Jordan Journal of Agricultural Sciences, Volume **1**, No. **1**, 2005

Growth and Physiological Responses of Tomato Landrace "Rohaba"(*Lycopersicon Esculentum* Mill.) to *in Vitro* Induced Water Deficit

Mai M. Abu- Shama, Rida A. Shibli, Khalil I. Ereifej and Ibrahim M. Makhadmah and Naseem M. Abed Alrahman*

ABSTRACT

Influence of *in vitro* induced water deficit upon vegetative growth and physiology of tomato landrace "Rohaba" (*Lycopersicon esculentum* Mill.), was investigated using 0.1, 0.2, 0.3 and 0.4 M sucrose, or 0, 0.1, 0.2 and 0.3 M sorbitol or mannitol as osmotic agents. Shoot height and shoot fresh weight were significantly reduced as sucrose was increased to 0.4 M. While a significant increase in shoot dry weight was obtained when sucrose levels were increased, rooting percentage and root number decreased. Root length was significantly increased at 0.3 M and reduced at 0.4 M sucrose. In the case of sorbitol, shoot height was significantly reduced at 0.3 M, shoot fresh and dry weight were significantly reduced as sorbitol levels were increased in the media. Rooting percentage, root number and root length were not affected at all by the sorbitol levels. When using mannitol, shoot height was significantly increased at 0.1 M and then significantly reduced at 0.2 M and 0.3 M. Shoot dry weight and fresh weight were reduced as mannitol levels were increased. Rooting percentage and root number were enhanced at 0.1 M. Root length was increased in response to different mannitol levels. The osmotic potential of tomato microshoots was significantly reduced (became more negative) as sucrose, sorbitol, or mannitol levels were increased. Tomato microshoots contents of N, K, Mg, Na and ash percentage were reduced significantly in response to increased levels of sucrose, sorbitol, or mannitol. Ca and P contents were not affected by elevated concentrations of osmoticums. Fiber content of tomato microshoots was not significantly affected at all by the sucrose levels, but a significant reduction in fiber content was obtained as sorbitol and mannitol levels were increased. Reducing sugar percentage, carbohydrate percentage and proline accumulation were increased and the protein (crude and soluble) and fat contents were reduced as the levels of sucrose, sorbitol and mannitol were increased.

KEYWORDS: *in vitro*, Lycopersicon, tomato, water deficit.

INTRODUCTION

Water is responsible for maintaining turgidity of cells (Spomer, 1995; Turner, 1981), nutrients uptake (Spomer, 1995) and other physiological and biochemical processes (Shibli, 1990). In many arid and semi-arid areas, water shortage has become a primary limitation of agricultural production (Al-Karaki and Al-Raddad, 1997) indicating the need for water conservation and utilization. Landraces and primitive forms of tomato, which are locally adapted, offer a good source for the improvement of the crop for

* Department of Plant Production, Faculty of Agriculture, Jordan University of Science and Technology, Irbid, Jordan. Received on 14/3/2005 and Accepted for Publication on 18/10/2005

both biotic and abiotic stress tolerances (Agong *et al*., 1997) including water status.

Plant tissue culture systems are employed to impose plant stresses of salinity and drought (Shibli *et al*., 1992, 2000). Media-induced water stress was used to investigate osmostress effects (Shibli *et al*., 1998; 1992) and to study plant water relations at the cellular and whole plant level (Bressan *et al*., 1982; Handa *et al*., 1982). Plant response to *in vitro* induced water deficit mimics those of *in vivo* conditions (Sawwan *et al*; 2000). In addition, *in vitro* culture provides a precise measurement of shoot and root growth under treatment effect (Shibli *et al*., 1992; 2000) and it has the benefit of microculture scale with rigorous treatment and environmental control (Shibli *et al*., 1992; Shibli and Smith, 1999).

Sorbitol (Sawwan *et al*., 2000; Shibli *et al*., 1992), mannitol (Shibli *et al*., 1992) and sucrose (Shibli, 1990) are suitable osmotic agents to impose artificial stress on plant specimens *in vitro*. The physico-chemical nature of the compounds used to impose artificial stress determines the effects on plant tissue (Gangopadhyay *et al*., 1997). Adding an osmotic agent to the medium would cause more negative water potential and consequently reduce growth (Shibli and Al-Juboory, 2002; Tahtamouni *et al*., 2001). Leaf loss and discoloration increased with the increase of osmoticum concentration (Tahtamouni *et al*., 2001). The negative osmotic potential of leaf cell sap and leaf dehydration, due to continuous accumulation of starch and carbohydrates, raises the osmostress conditions (Shibli *et al*., 1992), which account for changes in leaf color. The survival percentage of "Nabali" Olive microshoots was reduced by increasing water deficit (Shibli and Al-Juboory, 2002). Organic and inorganic solute accumulation caused cell adjustment of osmotic potential under stress conditions (Tschaplinski *et al*., 1995). The osmotic concentration of the medium affects the growth rate and morphogenic structures of cells and tissues (Shibli *et al*., 1992) and also influences all organic and inorganic constituents of the medium.

This study was conducted to study the growth, mineral uptake, osmotic adjustment and tissue content of carbohydrates, fiber, fat, protein and proline of "Rohaba" tomato microshoots under *in vitro* induced water deficit.

Materials and Methods

Establishment of *in Vitro* Mother Stock Cultures

In vitro cultures of tomato landrace "Rohaba" were established from seeds. Seeds which were obtained from the National Center for Agricultural Research and Technology Transfer (NCARTT) genebank, were surface sterilized with 70% alcohol for 30 seconds, followed by 3.5% sodium hypochlorite treatment for 15 minutes and then rinsed with sterilized distilled water for 3 times. Sterilized seeds were inoculated to ¼ strength MS medium (Murashige and Skoog, 1962) free of growth regulators for germination. Shoot tips were excised aseptically and were inoculated on MS mediums (pH 5.8) containing 30 gL^{-1} sucrose for 3 weeks to establish adequate stocks of microshoots before initiating experiments.

Microshoot Growth Responses to Water Deficit

Microshoots (3 cm Long) were subcultured to solid MS proliferation medium in 250 ml Erlenmeyer flask. Media contained 0.1, 0.2, 0.3, 0.4 M sucrose, or 0, 0.1, 0.2, 0.3 M, sorbitol or mannitol. Media in all sorbitol and mannitol treatments contained 30 gL^{-1} sucrose. After 6 weeks, data were collected for shoot height, number of roots, root length and shoot fresh weight. Shoots were oven dried to a constant weight at 70 ºC and the dry weight was recorded.

Osmotic potential of leaf cell sap samples were measured using a Wescore-5500 Vapor Pressure Osmometer (Shibli and Al-Juboory, 2002; Shibli and Smith, 1999). Shoots were dried to a constant weight at 70 °C and then grounded using a small mortar to pass 1.0 mm sieve size. Then samples of 0.5g of well-mixed, dry

and ground plant material were weighed and ignited in Muffle Furnace at 550 °C and the ash was dissolved in 5 ml of 2N HCl for determination of Na, K, P, Ca and Mg. Na and K were analyzed using flame photometer (Corning, M410) and Ca and Mg concentrations were determined using atomic absorption spectroscopy (Elmer-Perkin, 2380). P was analyzed using Vanadate-Molybdate Yellow method by Spectrophotometer (Spectronic 20 D) (Chapman and Pratt, 1961). Analysis of total nitrogen was performed with Micro-Kjeldahl digestion procedure (Bulman and Smith, 1993). Crude protein content was determined by multiplying the total nitrogen by a factor of 6.25 (Bulman and Smith, 1993). Proline concentration was determined using a standard curve and calculated on fresh weight basis as described by Bates *et al*. (1973). Moisture, ash, crude fat, crude fiber, reducing sugar and total carbohydrate were analyzed according to AOAC methods (1984).

Statistical Analysis

Treatments in the experiments were arranged in a Completely Randomized Design (CRD), with three replications. Collected data were subjected to the analysis of variance (ANOVA) using SAS program (Statistical Analysis System, 1998). Means were separated according to the Least Significant Difference (LSD) at 0.05 level.

Results and Discussion

Growth and Rooting

Height and fresh weight of tomato shoots were not affected with the increase of sucrose up to 0.3 M and then reduced significantly with 0.4 M sucrose. However, shoot dry weight was increased with the increase in sucrose concentration up to 0.3 M (Table 1) indicating the inability of sucrose at 0.3 M to serve as an osmoticum. Sucrose may be metabolized and consumed as a carbon source by the plant resulting in subsequent concentration reduction in the media (Chong and Pua, 1985; Hyndman *et al*., 1982, Shibli, 1990; Shibli *et al*., 1992). Chong and Pua (1985) found that increasing media sucrose up to 0.14 M is an optimal level for Ottawa apple shoot length and number and then shoot height was declined with sucrose increase above 0.14 M. Similar results were obtained by Lipavska and Vreugdenhil (1996), Allen *et al*. (1987) on young guayale (*Parthenium argentatum)* Gray plants. A significant increase in dry shoot weight was obtained when sucrose level was increased (Table 1). Similarly, increasing media sucrose concentration to 0.26 M yielded a significant increase of jojoba zygotic embryo dry weight and wax ester production in both liquid and semisolid media (Wang and Janick, 1986).

Increased sorbitol concentration from 0 to 0.2 M did not affect shoot height of tomato, whereas significant reduction was observed at the 0.3 M sorbitol (Table 1). Increasing media incorporated sorbitol decreased shoot height of Ottawa apple (Chong and Pua, 1985), chrysanthemum (Shibli, 1990; Shibli *et al*; 1992) and Nabali olive (Shibli and Al-Juboory, 2002).

There was a significant increase in shoot height and fresh weight of tomato at 0.1 M mannitol (Table 1) indicating that this level of mannitol was inactive osmoticum. Height and fresh and dry weights of tomato shoots were significantly reduced at 0.2 M or 0.3 M mannitol (Table 1), which could be explained that plants their turgidity under water deficit conditions, then the cells become unable to multiply, divide and expand or absorb essential materials for their normal growth and development (Hopkins, 1995). Similar results were obtained on *Chrysanthemum morifolium* Ramat (Shibli *et al*., 1992), Marianna 2624 prunus cuttings (Rajashekar *et al*., 1995) and African violet microcultures (Sawwan *et al*., 2000).

Rooting percentage and root number were not affected by different sucrose or sorbitol levels as compared to control (Table 1). Root length was not affected at 0.2 M sucrose as compared to the control and

(0.1 M). At 0.3 M sucrose, root length was significantly increased, whereas root length was significantly reduced at 0.4 M (Table 1). Similarly, Hyndman *et al*. (1982), reported that sucrose concentrations of 0.14 - 0.26 M caused longer cultured rose root growth than the lower concentrations of 0-0.08 M. Also, Shibli *et al*. (1992) found that rooting response of chrysanthemum plants was uniformly enhanced by the presence of sucrose in the medium. The increase in the root length at 0.3 M and the decrease at 0.4 M indicated that root growth continues up to optimal conditions but decreases gradually under adverse conditions.

Rooting percentage root number and root length were not significantly affected at all sorbitol levels (Table 1). Maintenance of insignificant difference in rooting percentage root number and root length in response to increased sorbitol levels would be an excellent strategic position for maintaining turgor, which contributes to drought resistance. In contrast, reduced root number and root length of Nabali olive microshoots at concentrations more than 0.075 M sorbitol in the medium had been reported (Shibli and Al-Juboory, 2002). Root length and root number of African violet microshoots were significantly reduced with increased sorbitol concentrations in the medium (Sawwan *et al*., 2000). Al-Karaki *et al*. (1995) reported that although roots of bean and sorghum plants were in a direct contact with the water stress inducing agent polyethylene glycol in the nutrient solution, shoots had more decrease in dry matter than roots. They explained that by differences in repartitioning of assimilates into roots versus shoots during reduced water availability.

The increase of rooting percentage and root number at 0.1 M mannitol and maintenance of insignificant differences at 0.2 M and 0.3 M (Table 1) is considered as a morphological adaptation to evade water deficit. A significant increase in root length was also observed at all levels of mannitol compared with the control; but each increase in mannitol over 0.1 M was associated with a significant decrease in root length (Table 1). Similarly, a major increase in root length was observed under drought stress conditions in a field experiment conducted by Hoogenboom *et al*. (1987). In contrast, total inhibition of rooting ability of different chrysanthemum cultivars by mannitol was reported by Shibli *et al*. (1992). Sawwan *et al*. (2000) found that an increased mannitol level significantly reduces root number of African violet plants.

Osmotic Potential

Osmotic potential of tomato microshoots was reduced (became more negative) as osmoticums concentrations were increased (Table 2). Increasing sucrose concentration from 0.087 M to 0.259 M significantly reduced the osmotic potential of different tested chrysanthemum cultivars (Shibli, 1990; Shibli *et al*., 1992). Sucrose had less influence on osmotic adjustment than sorbitol or mannitol since sucrose serves as a cell metabolite more than being as an osmoticum (Shibli *et al*., 1992). Shibli and Al-Juboory (2002) reported that sorbitol induced osmotic stress significantly reduced cell sap osmotic potential of Nabali olive (*Olea europea*). Present results (Table 2) are similar to those of Sawwan *et al*. (2000), where sorbitol and mannitol induced water deficit reduced the osmotic potential of African violet microcultures. Organic and inorganic solute accumulation was described as a reason behind the cell adjustment of osmotic potential under stress conditions (Ben-Hayyim, 1987; Handa *et al*., 1983; Sanchez *et al*., 1998; Tschaplinski *et al*., 1995). Osmotic adjustment has been described as a mechanism of many plants to tolerate or adapt to stress conditions (Allen *et al*., 1987; Shibli *et al*., 1992; Tschaplinski *et al*., 1995).

Mineral Uptake

In general, there was a significant decrease in N, K, Mg and Na content in tomato microshoots as concentration of osmoticum was increased in the media

(Table 2). According to Chipman *et al*. (2001), leaf nitrogen concentration of Delta pine soybean plants decreased during water stress. The authors attributed this decline to the remobilization of N in response to drought. Similarly, Tanguilig *et al*. (1987) reported that under water stress conditions, the uptake of N decreased in soybean plants. Present results are consistent with those of Dambrine *et al*. (1993) where a decrease in K concentration occurred in the dry plot of spruce plants as compared with the control. Volaire *et al*. (1988 a,b) found that Mg and Na concentrations were reduced in response to prolonged drought in perennial forage grasses.

Effects of the different osmoticum concentrations (Table 2) on P and Ca contents of tomato microshoots were insignificant except at 0.3 M sucrose, where a significant reduction in P was observed. Fawcett and Quirk (1962) concluded that the rate of P absorption by wheat plants was not affected by increasing water stress until the plants were subjected to wilting, absorption then decreased markedly. The present results (Table 2) are in harmony with those of Volaire and Thomas (1995) on drought tolerant cocksfoot cultivars (*Dactylis glomerata* L.). Maintenance of good P level might indicate some drought tolerance of "Rohaba" tomato through enhancing or maintaining good root growth under such conditions.

That Ca was not affected by the different levels of osmoticums (Table 2). This is in general agreement with earlier findings (Schier and Mcquattie, 2000) where water stress on pitch pine seedlings grown in a nutrient solution did not affect foliar Ca concentration. However, Alam (1994) reported that drought results in low uptake of anions and greater uptake of divalent cations (as Ca) compared to monovalent cations; the potential of roots to absorb nutrients in water stressed plants might be reduced because of decreased mineral demand due to reduced growth.

Chemical Analysis

Protein Content

Crude and soluble protein contents of tomato microshoots decreased significantly as the concentration of the three osmoticums were increased (Table 3). Soluble protein content was significantly increased at 0.2 M sucrose, but was significantly reduced at the two higher levels of this osmoticum. Elevated sorbitol and manitol concentrations also reduced soluble proteins (Table 3). Lazcano-Ferrat and Lovatt (1999) found that reduced vegetative growth of *Phaseolus* plants under water deficit stress was accompanied by a significant decrease in leaf protein concentration with unknown metabolic basis for this decrease. Also, mung bean seedlings showed a decrease in protein level when raised under increasing water deficits (Kumar and Singh, 1991). A similar finding had been reported by Handa *et al*. (1983), where soluble protein levels in cultured tomato cells declined in the adapted cell lines in response to polyethylene glycol induced osmotic stress. The decreased level of total as well as soluble protein contents in water stressed tissues appeared to be due to more degradation of proteins as well as overall inhibition of protein synthesis under water stress (Kumar and Singh, 1991).

Fat Content

Fat content of tomato microshoots was significantly reduced in response to elevated osmoticums concentrations (Table 3). This result is in conformity with earlier results wherein drought stress induced a sharp decrease in lipid content of rape (Dakhma *et al*., 1995) and safflower plants (Hamrouni *et al*., 2001).

Fiber Content

While sucrose had no effect on fiber content of tomato microshoots, increased levels of both sorbitol and mannitol reduced their fiber content. Peterson *et al*. (1992) reported reduction of fiber content of investigated legume plants under drought conditions and therefore, enhancing quality due to lower fiber concentrations in leaves and stems without changes in composition of the fiber fraction. Reduction in fiber content of Russian thistle plants was also reported in response to salinity

stress (Fowler *et al*., 1992).

Ash Content

Ash content was significantly reduced as concentrations of the three osmoticums increased (Table 4). These effects are in contrast with the effects of salinity induced water deficit on ash percent of African violet (Shibli *et al*., 2001). Ash content was also increased in saltbush plants grown in nutrient solution in response to salinity stress (Welch, 1978).

Reducing Sugar and Proline Percentages

Carbohydrate percentage reducing sugar percentage and prolines significantly increased in tomato microshoots in response to elevated osmoticum concentrations (Table 4). The present result supports that of Sanchez *et al*. (1998) where soluble carbohydrate concentration increased 5 to 7 times in water stressed pea cultivars. According to these authors, soluble carbohydrates play an important role in osmotic adjustment and turgor maintenance. The increase in soluble sugars and carbohydrate content can be attributed to smaller translocation from the leaf, slower consumption due to reduced growth and other changes such as starch hydrolysis (Kameli and Losel, 1996). Similarly, carbohydrate concentration increased in apple shoots in response to sorbitol induced osmotic stress (Karhu, 1997). Kerepesi and Galiba (2000) reported that drought tolerant wheat genotypes accumulated more carbohydrates than did sensitive genotypes.

Glucose and fructose formation rates were clearly higher in stressed wheat plants of the tolerant varieties (Kerepesi *et al*., 1998). An increase in reducing sugar levels in cells of tomato (Handa *et al*., 1982) and tobacco plants (Bressan *et al*., 1982; Iraki *et al*., 1989) adapted to polyethylene glycol induced osmotic stress had also been reported. On the other hand, the increase in reducing sugar content attributed to fructan hydrolysis in perennial rye grass and tall fescue basal tissues grown under drought conditions was reported (Karsten and Macadam, 2001; Spollen and Nelson, 1994). The increased sugar induced by water stress is significantly correlated to osmotic adjustment and turgor maintenance (Sanchez *et al*., 1998).

Kumari and Veeranjaneyulu (1996) reported that proline content of mulberry plants leaves and roots increased when water stress increased from mild to severe. Also Al-Karaki *et al*., (1996), found that as leaf water potential of bean and sorghum plants became more negative, proline accumulated in the leaves. Under drought stress compatible solutes such as proline can be accumulated to contribute both to turgor maintenance and the protection of macromolecular structure against the destabilizing effects of decreasing water activity (Arndt *et al*., 2000). Accumulation of proline under drought stress might be due to reduced rate of catabolism (Rhodes *et al*., 1986). It has been demonstrated that drought induces transcripts encoding Δ- pirroline- 5- carboxylate- synthetase (P5CS), which catalyses the first two steps in proline biosynthesis from glutamate (Sanchez *et al*., 1998; Somal and Yapa, 1998; Yoshiba *et al*., 1995).

Conclusions

The osmotic stress contributed by sucrose up to 0.3 M was overshadowed by its metabolism and consumption as a carbon source by the plant. Increasing sucrose concentration up to 0.4 M inhibited the growth of "Rohaba" tomato microshoots as indicated by decreased height, fresh shoot weight and root length. Microshoot height was decreased only at 0.3 M sorbitol. While fresh and dry shoot weights were decreased at all sorbitol levels, rooting percentage, root length and root number were not affected. Mannitol induced osmotic stress caused a reduction in "Rohaba" microshoot height and fresh and dry shoot weights. Different mannitol levels did not affect root growth parameters. The osmotic potential of tomato microshoots was significantly reduced (become more negative) as sucrose, sorbitol or mannitol levels were increased. Osmotic adjustment of "Rohaba" tomato can be considered as a mechanism of plant tolerance or adaptation to the induced water deficit stress conditions. The concentrations of N, K, Na, Mg and ash were reduced in response to elevated sucrose, sorbitol, or

mannitol levels. The elevated levels of any osmoticum did not affect Ca and P contents. Maintenance of good P content in "Rohaba" microshoots might be considered as a drought tolerance index. Changes in "Rohaba" tomato growth in sucrose, sorbitol or mannitol supplemented media were accompanied by some physiological and biochemical tolerance mechanisms and osmotic adjustment which might explain some reasons behind "Rohaba" tomato tolerance to the *in vitro* induced water deficit by the three osmoticum. This was evident by the accumulation of reducing sugar, carbohydrate and proline and the reduction in crude protein, soluble protein, fiber (in sorbitol and mannitol supplemented media) and fat contents in response to increased levels of these osmoticums. Finally, it can be concluded that *in vitro* culture could be a sufficient alternative for studying tomato physiological responses and tolerance to water deficit.

Table 1. Influence of osmoticums on microshoot height, fresh weight, dry weight, rooting percentage, root number and root length of in vitro grown "Rohaba" tomato (Lycopersicon esculentum Mill.)

Osmoticum	Concentration (M)	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Rooting (%)	Root number	Root length (cm)
Sucrose	0.1	7.35 a*	7.05 a	0.43 c	100.00 ab	13.49 ab	6.95 b
	0.2	7.44 a	7.78 a	0.63 b	153.07 a	20.65 a	8.21 ab
	0.3	6.73 a	6.89 a	0.75 a	126.33 a	17.04 a	8.89 a
	0.4	4.68 b	4.66 b	0.63 b	57.67 b	7.78 b	5.23 c
Sorbitol							
	0	7.35 a	7.05 a	0.43 a	100.00 a	13.49 a	6.95 a
	0.1	6.88 a	3.94 b	0.44 a	145.98 a	19.69 a	6.53 a
	0.2	6.16 a	2.96 bc	0.35 b	121.58 a	16.39 a	5.66 a
	0.3	3.92 b	1.78 c	0.28 c	96.91 a	13.07 a	5.26 a
Mannitol							
	0	7.35 b	7.05 a	0.43 a	100.00 b	13.49 b	6.95 d
	0.1	8.26 a	3.66 b	0.45 a	155.02 a	20.91 a	12.38 a
	0.2	5.56 c	2.21 c	0.34 b	116.70 b	15.74 b	10.91 b
	0.3	4.25 d	1.59 c	0.25 c	100.42 b	13.54 b	8.99 c

*Means within columns for each osmoticum having different letters are significantly different according to LSD at 0.05 level.

Table 2. Influence of osmoticums on microshoot osmotic potential and N, K, Mg, Na, P and Ca contents of in vitro grown"Rohaba" tomato (Lycopersicon esculentum Mill.).

Osmoticum	*Conc. (M)*	*Osmotic Potential (MPa)*	*N (%)*	*K (%)*	*Mg (%)*	*Na (%)*	*P (%)*	*Ca (%)*
Sucrose	0.1	-0.6 a	9.18 a*	3.68 a	0.32 a	1.14 a	0.40 a	0.14 a
	0.2	-0.66 b	8.05 b	3.09 b	0.30 ab	0.86 b	0.40 a	0.28 a
	0.3	-0.9 c	6.70 c	2.45 c	0.28 bc	0.72 bc	0.33 b	0.22 a
	0.4	-1.068d	6.53 c	2.10 d	0.25 c	0.62 c	0.35 ab	0.18 a
Sorbitol								
	0	-0.59 a	9.18 a	3.68 a	0.32 a	1.14 a	0.40 a	0.14 a
	0.1	-0.80 b	7.82 b	3.06 b	0.14 b	0.78 b	0.41 a	0.15 a
	0.2	-1.00 c	7.26 bc	2.57 c	0.15 b	0.66 bc	0.40 a	0.14 a
	0.3	-1.4 d	6.70 c	1.82 d	0.13 b	0.55 c	0.38 a	0.14 a
Mannitol								
	0	-0.5 a	9.18 a	3.68 a	0.32 a	1.14 a	0.40 a	0.14 a
	0.1	-1.2 b	7.88 b	2.63 b	0.15 b	0.74 b	0.39 a	0.15 a
	0.2	-1.3 c	7.43 c	2.02 c	0.13 bc	0.62 b	0.39 a	0.15 a
	0.3	-1.5 d	7.38 c	1.85 c	0.12 c	0.61 b	0.40 a	0.14 a

*Means within columns for each osmoticum having different letters are significantly different according to LSD at 0.05 level.

Table 3. Influence of osmoticums on microshoot contents of crude and soluble protein, fat and fiber of *in vitro* grown "Rohaba" tomato (*Lycopersicon esculentum* Mill.)

Osmoticum	*Concentration (M)*	*Crude protein (%)*	*Soluble protein (mg/g)*	*Fat (%)*	*Fiber (%)*
Sucrose	0.1	60.08 a*	22.75 a	0.386 a	10.58 a
	0.2	52.97 b	24.76 a	0.256 bc	11.71 a
	0.3	43.54 c	21.46 bc	0.270 b	10.98 a
	0.4	42.45 c	19.83 c	0.213 c	9.27 a
Sorbitol	0	60.08 a*	22.75 a	0.386 a	10.58 a
	0.1	50.78 b	21.00 ab	0.230 c	10.17 a
	0.2	47.00 bc	18.20 bc	0.280 b	7.27 b
	0.3	43.37 c	17.52 c	0.266 bc	6.49 b
Mannitol	0	60.08 a*	22.75 a	0.386 a	10.58 a
	0.1	51.20 b	15.89 b	0.140 b	10.15 a
	0.2	48.90 c	15.68 b	0.126 b	7.08 b
	0.3	48.26 c	14.93 b	0.133 b	5.93 b

*Means within columns for each osmoticum having different letters are significantly different according to LSD at 0.05 level.

Table (4). Influence of osmoticums on microshoot ash percentage, carbohydrate percentage reducing sugar and proline of *in vitro* grown "Rohaba" tomato (*Lycopersicon esculentum* Mill.)

Osmoticum	*Concentration (M)*	*Ash (%)*	*Carbohydrate (%)*	*Reducing sugar (%)*	*Proline (µmole/g)*
Sucrose	0.1	12.51 a	12.05 c	9.63 c*	2.12 c
	0.2	9.05 b	21.04 b	14.75 b	2.34 c
	0.3	7.58 bc	34.94 a	15.56 b	8.16 b
	0.4	6.89 c	36.32 a	18.03 a	10.27 a
Sorbitol					
	0	12.51 a	12.05 c	9.63 c	2.12 d
	0.1	8.91 b	26.22 b	12.15 b	3.03 c
	0.2	6.89 c	35.15 a	20.46 a	7.54 b
	0.3	6.18 c	40.37 a	21.54 a	8.95 a
Mannitol					
	0	12.51 a	12.05 d	9.63 c	2.12 d
	0.1	8.51 b	26.27 c	15.47 b	6.71 c
	0.2	8.62 b	32.27 b	21.57 a	9.93 b
	0.3	7.10 c	35.39 a	20.70 a	16.25 a

*Means within columns for each osmoticum having different letters are significantly different at 0.05 level.

REFERENCES

Agong, S.G., Schittenhelm S. and Friedt, W.1997. Assessment of Tolerance to Salt Stress in Kenyan Tomato Germplasm. *Euphytica*; 95: 369- 379.

Alam, S.M. 1994. Nutrient Uptake by Plants under Stress Conditions. In: Handbook of Plant and Crop Stress. Edited by M. Pessarakli. University of Arizona, Tucson, Arizona. Pp. 236- 240.

Al-Karaki, G.N. and Al-Raddad, A., 1997. Effects of Arbuscular Mycorrizal Fungi and Drought Stress on Growth and Nutrient Uptake of Two Wheat Genotypes Differing in Drought Resistance. *Mycorrhiza*; 7: 83-88.

Al-Karaki, G.N., Clark, R.B. and Sullivan. C.Y. 1996. Phosphorus Nutrition and Water Stress Effects on Proline Accumulation in Sorghum and Bean. *J. Plant Physiol*., 148: 745- 751.

Allen, S.G., Nakayama, F.S., Dierigand, D.A., Rasnick, B.A. 1987. Plant Water Relations, Photosynthesis and Rubber Content of Young Guayule Plants During Water Stress. *Agron. J*.; 79: 1030- 1035.

AOAC (Association of Official Analytical Chemists). 1984. *Official Methods of Analysis*. 14th Ed. Washington, DC, USA.

Arndt, S.K., Wanek, W., Cliffordand, S.C., Popp, M. 2000. Contrasting Adaptations to Drought Stress in Field-Grown *Ziziphus Mauritiana* and *Prunus Persica* Trees: Water Relations, Osmotic Adjustment and Carbon Isotope Composition. *Aust. J. Plant Physiol*., 27: 985-996.

Bates, L.S. 1973. Rapid Determination of Free Proline for Water Stress Studies, *Plant Soil*., 39: 205- 207.

Ben-Hayyim, G. 1987. Relationship between Salt Tolerance and Resistance to Polyethylene Glycol- Induced Water Stress in Cultured Citrus Cells, *Plant Physiol*.; 85: 430-433.

Bressan, R.A., Handa, A.K., Handa, S. and Hasegawa, P.M. 1982. Growth and Water Relations of Cultured Cells after Adjustment to Low External Water Potentials. *Plant Physiol*. 70: 1303-1309.

Bulman, P. and Smith, D. L.1993. Grain Protein Response of Spring Barely to High Rate and Post Anthesis Application of Fertilizer Nitrogen. *Agron. J*., 85: 1109-1113.

Chapman, H.D. and Pratt, P.F. 1961. Methods of Analysis for Soil, Plant and Water. University of California, Riverside, CA. Pp.169- 170.

Chipman, R.B., David Raper J.C. and Patterson R.P.2001. Allocation of Nitrogen and Dry Matter for Two Soybean Genotypes in Response to Water Stress During Reproduction Growth. *J. Plant Nutr*.; 24: 873-884.

Chong, C. and Pua, E.C. 1985. Carbon Nutrition of Ottawa

Three Apple Rootstocks During Stages of *In Vitro* Propagation. *J. Hort. Sci*.; 60: 285- 290.

Dakhma, W.S., M. Zarrouk and A. Cherif. 1995. Effects of Drought- Stress on Lipids in Rape Leaves. *Phytochemistry- Oxford*; 40: 1383- 1386.

Dambrine, E., N. Carisey, B. Pollier and A. Granier. 1993. Effects of Drought on the Yellowing Status and the Dynamics of Mineral Elements in the Xylem Sap of Declining Spruce (*Picea Abies* L.). *Plant Soil*; 150: 303- 306.

Fawcett, R.G. and J.P. Quirk. 1962. the Effect of Soil- Water Stress on the Absorption of Soil Phosphorus by Wheat Plants. *Aust. J. Agric. Res*.; 13: 193.

Fowler, J.L., J.H. Hageman, K.J. Moore, M. Suzukida, H. Assadian and M. Valenzuela. 1992. Salinity Effects on Forage Quality of Russian Thistle. *J. Range Manage*.; 45: 559- 563.

Gangopadhyay, G., S. Basu, B.B. Mukherjee and S. Gupta. 1997. Effects of Salt and Osmotic Shocks on Unadapted and Adapted Callus Lines of Tobacco. *Plant Cell Tiss. Org. Cult*.; 49: 45-52.

Hamrouni, I., H. Ben- Salah and B. Marzouk. 2001. Effects of Water Deficit on Lipids of Safflower Aerial Parts. *Phytochemistry- Oxford*; 58: 277- 280.

Handa, A. K., R. A. Bressan and P. M. Hasegawa. 1982. Characteristics of Cultured Tomato Cells after Prolonged Exposure to Medium Containing Polyethylene Glycol. *Plant* Physiol; 69: 514-521.

Handa, S., R.A. Bressan, A.K. Handa, N.C. Carpita and P.M. Hasegawa. 1983. Solute Contributing to Osmotic Adjustment in Cultured Plant Cells Adapted to Water Stress. *Plant Physiol*.; 73: 834-843.

Hoogenboom, G., M.G. Huck and C.M. Peterson. 1987. Root Growth Rate of Soybean as Affected by Drought Stress. *Agron. J*.; 79:607- 614.

Hopkins, G.W. 1995. Physiology of Plants under Stress, in: Introduction to Plant Physiology. Wiley and Sons, Inc, NY. Pp. 424- 431.

Hyndman, S.E., P.M. Hasegawa and R.A. Bressan. 1982. The Role of Sucrose and Nitrogen in Adventitious Root Formation on Cultured Rose Shoots. *Plant Cell Tiss. Org. Cult*.; 1: 229- 238.

Iraki, N.M., Bressan R.A., Hasegawa P.M. and Carpita. N.C. 1989. Alterations of the Physical and Chemical Structure of the Primary Cell Wall of Growth-Limited Plant Cells Adapted to Osmotic Stress. *Plant Physiol*., 91: 39- 47.

Kameli, A. and Losel, D. M. 1996. Growth and Sugar Accumulation in Durum Wheat Plants under Water Stress. *New Phytol*., 132:57- 62.

Karhu, S.T. 1997. Sugar Use in Relation to Shoot Induction by Sorbitol and Cytokinin in Apple. *J. Amer. Soc. Hort. Sci*., 122: 476- 480.

Karsten, H.D. and Macadam, J.W. 2001. Effect of Drought on Growth, Carbohydrates and Soil Water Use by Perennial Ryegrass, Tall Fescue and White Clover. *Crop Sci*., 41: 156-166.

Kerepesi, I. and Galiba, G. 2000. Osmotic and Salt Stress-Induced Alteration in Soluble Carbohydrate Content in Wheat Seedlings. *Crop Sci*.; 40: 482-487.

Kerepesi, I., Galiba, G. and Banyai, E. 1998. Osmotic and Salt Stresses Induced Differential Alteration in Water-Soluble Carbohydrate Content in Wheat Seedlings. *J. Agric. Food Chem*., 46: 5347- 5354.

Kumar, P.K. and Singh, R.A. 1991. Germination and Metabolism in Susceptible and Tolerant Mung Bean Genotypes under Moisture Stress. *Indian J. Plant Physiol*., 34: 267.

Kumari, B.D.R. and Veeranjneyulu, K. 1996. Changes in Leaf Water Potential, Osmotic Adjustment and Proline Metabolism in Mulberry During Water Stress. *Isr. J. Plant Sci*., 44: 135- 141.

Lazcano-Ferrat, I. and Lovatt, C. J. 1999. Relationship Between Relative Water Content, Nitrogen Pools and Growth of *Phaseolus Vulgaris* L. and *P. Acutifolius* A. Gray During Water Deficit. *Crop Sci*., 39: 467- 475.

Lipavaska, H. and Vreugdenhil, D. 1996. Uptake of Mannitol from Media by *In Vitro* Grown Plants. *Plant Cell Tiss. Org. Cult.*; 45: 103-107.

Murashige, T. and Skoog, F. 1962. A Revised Medium for Rapid Growth and Bioassay with Tobacco Cultures. *Physiol. Plant*., 15: 473- 496.

Peterson, P.R., Sheaffer, C.C. and Hall, M.H. 1992. Drought Effects on Perennial Forage Legume Yield and Quality. *Agron. J*.; 84(5): 774- 779.

Rajashekar, G., Palmquist, D. and Ledbetter, C.A. 1995. *In Vitro* Screening Procedure For Osmotic Tolerance In Prunus. *Plant Cell Tiss. Org. Cult*. 41: 159-164.

Rhodes, D., Handa, S. and Bressan, R.A. 1986. Metabolic Changes Associated with Adaptation of Plant Cells to Water Stress. *Plant Physiol*. 82: 890- 903.

Sawwan, J., Shibli, R.A., Swaidat, I. and Tahat, M. 2000. Phosphorus Regulates Osmotic Potential and Growth of African Violet Under *In Vitro* Induced Water Deficit. *J. Plant Nutr*.; 23: 759-771.

Schier, G.A. and Mcquattie, C.J. 2000. Effect of Water Stress on Aluminium Toxicity in Pitch Pine Seedlings. *J. Plant. Nutr*. 23(5): 637- 647.

Shibli, R.A. and Al-Juboory, K. 2002. Comparative Responses of "Nabali" Olive Microshoot, Callus and Suspension Cell Cultures to Salinity and Water Deficit. *J. Plant Nutr*. 25: 61-74.

Shibli, R.A. And Smith, M.A.L. 1999. Capacity For Biocompatibility Assessment In Tissue Culture And Hydroponic Systems. *J. Plant Nutr*. 22: 1529-1540.

Shibli, R.A. 1990. Physiological Studies of *In Vivo* And *In Vitro* Water Requirements and Water Relations of *Chrysanthemum Morifolium* Ramat. Ph. D. Thesis. University of Illinois, Urbana- Champaign, USA.

Shibli, R.A., Sawwan, J., Swaidat, I. and Tahat, M. 2001. Increased Phosphorus Mitigates The Adverse Effects Of Salinity In Tissue Culture. *Commun. Soli Sci. Plant Anal*.; 32: 429- 440.

Shibli, R.A., M. Munir, Ein, A.A. and Shatnawi, M. 2000. Growth and Micronutrient Acquisition of Some Apple Varieties in Response to Gradual *In Vitro* Induced Salinity. *J. Plant Nutr*. 23: 1209-1215.

Shibli, R.A., Suwwan, M.A. and Eriefej, K.I. 1998. Response of TYLCV Tolerant Tomato to NaCl Salinity Stress Vegetative Growth and Nutrient Uptake. *Dirasat*; 25: 89-104.

Shibli, R.A., Smith, M.A.L. and Spomer, L.A. 1992. Osmotic Adjustment and Growth Responses of Three *Chrysanthemum* Ramat. Cultivars to Osmotic Stress Induced *In Vitro*. *J. Plant Nutr*. 15: 1373-1381.

Spollen, W.G. and Nelson, C.J. 1994. Response of Fructan to Water Deficit in Growing Leaves of Tall Fescue. *Plant Physiol*., 106: 324- 336.

Spomer, L.A. 1985. Techniques For Measuring Plant Water. *Hort. Sci*. 20: 1021- 1028.

Tahtamouni, R. W., Shibli, R. A. and Ajlouni, M. M. 2001. Growth Responses and Physiological Disorders in Wild Pear (*Pyrus Syriaca* Boiss) During Slow-Growth *In Vitro* Preservation on Osmostressing Media. *Plant Tiss. Cult*. 11: 15-23.

Tanguilig, V.C., Yambao, E.B., Toole, J.C.O. and S.K. Dedatta. 1987. Water Stress Effects on Leaf Elongation, Leaf Water Potential, Transpiration and Nutrient Uptake of Rice, Maize and Soybean. *Plant Soil*; 103: 155.

Tschaplinski, T.J., Gebre, G.M., Dahl, J.E., Robert, G.T. and Tuskan, G.A., 1995. Growth and Solute Adjustment of Calli *Populus* Clones Cultured on Nutrient Medium Containing Polyethylene Glycol. *Can. J. Res.* 25: 1425-1433.

Turner, N.C. 1981. Techniques and Experimental Approaches for the Measurement of Plant Water Status. *Plant Soil*; 58: 339- 366.

Volaire, F. and Thomas,. H. 1995. Effects of Drought on Water Relations, Mineral Uptake, Water- Soluble Carbohydrate Accumulation and Survival of Two Contrasting Populations of Cocksfoot (*Dactylis Glomerata* L.). *Annals of Botany*; 75: Pp. 513- 524.

Volaire, F., Thomas, H. and Lelievre, F. 1988a. Survival and Recovery of Perennial Forage Grasses under Prolonged Mediterranean Drought. I: Growth, Death, Water Relations And Solute Content In Herbage And Stubble. *New Phytol*. 140:439-449.

Volaire, F., Thomas, H., Bertagne, N., Bourgeois, E., Francoise-Gautier, M. and Lelievre, F. 1988b. Survival and Recovery of Perennial Forage Grasses under Prolonged Mediterranean Drought. Π: Water Stress, Solute Accumulation, Abscisic Acid Concentration and Accumulation of Dehydrin Transcripts in Bases in Immature Leaves. *New Phytol*. 140: 451- 460.

Wang, Y.C. and Janick, J. 1986. Sucrose Concentration and Osmolarity as Factors Affecting *In Vitro* Wax Accumulation In Jojoba Embryos. *Hort. Sci*, 21: 1048-1049.

Welch, B.L. 1978. Relationships of Soil Salinity, Ash and Crude Protein in *Atriplex Canescens*. *J. Range Manage*.; 31: 132- 133.

Yoshiba, Y., Katagiri, T., Ueda, H., Mizoguchi, T., Yamaguchi Shinozaki, K., Wada, K., Hanada, Y. and Shinozaki, K. 1995. Correlation Between the Induction of A Gene for Δ- Pirroline- 5- Carboxylase- Synthetase and the Accumulation of Proline in *Arabidopsis Thaliana* under Osmotic Stress. *Plant J*. 7: 751- 760.

النمو والاستجابة الفسيولوجية لنبات البندورة (*Lycopersicon esculentum* Mill.) إلى الشد المائي داخل الأنابيب

مي أبو شمعة, رضا شبلي, خليل عريفج, إبراهيم مخادمة، نسيم عبد الرحمن*

ملخـص

تمت دراسة تأثير الشد المائي على نمو وفسيولوجية نبات البندورة "الصنف البلدي: رحابا" المزروع داخل الانابيب. تم تعريض النباتات المزروعة بواسطة الأنسجة الى الشد المائي باستخدام التراكيز 0.1 , 0.2, 0.3, 0.4 مولار من السكروز أو 0.0, 0.1, 0.2, 0.3 مولار من السوربيتول أو المانيتول.

في تجربة السكروز لوحظ أن طول الساق وكذلك الوزن الرطب للمجموع الخضري قد تناقصا بشكل معنوي على تركيز 0.4 مولار. وفي حين زاد الوزن الجاف بدرجة معنوية, نقصت نسبة التجذير وعدد الجذور بزيادة تراكيز السكروز. أما طول الجذور فقد زاد بشكل معنوي عند تركيز 0.3 مولار وقل عند التراكيز الأعلى.

وفي تجربة السوربيتول نقص طول الساق بشكل معنوي عند تركيز 0.3 مولار, ونقص الوزن الرطب والوزن الجاف لنبات البندورة بشكل معنوي بازدياد تراكيز السوربيتول. ولم تتأثر نسبة التجذير وعدد الجذور بزيادة تراكيز السوربيتول. وفي تجربة المانيتول فقد تبين أن طول الساق زاد بشكل معنوي عند تركيز 0.1 مولار وقل عند التراكيز الأعلى. وعند زيادة تركيز المانيتول نقص الوزن الرطب والوزن الجاف للبندورة بشكل معنوي.وفي حين زادت نسبة التجذير وعدد الجذور عند تركيز 0.1 مولار مانيتول, زاد طول الجذور مع زيادة تركيز المانيتول.

ونقص الضغط الاسموزي لنبات البندورة بشكل معنوي بزيادة تركيز المادة الاسموزية (السكروز أو السوربيتول أو المانيتول). ونقصت تراكيز النيتروجين والبوتاسيوم والصوديوم والمغنيسيوم وكذلك نسبة الرماد في نبات البندروة عند زيادة تراكيز المادة الاسموزية. ولم يتأثر محتوى النبات من الألياف بشكل معنوي عند زيادة تركيز السكروز, ولكنه نقص بشكل معنوي بازدياد تراكيز السوربيتول أو المانيتول. أما نسبة السكريات المختزلة ونسبة الكربوهيدرات ومحتوى البرولين فقد زادت بشكل معنوي بازدياد تركيز المادة الاسموزية في الوسط الغذائي, أما محتوى البروتين الخام والبروتين الذائب في الماء والدهون فقد تناقصت بشكل معنوي مع زيادة تركيز أي من المواد الاسموزية. وهذا يدل على أن صنف البندورة "رحابا" قادر على تحمل الجفاف عن طريق التعديل الاسموزي.

* قسم الإنتاج النباتي، كلية الزراعة، جامعة العلوم والتكنولوجيا الأردنية، اربد، الأردن ، تاريخ استلام البحث 2005/3/14، وتاريخ قبوله 2005/10/18.