, Rubinette and Cox Orange. Cox Orange was highly affected by water stress. Rubinette, a cross between Golden Delicious and Cox Orange, showed a moderate tolerance. Elstar which is a progeny of the cross between Golden Delicious and Ingrid Marie, can be classified as tolerant, while Golden Delicious was the most tolerant variety in the test of seedlings.

Keywords: Apple seedlings, Chlorophyll, Fluorescence, *Malus domestica*, PEG, Plant growth, Proline, Water-stress,

Introduction

Drought is a serious problem that affects many regions of the world, decreasing the photosynthetic rate of crops and limiting the productivity worldwide. Therefore, water availability is an essential factor influencing agriculture. Plant growth and photosynthesis are two of the most important processes abolished, partially or completely, by water stress (Kramer and Boyer.1995; Ben-Rouina, *et al*., 2006), and both of them are major causes of decreased crop yield.

Drought stress in plants occurs when evaporative demand exceeds water uptake. Deficit water budgets lead to numerous physiological alterations, both in the long and the short-term. Long-term drought responses include root to shoot rations (Chaves *et al*. 2002), and/or reduced leaf area (Whalley *et al*., 1998). Short-term responses include altered stomatal function (Stewart *et al*., 1995), and/or osmotic adjustment (Dami and Hughes, 1997). Tolerance to drought is therefore a complex phenomenon in which different traits are involved. Among characteristics that putatively confer drought tolerance, osmotic adjustment has received increasing impact during recent years. Associations between osmotic adjustment and grain yield under water stress in different plants (Morgan, 1995; Santamaria *et al*. 1990; Rodriguez *et al*. 1992; Sancheza *et al*. 2004) have been reported. However, the utility of osmotic adjustment as a trait of drought tolerance is open to debate.

Drought can be simply defined as a period of below normal precipitation that limits plant productivity in a natural or agricultural system. The decrease of water availability in the soil can be quantified as a decrease (Kramer and Boyer, 1995). The physiological mechanisms involved in cellular and whole plant responses to water stress therefore generate considerable interest and are frequently reviewed (Neuman, 1995; Turner, 1997).

Higher plants, including apple trees, have evolved a drought resistance mechanism to struggle against adverse environmental conditions. For example, the plants can improve their osmoregulation ability to strengthen their tolerance to drought. This suggests that the drought resistance of fruit trees can be improved by enhancing the ability of plants' osmoregulation. Proline is one kind of hydrophilic substance and efficient osmoregulator, which can prevent plant cells from dehydrating during drought (Nayer and Reza, 2008a).

The objective of the present work was to study the morphological and physiological responses of apple seedlings of different cultivars to short-term water stress by irrigation with water and different osmotic potential of PEG 6000, to establish critical water levels for plant growth and photosystem II and development of chlorosis. Therefore, we examined whether these responses are osmotic or turgor pressure effects, indicated by accumulation of proline and the photosynthesis parameters as chlorophyll a, fluorescence and chlorophyll value in apple leaves.

Materials and Methods

Experiments were performed on three month old seedlings of different apple cultivars (*Malus domestica* Borkh. Cv. Rubinette, *Golden Delicious*, Elstar and Cox Orange) which were grown in green house at 25 °C. The plants were grown in the professional soil type ED 73, which contents all mineral elements for nutrition. The plants with similar growth vigor and well watered were equally used in the experiment. The plants were subjected to different water stress treatments:

1- Control: the seedlings were irrigated 3 times a week.

2- Water stress 1: the seeddlings were irrigated one time at 10 days.

3- Water stress 2: the seedlingss were irrigated one time at 20 days.

4- water stress 3 induced by PEG 6000: the seedlingss were irrigated with PEG solution (-0,7 Mpa osmotic potential).

5- water stress 4 induced by PEG 6000: the seedlingss were irrigated with PEG solution (-1.6 Mpa. osmotic potential).

The weight of soil during twenty days of different water stress arrangements, which were used in this experiment, were demonstrated in the following diagram (Fig. 1).

After different treatments of water stress the following measurements were studied:

- Plant Growth

The effect of water stress on morphological characteristics, like plant length, leaf area and leaves number per plant were measured.

- Chlorophyll of fluorescence

Chlorophyll a fluorescence was measured with a portable fluorometer type PAM 2000 following the method of Schreiber (1986). Measurements were made in the field on overcast mornings on 20 mi dark-adapted grape leaves using leaf clips. For the determination of ground fluorescence (Fo), leaves were illuminated by a low light intensity of 0.1 µmol m^{-2} s^{-1}, followed by a saturation pulse of 1800 µmol m^{-2} s^{-1} for 0.6 s at the leaf surface to allow the determination of maximum fluorescence (Fm), where PS II reaction centres are closed (primary quencher Qa reduced). Optimum quantum yield in the dark adapted state (Fv/Fm) was calculated as:

$$(Fv/Fm) = (Fo-Fm)/Fm$$

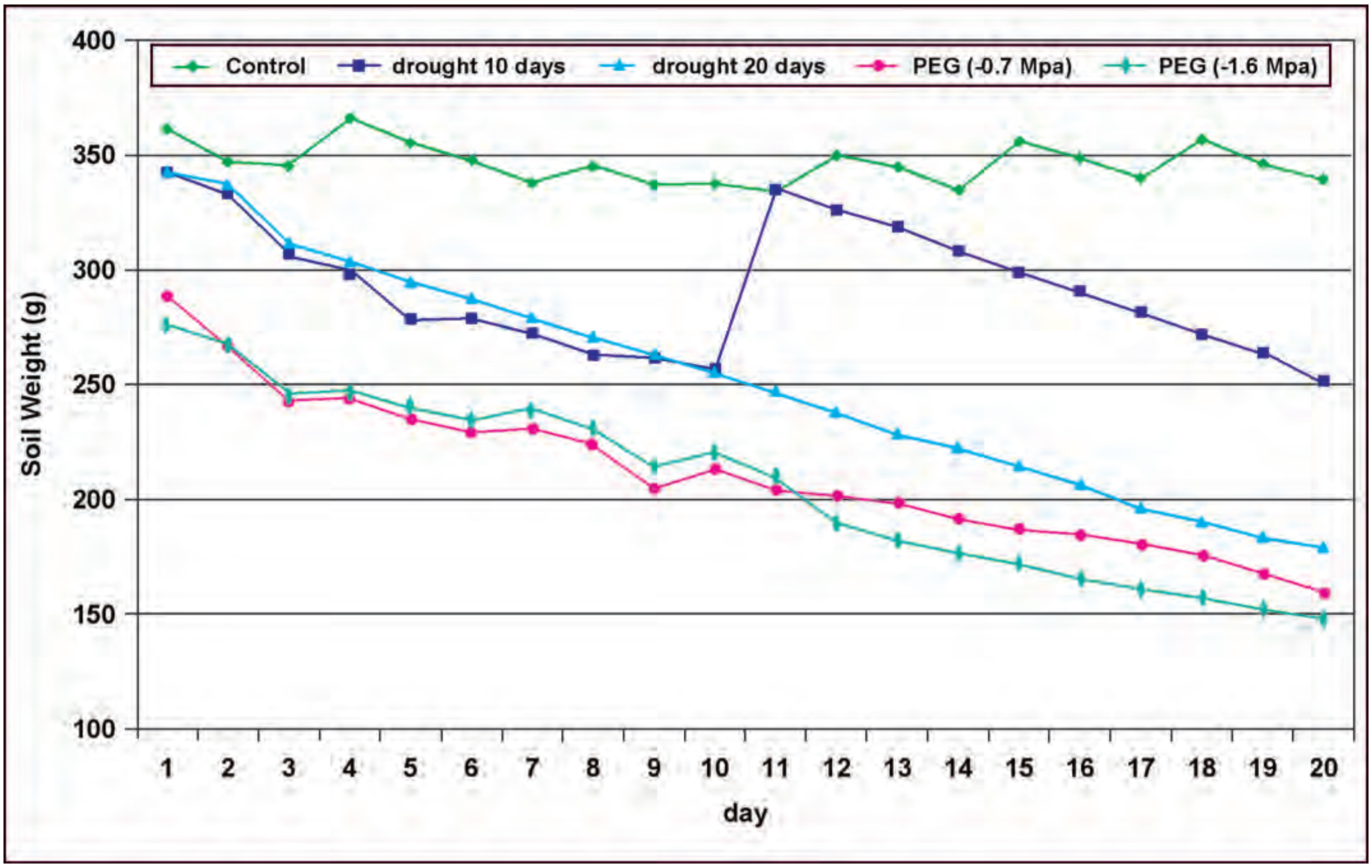

Fig. 1. the weight of soil (g) during twenty days of different water stress arrangements.

- Chlorophyll value

The chlorophyll value in apple leaves were measured with the portable chlorophyll meter SPAD-502, which is a non-invasive, portable diagnostic tool that measures the greenness or relative chlorophyll content in leaves. The SPAD 502 determines the relative amount of chlorophyll present by measuring the absorbance of the leaf in two wavelength regions. (Porro *et al.* 2001 ; Netto *et al*. 2005).

- Proline content

Free proline accumulation was determined using the method of Bates, *et al.* (1973). Leaf samples were harvested and were immediately frozen in liquid nitrogen. 0.03 gram dry weight of leaves was homogenized with 2 ml of 3% sulfosalicylic acid and mixed for 3x 15 sec., the homogenate was centrifuged at 14000 g for 10 min. The supernatant was treated with 1 ml of acetic acid and 1 ml of ninhydrin, and boiled at 100°C for 1h. After cooling the mixture, 2 ml of toluene was added, and well mixed. The chromophore containing toluene was separated and absorption at 520nm was read, using toluene as a blank. Proline concentration was calculated using L-proline for the standard curve. Contents of proline were expressed as mmol/g^{-1}.DW.

Statistics

The experimental data ware analyzed with the SAS (Statistical Analysis Software). Data were tested with ANOVA for normal distribution and variance homogeneity and were compared by either Duncan Multiple range test, in case the variances were homogeneously distributed. A probability level of 5% was accepted to indicate significant differences.

Results

The statistical analysis of different parameters showed different effects of water stress treatments on apple cultivars, Tab. 1 determined the statistical analysis of the parameters, which were suggested to investigate the effect of water deficit and PEG 6000 on morphological and physiological characteristic.

Effect of water stress on the morphological characteristic

I- Plant length

The result showed a significant difference between treatments and between varieties. Plant length was reduced by treatment with PEG 6000 of both osmotic potentials (-0.7 and -1.6 Mpa) (5.04 and 5.22 cm) in all varieties, compared to the control (6.20 cm) and after 10 and 20 days water stress (6.26 and 6.48 cm). There was no significant difference between water deficit plants compared to the control (Tab. 2). Rubintte plants showed significantly the highest length (6.71 cm) compared to the other varieties (Elstar 5.97 cm, Golden Delicious 5.66 cm and Cox Orange 5.01 cm). Rubinette and Cox Orange showed no significant difference between all water stress treatments compared to the control (Tab. 1), but plant length was significantly reduced in both Golden Delicious and Elstar by water stress with PEG (Tab. 2).

Tab. 1. The statistical analysis of tested parameters.

		plant length		leaves area		leaves number		Water content	
Source	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Treatment	4	5.97	0.0002	3.23	0.0126	5.80	0.0003	17.22	<0.0001
Varieties	3	8.59	<0.0001	5.60	0.0009	0.89	0.4479	2.61	0.0546
Var. x Treat.	12	1.36	0.1953	1.55	0.1029	1.22	0.2762	0.90	0.5507

		Fluorescence Fv/Fm		Chlorophyll value		proline	
Source	DF	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Treatment	4	0.82	0.5121	2.23	0.0667	4.31	0.0022
Varieties	3	5.16	0.0018	0.61	0.6083	1.37	0.2522
Var. x Treat.	12	1.16	0.3128	1.77	0.0534	0.90	0.5468

Tab. 2. Effect of water stress on plant length (cm) of different apple cultivars *(Malus domestica* Borkh. cv.*)*.

Treatment \ Varieties	Rubinette	Golden Delicious	Elstar	Cox Orange	Average
Control	6.56 [a]	6.54 [a]	6.77 [a]	4.94 [a]	6.20 [a]
10 days stress	7.57 [a]	5.73 [ab]	6.39 [a]	5.37 [a]	6.26 [a]
20 days stress	7.11 [a]	6.7 [a]	7.11 [a]	4.97 [a]	6.48 [a]
PEG (-0.7Mpa)	6.06 [a]	4.36 [c]	4.3 [b]	5.44 [a]	5.04 [b]
PEG (-1.6Mpa)	6.27 [a]	4.98 [bc]	5.3 [ab]	4.31 [a]	5.22 [b]
Average	6.71 [a]	5.66 [bc]	5.97 [b]	5.01 [c]	

II- Leaf area

Analysis of variance revealed that there was a significant effect of water stress on leaf area of apple seedlings, water stress induced by PEG (-1.6 Mpa) showed a significant decrease of leaf area (12.97 cm^2) compared to the control (15.02 cm^2), other water stress treatments (10, 20 days drought and -0.7 Mpa) were not significantly different (15.4, 16.03 and 13.8 cm^2) compared to the control (Tab. 3).

Cox Orange demonstrated the lowest leaf area (12.47 cm^2) compared to Rubinette (15.23 cm^2), Golden Delicious (15.63 cm^2) and Elstar (15.23 cm^2) (Tab. 3). Rubinette and Cox Orange showed no significant difference between water stress treatments, water stress by PEG (-0.7 and -1.6 Mpa) revealed a significant decrease of leaf area in both Golden Delicious (14.11 and 13.97 cm^2) and Elstar (12.83 and 13.13 cm^2) compared to the control (17.32 and 17.57 cm^2) (Tab. 3).

III- Leaves number

The results showed no significant difference between varieties in leaves number. After 10 and 20 days water stress, plants showed no significant effect on leaves number (7.69 and 7.64) compared to the control (7.64) (Tab. 4), while water stress induced by PEG 6000 at both concentration (-0.7 and -1.6 Mpa) reduced significantly leaves number in apple seedlings (7 and 6.75), compared to the control (7,64) (Tab. 4).

Tab. 3. Effect of water stress on leaves area (cm^2) of different apple cultivars *(Malus domestica* Borkh. cv.*)*.

Varieties / Treatment	Rubinette	Golden Delicious	Elstar	Cox Orange	Average
Control	13.57 [a]	17.32 [a]	17.57 [a]	11.63 [ab]	15.02 [ab]
10 days stress	16.62 [a]	15.34 [ab]	15.10 [ab]	14.53 [a]	15.4 [ab]
20 days stress	16.91 [a]	17.42 [a]	17.54 a	12.23 [ab]	16.03 [a]
PEG (-0.7Mpa)	17.03 [a]	14.11 [b]	12.83 [b]	11.20 [b]	13.8 [bc]
PEG (-1.6Mpa)	12.04 [a]	13.97 [b]	13.13 [b]	12.77 [ab]	12.97 [c]
Average	15.23 [a]	15.63 [a]	15.23 [a]	12.47 [b]	

Tab. 4. Effect of water stress on number of plant leaves of different apple cultivars (*Malus domestica* Borkh. cv.).

Varieties / Treatment	Rubinette	Golden Delicious	Elstar	Cox Orange	Average
Control	7.43 [a]	8.0 [a]	7.57 [a]	7.57 [a]	7.64 [a]
10 days stress	7.57 [a]	7.43 [ab]	7.57 [a]	8.14 [a]	7.69 [a]
20 days stress	7.14 [a]	8.14 [a]	7.43 [a]	7.86 [a]	7.64 [a]
PEG (-0.7Mpa)	7.57 [a]	7.29 [b]	6.71 [b]	6.43 [b]	7.0 [b]
PEG (-1.6Mpa)	6.57 [b]	6.57 [c]	6.57 [b]	7.29 [b]	6.75 [b]
Average	7.26 [a]	7.49 [a]	7.17 [a]	7.46 [a]	

Effect of water stress on the physiological characteristic

I- Water content (WC) of apple leaves

Analysis of variance of water content revealed a significant effect of water stress on apple seedlings, Cox Orange showed the lowest (WC) in the leaves (71.1%) compared to (Rubinette 72.9% and Golden Delicious 72.5%), while Elstar WC was not significantly affected. Twenty days of water stress (72.2%), and water stress induced by PEG (-0.7 and -1.6 Mpa) showed a significant decreased in WC (69.8% for both) of apple leaves compared to the control (74.8%) and to 10 days water stress (73.9%) (Tab. 5).

Rubinette and Golden Delicious showed a significant difference in water content between the control and water stressed plants for 20 days (73.2% and 72,5%) respectively. Treatments with PEG (-0,7 and -1,6 Mpa) decreased highly WC in Rubinette, Golden Delicious, Cox Orange and Elstar varieties (Tab. 5).

II- Chlorophyll of fluorescence

Photochemical yield or efficiency (Fv/Fm), measured *in-situ* on 20 min. dark-adapted apple leaves, indicated a significant decrease of chlorophyll fluorescence of some varieties, it was 0.807 in Golden Delicious and Elstar decrease to 0.803 in Rubinette and 0.801 in Cox Orange (Tab. 6). The different water stress treatment showed no significant difference in all apple cultivars (Tab. 6).

Tab. 5. Effect of water stress on leaves water content (%WC) of different apple cultivars (*Malus domestica* Borkh. cv.).

Varieties / Treatment	Rubinette	Golden Delicious	Elstar	Cox Orange	Average
Control	76.1 [a]	75.1 [a]	74.4 [a]	73.6 [a]	74.8 [a]
10 days stress	74.8 [ab]	73.9 [ab]	73.8 [a]	73.1 [a]	73.9 [a]
20 days stress	73.2 [bc]	72.5 [bc]	71.9 [a]	71.3 [ab]	72.2 [b]
PEG (-0.7Mpa)	71.1 [cd]	70.8 [cd]	67.7 [b]	69.6 [ab]	69.8 [c]
PEG (-1.6Mpa)	69.3 [d]	70.3 [d]	71.9 [a]	67.7 [b]	69.8 [c]
Average	72.9 [a]	72.5 [a]	71.9 [ab]	71.1 [b]	

Tab. 6. Effect of water stress on chlorophyll fluorescence (Fv/Fm) of different apple cultivars (*Malus domestica* Borkh. cv.).

Varieties / Treatment	Rubinette	Golden Delicious	Elstar	Cox Orange	Average
Control	0.801 [a]	0.809 [a]	0.807 [a]	0.808 [a]	0.806 [a]
10 days stress	0.804 [a]	0.808 [a]	0.806 [a]	0.806 [ab]	0.806 [a]
20 days stress	0.803 [a]	0.805 [a]	0.809 [a]	0.801 [b]	0.8.4 [a]
PEG (-0.7Mpa)	0.803 [a]	0.806 [a]	0.804 [a]	0.807 [ab]	0.805 [a]
PEG (-1.6Mpa)	0.803 [a]	0.805 [a]	0.806 [a]	0.803 [ab]	0.804 [a]
Average	0.803 [b]	0.807 [a]	0.807 [a]	0.801 [b]	

III- Chlorophyll value

Chlorophyll value in the apple leaves showed no significant difference between varieties. After 20 days of water stress and PEG (-1.6 Mpa) there was no siginifcant decrease of Chlorophyll value in the leaves (26.6 and 27.7) (Tab. 7).

Rubintte, Elstar and Cox Orange showed no significant difference between all water stress treatments and control. Golden Delicious showed a significant reduction of chlorophyll value after 20 days of water stress (25.18) compared to the control (28.97) while, the reduction in the other treatments was not significant (Tab. 7).

IV- Effect of water stress on proline accumulation in apple leaves

20 days stressed plants and those subjected to (-0,7 and -1,6 Mpa) PEG showed proline accumulation in the leaves (1.64, 1.74 and 1,56 mmol/g DW) compared to the control and 10 days stressed plants (Tab. 8). Rubinette leaves showed a significant accumulation of proline when the plants stressed for 20 days compared to the control and other treatments. Golden Delicious and Elstar showed the best tolerance to water stress, there was no significant difference in proline accumulation between control and the water stress induced by water deficit or by PEG, both varieties indicated the lowest proline content in the leaves by PEG water stress compared to Rubinette and to high proline accumulation in Cox Orange (Tab. 8).

Discussion

Water stress is one of the most important problems that restrict cultivation of crops in arid and semi-arid regions, it causes adverse effects on plant growth and productivity (Amri and Shahsavar, 2010).

Water stress reduced leaf water status of apple leaves measured as WC which may due to water deficit and by PEG-6000 which induced osmotic effect resulting in dehydration at tissue level and reduced the water in the tissues (Davies, and Lakso, 2006).

Zhang *et al.*, (2010) showed that water stress for one month on apple seedlings of Fugi/M.9EML led to a decrease of plant length, number of leaves and leaf area compared to the control, in addition to a decrease in relative water content (RWC) and photosynthesis.

Tab. 7. Effect of water stress on chlorophyll value of different apple cultivars (*Malus domestica* Borkh. cv.).

Varieties / Treatment	Rubinette	Golden Delicious	Elstar	Cox Orange	Average
Control	28.30 a	28.97 ab	27.1 a	27.66 ab	28.01 a
10 days stress	26.15 a	29.41 a	28.02 a	28.87 a	28.12 a
20 days stress	26.60 a	25.18 c	28.11 a	26.50 b	26.60 b
PEG (-0.7Mpa)	28.53 a	28.5 ab	27.35 a	28.12 ab	28.13 a
PEG (-1.6Mpa)	26.84 a	26.58 bc	28.61 a	28.77 a	27.70 ab
Average	27.28 a	27.73 a	27.84 a	28.00 a	

Tab. 8. Effect of water stress on Proline accumulation (mmol/g DW) in leaves different apple cultivars (*Malus domestica* Borkh. cv.).

Treatment \ Varieties	Rubinette	Golden Delicious	Elstar	Cox Orange	Average
Control	0.89 [b]	0.94 [ab]	0.92 [a]	0.93 [c]	0.92 [c]
10 days stress	0.95 [b]	0.81 [b]	1.25 [a]	1.47 [abc]	1.12 [bc]
20 days stress	2.22 [a]	1.34 [ab]	1.50 [a]	1.25 [bc]	1.57 [ab]
PEG (-0.7Mpa)	1.63 [ab]	1.31 [ab]	1.57 [a]	2.05 [ab]	1.64 [a]
PEG (-1.6Mpa)	1.71 [ab]	1.66 [a]	1.23 [a]	2.36 [a]	1.74 [a]
Average	1.48 [a]	1.21 [a]	1.29 [a]	1.61 [a]	

Proline, which increases proportionately faster than other amino acids in plants under water stress, has been suggested as an evaluating parameter for irrigation scheduling and for selecting drought resistance varieties. The capacity for osmotic adjustment, via the accumulation of proline, during stress has been also found in leaves of grapevine (Shultz and Matthews, 1993) and in apple trees (Lakso *et al*., 1984). However, accumulation of proline under stress conditions seems to be species and cultivar dependent. In fact, in many plants, under stress conditions, it have stated that proline accumulation correlated with stress tolerance and its concentration is generally higher in stress tolerant than in stress sensitive plants (ASHRAF and FOOLAD, 2007). (SOFOA *et al*., 2004) showed on Olive leaves, a proline concentration between (0.5 µmol/mg DW) in the control leaves and (1.59 µmol/mg DW) in water stressed olive leaves after 20 days of water stress. Yang and Huang (1994) suggested that the concentration of free proline in apple trees increased significantly under water stress.

Lotfi, *et al*., (2010) indicated in study on Walnut that water stress by PEG 6000 which produce water potentials of 0 Mpa (control), –0.10 MPa, –0.50 MPa, –0.75 MPa, –1.00 MPa, –1.50 MPa and –2.00 MPa, increased free proline levels in response to water stress which were higher in drought-tolerant genotypes than in sensitive ones. As a consequence, proline concentrations could be used as a biochemical marker of water stress in plants.

Leaf photosynthetic capacity such as chlorophyll content, ribulose bisphosphate carboxylase/oxygenase (Rubisco) activity and efficiency of light reactions of photosynthesis, can all be influenced by salinity and water stress (Perti *et al*., 2000, Munns, 2002). In addition, stomatal limitation of photosynthesis may play an important role. In the present experiment, chlorophyll fluorescence of apple leaves was not affected by water stress. The osmotic effects of PEG solutions caused photoinhibition in apple leaves. A decrease of net photosynthesis rate occurs frequently with a reduction of stomatal conductance (Tolker *et al*., 1999; Santiago, et al., 2000), which appear as a consequence of osmotic stress (Feldina *et al*., 1994). The different responses of apple cultivars to water stress depends of status of apple seedlings and its concentration of mineral nutrients in the leaves, especially the concentration of calcium, which plays a big role in the wall structure and water stress tolerance (Ming *et al*. 2003). The biochemical protection of cells under water stress depends of the stage of apple leaves and stress period (after 23 days of drought and at −2.0 MPa)

(Sircelj *et al*., 2005).

The rate at which water stress is imposed affects plant ability to adapt its physiology to compensate the reduction in water availability (Kang and Zhang, 2004). The intensity of water stress treatment based commonly on plant response such as incipient wilting (Stewart *et al*., 1995), leaf water potential (Edwards and Dixon, 1995), or stomatal closure (Patakas and Noitsakis, 1999). Water stress induced by PEG-6000 or sodium chloride decreased seeds germination and length of roots and shoots with increasing of PEG or NaCl concentrations or osmotic potential -1,03 Mpa (Nayer and Reza, 2008b). Our results showed as well that the osmotic potential of PEG 6000 -1,6 Mpa decreased the morphological *characteristic*s in apple seedlings.

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