International Journal of Agricultural Technology 2015 Vol. 11(1):143-164
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Morphological and Physiological Changes in Two *Triticium aestivum* Cultivars Differing in Water Stress Tolerance

Abdalla, M. M.*

Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt.

Abdalla, M. M. (2015). Morphological and physiological changes in two *Triticium aestivum* cultivars differing in water stress tolerance. International Journal of Agricultural Technology 11(5):143-164.

Abstract Regular depriving two *Triticum aestivum* cultivars of water for either 1, 3 or 6 days then irrigating them throughout 32 days , progressively declined the shoot growth including (linear growth , number and area of leaves, fresh and dry weights of shoots) , the percentage of relative water content (%RWC) , photosynthetic and transpiration rates, the contents of both chlorophyll a+b and total chlorophyll and each of auxins , gibberellins , and cytokinins of leaves. It also drastically decreased the values of polysaccharides, total sugars (TS), phosphorus , potassium , magnesium ,calcium and the activity of catalase in the shoots and roots of both varieties. Reversibly, water stress stimulated the linear growth and number of adventitious roots, and their fresh and dry weights. Additionally, it raised the contents of absicisic acid (ABA), carotenoids, direct reducing sugars (DRS), proline, sucrose, sodium , iron and the activities of both hydrolytic (invertase, α and β-amylase) and oxidative enzymes (peroxidase, polyphenol oxidase and IAA- oxidase) in the shoots and roots of both varieties above those of the untreated controls. However, these changes were more pronounced in the sensitive varieties and with the period and severity of drought.

Keywords: growth criteria, gas exchange, enzymes, metabolites, phytohormones.

Abbreviations: abscisic acid (ABA), cytokinins (CK), direct reducing sugars (DRS), percentage relative water content (%RWC.), photosynthetic rate (Pn), total sugars (TS) , transpiration rate (E), water stress (WS)

Introduction

Drought is one of the major abiotic stresses affecting plants; more than 50% of the Earth's surface area including the vast majority of agricultural land, is vulnerable to drought (Kogan, 1997; Hubbard *et al.*, 2010). Drought- induced crop losses have a significant economic impact, which is predicted to increase with global climatic change (Marris, 2008; Battisti and Naylor, 2009).

* **Coressponding author:** Abdalla, M. M.; **Email**: messam_9156@hotmail.com.

Accordingly, the future food demand for rapidly increasing population pressures is likely to further aggravate due to the effects of drought (Farooq *et al.,* 2005). Plants vary greatly in their capability to tolerate stress conditions, hence some of them are unable to endure stress so wilt and die (sensitive plants), while others can tolerate stress by undergoing certain physiological changes in their tissues which thus maintain their cell water potential and turgidity at normal level inspite of soil drought (tolerant plants) (El-Telwany,1987;Estrada- Campuzano *et al.*, 2008; Farooq *et al.,* 2009) .Water stress (WS) greatly reduces the linear growth of shoots, the time of leaf emergence on the plants, their rate of growth , number , leaf area and fresh and dry weights (Kawakami *et al.*, 2006; Manikavelu *et al.*, 2006; Zeid and Shedeed, 2006; Farooq *et al.*, 2009). Reversibly, water stress positively stimulated the root growth rate, their number, the root/ shoot growth ratio and their fresh and dry weights (Kawakami *et al.*, 2006; Zeid and Shedeed, 2006; Quach *et al.*, 2014). Various studies have revealed that increasing duration and severity of stress, decreased the photosynthetic (Pn)and transpiration rates (E) and percentage relative water content (% RWC) using different plant species (Sanchez-Blanco *et al.*, 2004; Egilla *et al.*, 2005; Farooq *et al.*, 2009; Merewitz *et al.*, 2011). Several investigations showed that water stressed leaves contain less amounts of chlorophyll a , b and total pigments and higher values of carotenoids as compared to untreated control ones(Farooq *et al.*, 2009; Merewitz *et al.,* 2011; Chutia and Borah, 2012) . Moreover, WS boosted the levels of glucose ,sucrose and fructose (Bajji *et al.*, 2001; Saeedipour, 2011; Vajdehfar, 2011; Sales *et al.*, 2013) while it reduced the amounts of starch and total sugar (TS) (Martinez *et al.*, 2004; Wu and Xia, 2006). Furthermore, several workers declared that WS caused the extensive accumulation of proline in all stressed plant organs especially in leaves as a consequence of increasing breakdown of proteins with simultaneous decline in its synthesis in addition to conversion of some of amino acids as ornithin, arginine and glutamic to proline (Bajji *et al*, 2001; Farooq *et al*, 2009; Vajdehfar, 2011). Contraversing effects of WS on the mineral contents were obtained depending on the type of plant and the extent of stress. Several investigations displayed that successive water withdrawal led to a decline in each of P, Na, K, Ca, Mg, Fe and Mo contents in some experimental plants (Razi and Sen, 1996; Farooq *et al.*, 2009), although it caused remarkable increases in the contents of Na, Fe, Mn and Cu in others (El-Tayeb, 2006; Yamaguchi *et al.*, 2010; Pirzard *et al.*, 2012). Drought induces the generation of reactive oxygen species (ROS) causing lipid peroxidation which leads to membrane injury , protein degradation , enzyme inactivation and the disruption of DNA strands (Farooq *et al.,* 2009). Plants protect cellular and subcellular systems from the cytotoxic effects of these

active oxygen radicals through both enzymatic and non-enzymatic antioxidant systems such as, peroxidase, IAA-oxidase, catalase, carotenoids, ascorbic acid and α-tocopherol (Munne-Bosch and Alegre, 2000; Awate *et al.*, 2014). Moreover, Vardhini *et al.* (2011) reported that polyphenol oxidase and IAA-oxidase increased remarkably in sorghum seedlings imposed under stress. WS has been found to influence the activity of α and β-mylases and invertase enzymes using different types of plants (Bialecka and Kepczynski, 2010; Saeedipour, 2011). Many workers explored that WS markedly reduced the contents of each of auxins, gibberellins and cytokinins (CK) and inhibit their production, while it reversibly raised the amounts of ABA, thus decreasing the capability of roots to absorb most of minerals nutrients from soil which led to a reduction in the rate of most vital processes that takes place in plants thus decreasing the rate of growth (Pospisilova *et al.*, 2005; Zhang *et al.*, 2007; Merewitz *et al.*, 2011; Vajdehfar *et al.*, 2011). ABA was reported to be one of the contributing factors which can overcome drought in plants (Hubbard *et al.*, 2010).

Therefore, the main target of the present study was to improve agricultural productivity within limited land and water resources and to detect the morphological and physiological tools underlying the differential tolerance of the two *T. aestivum* cultivars to WS.

Materials and methods

Plant material and treatment

The grains of 4 *Triticum aestivum* cultivars (Fairy 8, Ucorogoa ,legemi, durum) were obtained from the Agricultural Research Centre , Ministry of Agriculture, Giza, Egypt. A preliminary experiment was carried out using the 4 different cultivars to: **1**-detect which is the most tolerant and which is the most sensitive to WS. **2**-determine the most favorable periods that can be applied for water withdrawl;1 , 2 , 3, 4, 6 or 8 days and which can influence growth.Grains of *T. aestivum* L. cv. Fairy 8 (relatively drought sensitive) and cv. Ucorogoa (relativity drought tolerant) were chosen for the present study. Eight sets, each of ten plastic pots (20 cm in diameter and 22 cm in depth) were then arranged, each pot was filled with 2.5 kg of a mixture of pitmos and garden soil in the ratio of 1:2 (w/w).Twenty selected grains from both varieties were cultivated in each pot. Initially, all pots were irrigated daily with 500 ml of water so as to keep the water content of each pot at 70% of the total holding capacity of the used soil, Then at the eighth day of plant growth, thinning was done so as to leave 15 uniform plants in each pot .In the meantime, 50 ml of Hoagland

solution were added to each pot and WS treatment was immediately applied as follows:

1-The first two sets were irrigated daily in both sensitive (S) and tolerant (T) cultivars (Cont. S & Cont. T).

2-The second two sets were irrigated after one day drought (1S &1T).

3-The third two sets were usually irrigated after 3 days from water withdrawal (4S & 4T).

4-The fourth two sets were irrigated after six days (at the seventh day) from water withdrawal (7S &7T).

When the plants were 32 days old & before their anthesis, the experiment was terminated as plants of the sensitive cultivar (7S) showed permanent wilting aspects and were near death.

At this stage, ten fresh replicates of all treatments were taken for measuring growth criteria and gas exchange rates. Fresh samples (3 replicates/ treatment) were also taken for determining both pigment, % RWC, enzyme activities & hormonal contents, while oven-dried samples were taken for estimating both carbohydrate, proline and mineral contents.

Measurement of gas exchange rates

Pn and E rates were measured using an open gas portable photosynthesis system (LI-6400, LICOR, BIO Sciences, USA). Measurements were performed on sunny days under natural light conditions and between 9.00h and 12.00h on the upper most fully expanded leaves of 10 plants randomly chosen per treatment and expressed on a leaf area basis (Renault *et al.,* 2001).

Chemical analysis

The% RWC was estimated according to the method adopted by Turner and Kramer (1980) using the following equation: -

%RWC = F.W. – D.W. X 100/ T.W - D.W.

Photosynthetic pigments, chlorophyll (a), chlorophyll (b) and carotenoids were determined using the spectrophotometric method recommended by Metzener *et al.* (1965). The different carbohydrate fractions were determined in the oven-dried plant materials. The method of extraction and clarification were similar to those described by Said and Naguib (1964). Direct reducing sugars (DRS) were determined following the anthrone method suggested by Umbrient *et al.* (1959). The total reducing sugars (TRS) were determined after hydrolysis of sucrose by sulfuric acid (1.5N). Then, the sucrose content was calculated from the difference between the TRS and DRS, whereas polysaccharides were estimated in the dry residue left after extraction of soluble sugars by the

anthrone method (Umbrient *et al.* 1959). Free proline content was estimated in plant tissues following the procedure of Bates *et al.* (1973). The method of extraction of minerals from plant tissues was essentially similar to that of Chapman and Pratt (1961). Phosphorus was determined following the method described by Humphries (1956). Sodium and potassium were estimated photometrically. Calcium, magnesium and iron were determined using atomic absorption spectrophotometer according to AOAC (1984). The method of extraction of enzymes from plant tissues was essentially similar to that adopted by Guerrier and Strullu (1990) with some modifications .IAA- oxidase enzyme was assayed following the method described by Darbyshire (1971), while each of catalase, peroxidase and polyphenol oxidase enzymes were assayed according to the procedure adopted by Kar and Mishra (1976). With regard to the invertase activity, it was assayed following the method adopted by Russel and Jimmy (1980). The activity of α-amylase was assayed according to the procedure adopted by Davis (1977) and it was represented as the decrease in optical density / minute/1g fresh weight, while the activity of β- amylase was determined following the method described by Malik and Singh (1980).

Hormonal analysis

For estimation of growth regulators, fresh samples of shoots of different plants were collected in cold redistilled 95% ethanol in glass stopper brown jars and kept in a deep freeze ready for further analysis process. The method of extraction was essentially adopted by Wasfy *et al.* (1974).The fraction of the ethanol extract was carried out according to the method described by Shindy and Smith (1975). The acidic fraction contain the acidic hormones (IAA, gibberellins and ABA) while the aqueous fraction comprised the CK .The growth promoters (auxins , gibberellins and CK) and the growth inhibitors (ABA) were estimated using High Performance Liquid Chromatography(HPLC) according to the method adopted by Muller and Hilgenberg (1986).

Statistical analysis

Morphological and gas exchange values were means ± standard error (SE) of 10 replicates while those of chemical and hormonal analysis were means ± (SE) of 3 replicates. Significant differences were calculated using student's (t) test. SPSS version 15 was performed for multiple comparisons.

Results and discussion

Growth parameters

By increasing the severity & duration of drought from one to seven days, the shoot length were correspondingly declined with the percentage of 41% and 56.5% (For var. Fairy 8) and 35.4% and 40.8% (for var. Ucorogoa) after 4 and 7 days from drought respectively (Table 1) . Similar results were obtained by Manikavelu *et al.* (2006), Zeid and Shedeed (2006), and Chutia and Borah (2012). Such decrease in shoot length in response to drought may be either due to the decrease in cell elongation resulting from water shortage which led to a decrease in each of cell turgor, cell volume and eventually cell growth and/or due to blocking up of xylem and phloem vessels thus hindering any translocation through (Sanchez-Blanco *et al.*, 2004; Taiz and Zeiger, 2006; Farooq *et al.*, 2009). Data obtained showed that there is an inverse relationship between increasing the severity of drought and the number and area of leaves formed on treated plants of both varieties (Table 1).Comparable results were detected by Koyro (2006), Manikavelu *et al.* (2006), Martin and Stephens (2006) and Zeid and Shedeed (2006).

The reduction in number of leaves due to WS can be attributed to its direct effect on cell division which arose from reduction in nucleic acid synthesis and/or enhancement of its breakdown (Ashraf *et al.*, 1996), or it may be due to the enhancement of leaf abscission which arose from hormonal imbalance (increased ABA and decreased IAA levels) in treated plants (Vajdehfar *et al.*, 2011), while the diminish in leaf area was attributed to the negative effect of WS on the rate of the cell elongation which resulted in leaves with a reduced cell volume and cell number (Kawakami *et al.*, 2006; Boutraa *et al.*, 2010). Current results revealed that WS negatively affected both the fresh and dry weights of the cultivars. Thus for instance, the percentage of fresh weights of shoots of S plants which suffered from thirsty for 1and 6 days were less by 22.1% and 61%, while the percentage reached 15.6% and 46.1% lower in the T ones respectively (Table 1). These decreases were directly proportional to the severity of drought and the variety of *Triticum* used. These results were appropriate with those of Mayak *et al.* (2004), Monti *et al.* (2006), Rahbarian *et al.* (2011), Vajdehfar *et al.* (2011) and Barnaby *et al.* (2013). The decrease in both fresh and dry weights of stressed shoots revealed the impact of water in maintaining cell turgidity and stimulating or regulating the photosynthetic enzymes which in turn, influence the CO_2 assimilation rate, dry matter production and the fresh weights (Farooq *et al.*, 2009). Plants subjected to low levels of stress (1S&1T) showed slight increases both in their root length and

their number of adventitious roots whereas those subjected to high levels of stress (4S, 7S, 4T &7T) registered high significant increases. These results were comparable to those of Manikavelu *et al.* (2006), Zeid and Shedeed (2006) and Quach *et al.* (2014).

Such increase in the linear growth of roots of both *Triticum* cultivars were attributed to the ability of plants to reduce the shoot/root ratio and water loss by transpiration and the roots to proliferate and elongate quickly in an attempt to

Table 1. Effect of water stress on the growth criteria of two *Triticum aestivum* cultivars . Values are means ± SD (n=10)

Treatment	Mean length of shoot /plant (cm)		Mean number of leaves/plant		Mean area of leaves /plant (cm^2)		Mean fresh weight of shoot/plant (g)		Mean dry weight of shoot/plant (g)		Mean length of root/plant (cm)		Mean number of adventitious roots/plant		Mean fresh weight of root/plant (g)		Mean dry weight of root/plant (g)	
	Sens.	**Tol.**	**Sens.**	**Tol.**	**Sens.**	**Tol.**	**Sens.**	**Tol.**	**Sens.**	**Tol.**	**Sens.**	**Tol.**	**Sens.**	**Tol.**	**Sens.**	**Tol.**	**Sens.**	**Tol**
Control	23 ±0.3	24.0 ±0.6	5.9 ±0.02	6.5 ±0.06	12.3 ±0.2	19.03 ±0.5	3.33 ±0.02	3.6 ±0.02	0.59 ±0.05	0.90 ±0.04	22.4 ±0.6	22.3 ±0.5	5.2 ±0.05	8.4 ±0.03	0.43 ±0.02	0.80 ±0.05	0.077 ±0.003	0.082 ±0.004
1	18 ±0.4	19.5 ± 0.3	3.6 ±0.01	5.6 ±0.03	10.03 ±0.5	12.02 ±0.6	2.6 ±0.01	3.04 ±0.02	0.42 ±0.03	0.81 ±0.05	23.3 ±0.4	23.7 ±0.6	5.9 ±0.06	9.8 ±0.07	0.5 ±0.06	0.96 ±0.07	0.083 ±0.006	0.091 ±0.005
4	13.6 ±0.2	15.5 ±0.6	2.9 ±0.02	3.9 ±0.01	7.0 ±0.3	7.78 ±0.3	1.86 ±0.02	2.63 ±0.04	0.32 ±0.01	0.45 ±0.03	24.8 ±0.3	25.0 ±0.2	7.3 ±0.04	10.0 ±0.03	0.59 ±0.05	0.99 ±0.04	0.076 ±0.008	0.098 ±0.003
7	10 ±0.3	14.2 ±0.5	2.4 ±0.05	3.7 ±0.02	5.45 ±0.4	6.70 ±0.2	1.30 ±0.02	1.94 ±0.04	0.27 ±0.02	0.38 ±0.01	26.1 ±0.4	26.9 ±0.7	8.1 ±0.05	11.9 ±0.04	0.67 ±0.02	1.42 ±0.06	0.086 ±0.007	0.114 ±0.005

***** The mean difference is significant at the 0.05 level

reach deeper levels in soil where underground water levels were found so as to absorb their needs which thus enable plants to survive properly irrespective of WS (Martin and Stephens, 2006; Barnaby *et al.*, 2013). Furthermore, results in table (1) showed that WS positively raised the percentage of root fresh weights to a maximum weight after 6 days of water withdrawal (77.8% for 7T and 55.8% for 7S). The above results were concurrent with those of Turkan *et al.* (2005). Treatment of both cultivars with the three levels of WS, in general, highly significantly raised the mean dry weights of root / plant over those of untreated ones (Table 1). These results were documented by Monti *et al.* (2006) and Boutraa *et al.* (2010).The increase in rate of growth ,fresh and dry weights of roots in response to drought depends mostly on the type of plant used , how it is effected by drought and their adaptability to severe conditions either by growing to deeper levels and branching extensively in the soil so as to draw all their water needs or by accumulating osmolytic substances in the stressed cells thus declining the osmotic potential of the cells to minimum levels (El-Telwany,1987; Sanchez-Blanco *et al.*, 2004; Farooq *et al.*, 2009).

Gas exchange rates, photosynthetic pigment and% RWC

It is clearly shown from table (2) that increasing the severity of drought negatively influenced the Pn and E rates and the contents of leaves of chlorophyll a +b and total pigments. It was also obvious that the sensitive variety (Fairy 8) intuited drought much more than the tolerant one. In contrast, the carotenoid content increased progressively with the increase of drought increments. These results were fortified by those of Farooq *et al.* (2009), Rahbarian *et al.* (2011), Chutia and Borah (2012) and Barnaby *et al.* (2013). Photosynthetic pigments play important roles in harvesting light. The contents of both chlorophyll a and b declined under WS (Farooq *et al.*, 2009). Carotenoids play fundamental roles as antioxidants which help plants to resist drought stress and hamper the photo-oxidation of chlorophyll molecule thus protecting and sustaining photochemical processes (Farooq *et al.*, 2009; Jaleel *et al.*, 2009). Such retardation in the content of photosynthetic pigment in response to WS was attributed to the ultra structural deformation of plastids including the protein membranes forming the thylakoids which in turn causes untying of photo system II which captures photons, so its efficiency declined, thus causing declines in electron transfer, ATP and NADPH production and eventually CO2 fixation processes (Zhang *et al.*, 2007; Rahbarian *et al.*, 2011). Water deficit induces oxidative stress due to the imbalance between light capture and its utilization and thus inhibit photosynthetic activity (Murthy *et al.*, 2012). Moreover, under WS plants close their stomata to avoid

further water loss which, in turn, decrease internal CO2 concentration, inhibit the activity of ribulose 1-5 biphosphate carboxylase/ oxygenase enzyme activity and ATP synthesis leading to a decrease in net photosynthetic rate (Dulai *et al.*, 2006). As to the % RWC of plant (Table2), it decreased progressively in differently treated plants of both varieties below the untreated ones. Such decline was much pronounced in S varieties (41.7%) than in the T ones (25.9%) due to subjection to severe stress (6 days of water withdrawal).

Table 2. Effect of water stress on the photosynthetic pigment , percentage relative water contents and gas exchange rate of leaves of two *Triticum aestivum* cultivar

Sensitive	Treatment	relative water content (%RWC)	Photo synthesis (µmol CO_2 m^{-2} s^{-1})	Transpiration (Trmmol m^{-2} s^{-1})	Chl.(a+b)	Carotenoids	Total Pigments
	Control	86.8±0.11	8.34 ±0.02	4.77 ± 0.02	1093.8 ± 2.2	296.6 ±1.2	1390.4 ± 3.2
	1	80.2 ±0.05	7.2 ±0.02	3.98 ± 0.01	682.3 ± 1.3	371.2 ± 0.8	1053.5 ± 1.7
	4	67.4 ±0.04	6.41± 0.03	3.14± 0.02	492.9 ± 0.9	390.8 ± 0.8	883.7 ± 1.5
	7	50.6 ±0.1	5.82 ±0.04	2.72 ± 0.01	385.1 ± 0.7	435.6 ± 0.5	820.7 ± 0.9
Tolerant	**Control**	90.6 ±0.12	9.28 ±0.03	5.82 ± 0.05	1155.0 ± 3.4	352.3 ± 1.3	1507.3 ± 3.7
	1	81.3 ±0.06	8.54 ±0.05	4.61 ± 0.03	775.8 ± 2.5	440.9 ± 1.2	1216.7 ± 3.1
	4	72.4 ±0.14	7.38 ±0.03	3.54± 0.02	542.2 ± 2.2	455.4 ± 1.4	997.6 ± 2.8
	7	67.1 ±0.23	6.45 ±0.02	3.05 ± 0.04	454.0 ± 1.7	462.4 ±1.6	916.4 ± 2.4

* The mean difference is significant at the 0.05 level. Values of photosynthetic pigments are means ± SD (n=3) and expressed as µg/g fresh weight, while values of gas exchange rates are means ±SD (n=10).

Our results were confirmed with those of Sanchez-Blanco *et al.* (2004), Egilla *et al.* (2005), Akram (2011) and Merewitz *et al.* (2011). Such variation in the response of both varieties to water deficit was attributed to the genetic ability of the T trait to reduce water loss by maintaining water use efficiency and undergoing certain modifications in their metabolic pathway thus declining

their osmotic and water potentials with a concomitant preliminary decrease in their (%RWC). If stress condition prevailed, stomata apertures were closed, Pn and E rates were declined while respiration rate increased so as to provide some hydrolysates as prerequisites for raising the osmotic potential thus increasing cell turgor and eventually growth was presumed once more.

Carbohydrate and proline contents of shoots and roots

It is evident from table (3) that the values of DRS extracted from the shoots & roots of both *Triticum* cultivars increased progressively by increasing the thirsty period above those amounts in daily irrigated plants although they were more augmented in T cultivars. In contrast to soluble sugars, the polysaccharides and TS values declined markedly in shoots and roots of both varieties in response to WS application. Such decline was most pronounced with increasing the severity of drought (Table 3). Concerning soluble sugars (DRS and sucrose), these results were compatible with those of Zhang *et al.* (2007), Vajdehfar *et al.* (2011), Al- Jebory *et al.* (2012) and Barnaby *et al.* (2013) using different plants . As to those of polysaccharides and TS they were consonant with the results of each of Martinez *et al.* (2004) and Wu and Xia (2006). The above decline in total insoluble and TS which accompanied an increase in soluble sugars was attributed to soil water deficiency which triggers certain chemical stimulus (mostly ABA) through xylem vessels to leaves of stressed plants which led to stomata closure, reduction of each of stomata conductance, CO2 concentration in leaf tissues, electron transport system, CO2 fixation, Pn rate and eventually quantity of photosynthates, thus causing decline in growth rates. These conditions, in the mean time, enhances some plants to increase their respiration rates as a prerequisite to produce both ATP (to activate stressed cells) and osmotic soluble substances (which reduces cell osmotic potential) thus increasing cell water uptake. Moreover, soluble carbohydrates are compatible solutes and osmolytes that have the potential to prevent cellular damage from ROS and thus protect plants from abiotic stress (Barnaby *et al.*, 2013). Our view was fortified by each of Sanchez-Blanco *et al.* (2004), Banon *et al.* (2006) and Farooq *et al.* (2009). Table (3) showed positive correlation between the proline amounts of shoots and roots and the severity and duration of drought as compared to untreated *Triticum* varieties. Over that, T cultivars contained more proline than S ones. These results were documented by Zhang *et al.* (2007), Gunes *et al.* (2008), Al- Jebory *et al.* (2012), Chutia and Borah (2012) and Barnaby *et al.* (2013) using different plants. Such increases in proline values with WS were attributed to one of the defense mechanisms which stressed plants led to reduce cell osmotic potential, thus increasing cell water uptake with concomitant increases in both cell activity and turgidity.

Moreover, Phutela *et al.* (2000) suggested that proline accumulated in tissues of stressed plants due to the increased rate of its synthesis by pyrroline-5-carboxylate synthetase and the decreased rate of its degradation by proline oxidase enzyme.

Table 3. Effect of water stress on the carbohydrate and proline contents of two*Triticum aestivum* cultivars

Shoot System	Sensitive	Treatment	Direct reducing sugars (DRS)	Sucrose	Poly-saccharides	Total Sugars	Proline
		Control	429.1 ± 4.2	399.4 ± 3.9	7369.5 ±0.88	8198.0 ±0.91	6.4 ± 0.04
		1	412.4 ± 2.4	521.7 ± 6.2	5576.7 ±1.7	6510.7 ±0.98	9.9 ±0.21
		4	451.4 ± 3.1	560.07± 4.7	3155.3 ±1.4	4166.8 ±1.77	11.9 ±0.6
		7	463.5 ± 4.2	634.8 ± 0.5	2620.0 ±0.9	3778.3 ±1.62	13.19 ±0.8
	Tolerant	**Control**	562.2 ± 2.2	598.8 ± 0.33	8706.7 ±2.3	9867.7 ±2.4	7.8 ± 0.05
		1	550.1 ± 4.1	594.5 ± 1.1	6884.5 ±2.0	8029.1 ± 2.3	8.9 ± 0.3
		4	564.1 ± 2.1	629.0 ± 0.8	4993.4 ±1.9	6186.1 ±1.88	9.8 ± 0.2
		7	602.1 ± 4.2	673.3 ± 0.7	3986.4 ±1.4	5261.8 ±1.5	11.1 ± 0.4
Root System	**Sensitive**	**Control**	202.8 ± 2.1	336.1 ± 3.9	6287.9 ±1.8	6846.7 ± 2.1	5.9 ± 0.71
		1	187.6 ± 2.3	399.2 ± 4.1	3843.5 ±1.4	4430.2 ±1.3	6.7 ± 0.3
		4	216.9 ± 2.6	416.8 ± 5.1	2785.4 ±0.81	3419.1 ±0.9	8.1 ±0.02
		7	252.3 ± 3.1	471.1 ± 1.3	2321.0 ±1.61	3044.4 ±1.6	10.2 ±0.6
		Control	278.0 ± 2.2	789.2 ± 4.3	8556.7 ±2.1	9623.9 ±2.1	5.4 ± 0.03
		1	266.4 ± 4.1	593.6 ± 1.06	6698.9 ±2.2	7558.9 ±2.4	6.1 ± 0.1
	Tolerant	**4**	296.6 ± 3.1	638.4 ± 2.7	3465.4 ±1.4	4400.4 ±1.5	7.5 ± 0.2
		7	394.7 ± 3.2	693.2 ± 0.6	2891.8 ±1.7	3979.7 ± 1.6	9.1 ±0.04

*The mean difference is significant at the 0.05 level. Values are means ± SD (n=3) and expressed as mg/100g dry weight (carbohydrates) or as m/g fresh weight (proline).

Mineral element contents of shoots and roots

It is apparent from table (4) that the Na and Fe levels increased progressively in both shoots and roots with the increase in period of water withdrawal from 1 to 6 days above those of untreated control. Reversibly, when the two varieties of *Triticum* were subjected to stress treatments, their K, Ca, Mg and P amounts, in general, decreased so as to reach their minimum values in both shoots and roots of plants irrigated every 7 days as being compared to those of unstressed plants (table 4). Notwithstanding that, the accumulation of both Na and Fe values, while the reduction of P, Mg, Ca and K amounts were

relatively utmost in S cultivars of *Triticum* than T ones. The increased accumulation of both Na and Fe as a consequence of drought treatment in both varieties of *Triticum* were harmonious with those of El-Tayeb (2006) and Yamaguchi *et al.* (2010).

Table 4. Effect of water stress on the mineral element contents of two *Triticum aestivum* cultivars

		Treatment	Sodium	Potassium	Phosphorus	Magnesium	Calcium	Iron
Shoot System	**Sensitive**	**Control**	291.2 ±1.1	521.2 ±1.4	2937.5 ±2.3	261.8 ±1.1	432.6 ±0.9	46.9 ±1.07
		1	356.5 ±1.2	391.8 ±1.07	1793.6 ±2.3	208.6 ±0.66	383.7 ±1.2	57.2 ±1.6
		4	494.6 ±1.3	357.2 ±1.6	1418.8 ±2.1	187.3 ±0.91	345.3 ±1.4	73.1 ± 0.86
		7	533.9 ±1.7	325.3 ±1.5	1396.3 ±1.4	124.1 ±0.99	325.3 ±1.3	79.1 ± 0.91
	Tolerant	**Control**	260.5 ±1.06	544.7 ±1.5	3150.0 ±2.1	290.1 ±1.5	527.9 ±1.1	27.7 ± 0.77
		1	313.5 ±1.1	491.8 ±1.07	2181.3 ±2.07	238.6 ±1.4	488.4 ±1.3	34.5 ± 0.61
		4	438.5 ±0.88	460.2 ±0.83	1975.0 ±1.9	211.4 ±1.3	451.2 ±1.4	49.6 ± 0.51
		7	487.7 ±0.91	403.5 ±1.2	1856.3 ±1.7	196.8 ±1.4	412.7 ±1.6	57.5 ±0.8
Root System	**Sensitive**	**Control**	334.6 ±1.06	255.3 ±0.5	2206.3 ±2.6	226.4 ±1.3	330.2 ±1.6	65.0 ±0.3
		1	364.2 ±1.7	233.6 ±0.8	1950.0 ±2.4	134.5 ±0.9	313.5 ±1.4	66.6 ±0.5
		4	601.3 ±2.1	183.5 ±1.1	1575.0 ±2.2	125.0 ±0.7	293.0 ±1.2	71.6 ±0.7
		7	633.6 ±1.1	155.6 ±0.4	1237.5 ±1.9	119.5 ±0.6	240.7 ±0.9	86.6 ±0.8
	Tolerant	**Control**	315.8 ±1.05	284.7 ±0.9	2306.3 ±2.3	243.2 ±0.7	387.2 ±1.2	60.1 ±0.5
		1	327.7 ±1.2	278.8 ±1.3	2262.5 ±1.7	181.4 ±0.66	360.5 ±1.4	62.2 ± 0.42
		4	565.8 ±0.9	248.2 ±0.7	2193.8 ±1.3	179.5 ±0.81	347.8 ±1.0	68.8 ±0.6
		7	602.3 ±1.03	225.9 ± 0.51	2081.3 ±1.04	136.4 ±0.5	304.2 ±0.9	79.2 ± 0.71

* The mean difference is significant at the 0.05 level. Value are means ± SD (n= 3) and expressed as mg/100g dry weight.

Such increase in Na values in response to drought treatments is considered as one of the safeguard mechanism which plants can lead in order to control osmotic pressure of stressed cells and tissues so as to raise their ability of water and solute uptake from soil (Morsy, 1996). The increased levels of Fe can be assigned to the corresponding increases in peroxidase activities in *Triticum* plants. At the other side , the decreased levels of each of K , P , Mg and Ca in response to stress in both varieties of *Triticum* were ascertained by the work of each of Koyro (2006), Wu and Xia (2006), Ali *et al.* (2008) and Farooq *et al.* (2009). Such reductions in the contents of these elements in different tissues were attributed primarily to soil water deficiency which markedly reduces the transpiration rate that lessen the flow rates of elements in soil , their absorption by stressed root cells and also its ability to translocate

through the xylem and different organs and tissues. This situation resulted in an interruption in the various metabolic pathways carried out by plants namely; respiration, Pn, biosynthesis of phospholipids, nucleic acids, plastids , enzymes etc , disorders in both plasma membrane permeability and stomata osmotic regulations , thus plants seized growth and eventually died (El-Telwany, 1987; Lindhauer, 2007; Farooq *et al.*, 2009).

Activities of certain hydrolytic and oxidative enzymes

Table 5. Effect of water stress on the activities of certain enzymes of shoots and roots of two *Triticum aestivum* cultivars

	Treatment		α-amylase (decrease in optical density in min.)	β-amylase (µg of maltose released/ g. FW/h)	Invertase (mg reducing sugar released/ g. FW/h)	Poly phenol oxidase	Peroxidase	Catalase	IAA-oxidase (µg of IAA-oxidized)
							(enzyme activity/g. FW/h)		
Shoot system	S	Control	3.3 ± 0.11	160 ± 1.6	409.5 ± 1.4	2.1 ± 0.01	1.9 ± 0.06	900 ± 1.7	557.5 ± 1.7
		1	2.6 ± 0.3	206.7 ± 0.9	493.8 ± 1.7	2.3 ± 0.03	2.1 ± 0.05	868 ± 1.5	615.4 ± 1.5
		4	2.3 ± 0.02	363.3 ± 1.5	525.7 ± 2.2	2.4 ± 0.06	2.3 ± 0.07	800 ± 1.9	634.6 ± 1.8
		7	1.99 ± 0.06	423.3 ± 1.6	593.8 ± 1.4	2.7 ± 0.08	2.9 ± 0.08	702 ± 2.8	689.2 ±`1.6
	T	Control	3.6 ± 0.3	133.3 ± 1.2	498.1 ± 1.6	1.9 ± 0.05	1.7 ± 0.04	1036 ± 1.6	462.6 ± 1.1
		1	3.4 ± 0.02	186.7 ± 0.9	510 ± 1.1	2.2 ± 0.14	1.90 ± 0.5	978 ± 2.4	500.2 ± 0.9
		4	3.04 ± 0.01	333.3 ± 2.1	595.2 ± 1.5	2.3 ± 0.01	2.0 ± 0.03	818 ± 2.2	586.5 ± 1.3
		7	2.8 ± 0.03	353.3 ± 1.7	681.9 ± 1.3	2.5 ± 0.04	2.2 ± 0.01	778 ± 2.4	614.6 ± 2.1
Root System	S	Control	2.1 ± 0.01	120.0 ± 0.6	380.9 ± 0.9	2.5 ± 0.04	3.2 ± 0.02	900 ± 2.3	458.3 ± 0.02
		1	1.9 ± 0.05	193.3 ± 0.8	419.1 ± 1.1	2.6 ± 0.03	2.6 ± 0.01	892 ± 3.1	517.3 ± 0.05
		4	1.7 ± 0.03	320.0 ± 0.7	442.8 ± 1.3	2.9 ± 0.05	2.3 ± 0.04	830 ± 2.1	571.9 ± 0.6
		7	1.2 ± 0.01	370 ± 0.5	490.5 ± 1.2	3.4 ± 0.06	2.1 ± 0.03	778 ± 1.7	624.1 ± 0.9
	T	Control	2.6 ± 0.04	106.7 ± 0.9	428.6 ± 0.5	1.9 ± 0.03	4.3 ± 0.04	1086 ± 2.5	382.5 ± 0.2
		1	2.2 ± 0.02	140.0 ± 1.8	485.2 ± 2.1	2.1 ± 0.01	3.2 ± 0.05	990 ± 2.3	436.6 ± 0.1
		4	2.02 ± 0.03	233.3 ± 0.4	561.9 ± 1.8	2.4 ± 0.2	2.8 ± 1.5	918 ± 2.8	459.4 ± 1.5
		7	1.7 ± 0.2	320 ± 1.7	666.7 ± 1.4	2.7 ± 0.3	2.7 ± 0.02	900 ± 1.6	593.2 ± 2.7

* The mean difference is significant at the 0.05 level. Values are means ± SD (n=3).

Data shown in the table (5) visualized that the activities of each of α and β amylases, invertase , peroxidase , poly phenol oxidase and IAA- oxidase enzymes were increased progressively by increasing the extent of stress so as to reach their maximum activities in the shoots and roots of weekly irrigated plants (7S and 7T) of both cultivars as being compared with those of untreated plant organs. However, the activity levels of these enzymes were obviously higher in T cultivars of *Triticum* than in S ones. At the other side, the catalase (in shoots and roots) and peroxidase (in roots only) activities were moderately and markedly declined in response to water stress in S and T cultivars respectively. The increased activities of hydrolytic enzymes (β- amylase, α-amylase & invertase) in response to water stress were in accordance with those of Bialecka and Kepczynski (2010) and Saeedipour (2011). Such increases in the activities of hydrolytic enzymes which accompanied the accumulation of both sucrose and reducing sugars and the decline in the values of polysaccharides in the shoots and roots of both *Triticum* cultivars due to WS was considered as one of the defense tools against stress since soluble sugar act as osmolytes that raises the negative osmotic potential of plants thus increasing its capability to absorb water from soil and retrieve cell turgidity.

On the other hand, the increased activity of oxidative enzymes (peroxidase , polyphenol oxidase and IAA–oxidase) and the reduction in catalase activity , in the present work , due to WS were consonant with those El-Tayeb (2006), Zhang *et al.* (2007), Farooq *et al.* (2009), Murthy *et al.* (2012) and Awate *et al* (2014). The increased activity of antioxidant enzymes due to stress in T cultivars more than S ones is of prime importance in the plant defense mechanism against drought by the rapid removal of ROS which arises from the imbalance between the decreased rate of CO_2 influx and assimilation in the cells and the increased production of spare electrons and therefore protect the cells, maintain plant growth and productivity (Turkan *et al*, 2005; Murthy *et al.*, 2012), or it may be imputed to the higher capacity of stressed plants and especially T cultivars to decompose toxic H_2O_2 which accumulates at higher levels due to reduction in rate of CO_2 fixation which, in turn, result from retardation in electron transport system or due to reduction in catalase activity (El-Tayeb, 2006; Murthy *et al.*, 2012).

Hormonal content

The hormonal content of leaves of both treated and untreated plants of both varieties are shown in Table (6). Application of different water regime to both cultivars of *Triticum* clearly decreased the contents of all growth promoting hormones (auxins, gibberellins, CK) below those of untreated plants. In contrast, the ABA levels were found to increase markedly with the increase

in both duration and severity of drought so as to reach maximum values in 7T &7S treated plants. Noteworthy that S cultivars contained less growth promoting hormones and more ABA than T ones in response to various WS treatments. These results are confirmed by those of Perales *et al.* (2005), Zhang *et al.* (2007), Farooq *et al.* (2009) and Vajdehfar *et al.* (2011). Phytohormones play vital roles in drought tolerance of plants; auxins enhance the proliferation of roots which is vital for drought tolerance. It also participated with calcium in the signaling mechanism of drought –induced proline accumulation (Farooq *et al*, 2009). Physiological damage caused by WS and stress signaling are closely associated with the endogenous level and balance of hormones (Yang *et al.*, 2002; Merewitz *et al.*, 2011). CK synthesis and transport are typically inhibited whereas its degradation is promoted under WS (Kudoyarova *et al.*, 2006; Farooq *et al.*, 2009; Merewitz *et al.*, 2011).

Table 6. Effect of water stress on the phytohrmonal contents of two *Triticum aestivum* cultivars

	Treatment	Auxins	Gibberellins	Cytokinins	ABA
Sensitive	**Control**	12.3 ±0.4	10.6 ±0.7	22.1 ±0.6	**10.1 ±0.3**
	1	9.5 ±0.3	8.9 ±0.4	20.6 ±0.8	**11.6 ±0.5**
	4	5.2 ±0.6	5.0 ±0.3	14.4 ±0.5	**13.8 ±0.4**
	7	2.2 ±0.2	3.4 ±0.3	7.8 ±0.4	**15.6 ±0.7**
Tolerant	**Control**	14.1 ±0.7	11.8 ±0.8	25.4 ±0.7	**11.3 ±0.2**
	1	13.6 ±0.4	10.4 ±0.4	22.7 ±0.4	**12.4 ±0.4**
	4	6.8 ±0.2	6.8 ±0.6	16.4 ±0.5	**15.2 ±0.6**
	7	2.7 ±0.3	3.9 ±0.2	9.7 ±0.3	**16.9 ±0.7**

* The mean difference is significant at the 0.05 level. Values are means ± SD (n=3) and expressed as mg/Kg. FW .

GA3 was suggested to participate in drought rhizogenesis as an adaptive strategy that occurs during drought (Farooq *et al.*, 2009).

When plants wilt, ABA levels typically rise and alters the relative growth rates of various plant parts. It triggers the events leading to stomata closure, reduction of transpiration and thus conserves plant water contents. Moreover, ABA activates the synthesis of specific protein kinases which cause gene expression signaling cascades that activates the synthesis and accumulation of antioxidants, osmoprotectants and solutes under acute drought stress (Farooq *et al.*, 2009; Hubbard *et al.*, 2010).

Conclusion and future prospects

Currently, it is concluded that water stress changes the hormonal balance in plants towards increasing both the levels of growth inhibitors as ABA and the activity of anti-oxidant enzymes while decreasing the values of growth promoting hormones which, in turn, forces treated plants and specially the T traits to change their mechanism and management strategies so as to curtail drought by the following means:

1- Stomata closure so as to reduce water loss, which, in turn, reduces gas exchange thus causing low CO_2 content, low photosynthetic rate and eventually low growth rate.

2-Extensive and deep soil-root growth and its efficient use thus increasing the rate of water-uptake, consonant with the decrease in leaf area to reduce the transpiration loss which thus compensate low soil water level (Farooq *et al.*, 2009).

3-Stimulating the activity of some hydrolytic enzymes (α and β amylases and invertase) so as to raise the values of some osmolytic substances as soluble sugars and amino acids as proline which increases the cell osmotic potential that leads to an increase in the cell water uptake and thus plants can endure drought (Wu *et al.*, 2005; Monti *et al.*, 2006; Farooq *et al.*, 2009).

However, valuable work should be done on the development of crop plants tolerant to drought stress which might be a promising approach that help in meeting the future food demands for rapidly increasing population pressures.

Acknowledgments

The author is thankful to the Biotechnology Department, National Research Center, Dokki, Giza, for performing mineral analysis by atomic absorption spectrophotometer and hormonal analysis by high performance liquid chromatography (HPLC). Sincere thanks and obligations are also extended to Dr. Raaifa A. Hassanein, Prof of Plant, Physiology, Botany Department, Faculty of Science, Ain Shams University, for her valuable advice and encouragement throughout this work.

References

Akram, M. (2011). Growth and yield components of wheat under water stress of different growth stages. Banglad. Journal of Agricultural Research 36:455- 468.

Ali, Q., Ashraf, M., Shahbaz, M. and Humera, H. (2008). Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea Mays* L.) plants. Pakistan Journal of Botany 40:211-219.

Al-Jebory, E. I. (2012). Effect of water stress on carbohydrate metabolism during Pisumsativum seedlings growth. Euphrates Journal of Agricultural Science 4:1-12.

AOAC (1984). Official methods of analysis of the association of official analytical chemists. 14th edition. Washington, D.C.

Ashraf, M. Y., Mazhar. H., Naqvi, L. and Khan, A. H (1996). Effect of water stress on total phenols, peroxidase activity, growth and yield of tomato. ActaHort 516:41-45.

Awate, P. D., Patil, M. S. and Gaikwad, D. K. (2014). Alleviation of oxidative damage by exogenous application of plant growth regulators on medicinally important oil yielding plant *Simarouba Glauca* Dc. under water sress conditions. Indian Journal of Medical Research 4:36-37.

Bajji, M., Kinet, J. and Lutts, S. (2002). The use of electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regulation 36:61-70.

Banon, S., Ochoa, J., Franco, J. A., Alarcon, J. J. and Sanchez-Blanco, M. J. (2006). Hardening of oleander seedlings by deficit irrigation and low air humidity. Environmental and Experimental Botany 56:36-43.

Barnaby, J. Y., Kim, M., Bauchan, G., Bunce, J., Reddy, V. and Sicher, R. C. (2013). Drought responses of foliar metabolites in three maize hybrids differing in water stress tolerance. Plos One 8:1-10.

Bates, L. S., Waldren, R. P. and Teare, I. D. (1973). Rapid determination of proline for water studies. Plant and Soil 39:205-207.

Battisti, D. S. and Naylor, R. L. (2009). Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323:240-244.

Bialecka, B. and Kepczynski, J. (2010). Germination, α-, β- amylase and total dehydrogenase activities of Amaranthuscaudatus seeds under water stress in the presence of ethephon or gibberellins A3. Acta Biologica Cracoviensia Series Botanica 52:7-12.

Boutraa, T., Akhkha, A., Al-Shoaibi, A. A. and Alhejeli, A. M. (2010). Effect of water stress on growth and water use efficiency (WUE) of some wheat cultivars (*Triticum durum*) grown in Saudi Arabia. Journal of Taibah University for Science 3:39-48.

Chapman, H. D. and Pratt, F. P. (1961). Method of analysis for soils, plants and waters. University of California, Division of Agricultural Sciences.

Chutia, J. and Borah, S. P. (2012). Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assm India II. Protein and proline status in seedlings under PEG induced water stress. American Journal of Plant Sciences 3:971-980.

Darbyshire, B. (1997). The effect of water stress on indole acetic acid oxidase in pea plants. Plant Physiology 47:65.

Davis, B. D. (1977). Occurrence of alpha-amylase in the axis of germinating peas. Plant Physiology 60:513-517.

Dulai, S., Molnar, I., Pronay, J., Csernak, A., Tarnai, R. and Molnar, L. (2006). Effects of drought on photosynthetic parameters and heat stability of PSII in wheat and in Aegilops species originating from dry habitats. Acta Biologica Szegediensis 50:11-17.

Egilla, J. N., Davies, J. F. T. and Boutton, T. W. (2005). Drought stress influences leaf water content, photosynthesis, and water use efficiency of Hibiscus rosasinesis at three potassium concentrations. Photosynthetica 43:135-140.

El-Tayeb, M. A. (2006). Differential response of two Viciafaba cultivars to drought: growth, pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity. Acta Agronomica Hungarica 54:25-37.

El-Telwany, K. A. (1987). Effect of soil drought on certain physiological aspects in plant. (Master's thesis). Ain Shams University.

Estrada-Campuzano, G., Miralles, D. J. and Slafer, G. A. (2008). Genotypic variability and response to water stress of pre- and post-anthesis phases in triticale. European Journal of Agronomy 28:171-177.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D. and Basra, S. M. A. (2009). Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development 29:185-212.

Guerrier, G. and Strullu, D. G. (1990). Development laxesembryonnaires de poispourvus de reserves. Canadian Journal of Botany 68:742-746.

Gunes, A., Inal, A., Adak, M. S., Bagci, E. G., Cicek, N. and Eraslan, F. (2008). Effect of drought stress implemented at pre- and post- anthesis stage some physiological as screening criteria in chickpea cultivars. Russian Journal of Plant Physiology 55:59-67.

Hubbard, K. E., Nishimura, N., Hitomi, K., Getzoff, E. D. and Schroeder, J. I. (2014). Early abscisic acid signal transduction mechanisms: newly discovered components and newly emerging question. Genes and Development 24:1695-1708.

Humphries, E. C. (1956). Mineral component and ash analysis. In peach, K. and Tracey, M. V. (Eds.), Modern methods of Plant Analysis. Berlin: Springer-Verlag. pp. 148

Jaleel, C. A., Manivannan, P., Wahid, A., Farooq, M., Al-Juburi, H. J., Somasundaram, R. and Panneerselvam, R. (2009). Drought stress plants: a review on morphological characteristics and pigments composition. International Journal of Agriculture and Biology 11:100-105.

Kar, M. and Mishra, D. (1976). Catalase, peroxidase and polyphenoloxidase activities during rice leaf senescence. Plant Physiology 57:315-322.

Kawakami, J., Iwama, K. and Jitsuyama, Y. (2006). Soil water stress and the growth and yield of the potato plants grown from microtubers and conventional seed tubers. Field Crop Research 95:89-96.

Kogan, F. N. (1997). Global drought watch from space. Bulletin of the American Meteorological Society 78:621-636.

Koyro, H. W. (2006). Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.) Environmental and Experimental Botany 56:136-146.

Kudoyarova, G. R., Vysotskaya, L. B., Cherkozyanova, A. and Dodd, I. C. (2006). Effects of partial root zone drying on the concentration of zeatin-type cytokinins in tomato (*Solanumlycopersicum* L.) xylem sap and leaves. Journal of Experimental Botany 58:161-168.

Lindhauer, M. G. (2007). Influence of K nutrition and drought on water relations on water relations and growth of sunflower (*Helianthus annuus* L.). Journal of Plant Nutrition and Soil Science 148:654-669.

Malik, C. P. and Singh, M. P. (1980). Plant Enzymology and Histo- Enzymology- A Text Manual. New Delhi-Ludhiana: Kalyani Publishers.

Manikavelu, A., Nadarajan, N., Ganesh, S. K., Gnanamalar, R. P. and Babu, R. C. (2006). Drought tolerance in rice: morphological and molecular genetic consideration. Plant Growth Regulation 50:121-138.

Marris, E. (2008). Water: More crop per drop. Nature 453:273-277.

Martin, P. J. and Stephens, W. (2006). Willow growth in response to nutrients and moisture on a clay landfill cap soil. Π: Water use. Bioresource Technology 97:449-458.

Martinez, J., Luttus, S., Schank, A., Bajji, M. and Kinet, J. (2004). Is osmotic adjustment required for water stress resistance in the Mediterranean shrub *Atriple xhalimus* L?. Journal of Plant Physiology 161:1041-1051.

Mayak, S., Tirosh, T. and Glick, B. R. (2004). Plant growth promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Science 166:525-530.

Merewitz, E. B., Gianfagna, T. and Huang, B. (2011). Photosynthesis, water use, and root viability under water stress as affected by expression of SAG12-ipt controlling cytokinin synthesis in Agrostisstolonifera. Journal of Experimental Botany 62:383-395.

Monti, A., Amaducci, M. T., Pritoni, G. and Verturi, G. (2006). Variation in carbon isotope discrimination during growth and at different organs in sugar beet (*Beta vulgaris* L.) Field Crops Research 98:157-163.

Morsy, A. A. (1996). Physiological Studies on Egyptian medicinal plants. (Master's thesis). Faculty of Science, Ain Shams University.

Muller, P. and Hilgenberg, W. (1986). Isomers of zeatin and zeatinriboside in club root tissue: evidence for trans- zeatin biosynthesis by plasma diophorabrassicae. Plant Physiology 66:245-250.

Murthy, S. M., Devaraj, V. R., Anitha, P. and Tejavathi, D. H. (2012). Studies on the activities of antioxidant enzymes under induced drought stress in in vivo and in vitro plants of *Macrotyloma uniflorum* (Lam.) Verdc. Recent Research in Science and Technology 4:34-37.

Munné-Bosch, S. and Alegre, L. (2000). Changes in carotenoids, tochopherols and diterpenes during drought and recovery and the biological significance of chlorophyll loss in *Rosmarinus officianalis* plants. Planta 210:925-931.

Perales, L., Arbona, V., Gómez-Cadenas, A., Carnejo, M. and Sanz, A. (2005). A relationship between tolerance to dehydration of rice cell lines band ability for ABA synthesis under stress. Plant Physiology and Biochem 43:786-792.

Pirzard, A., Darvishzadeh, R., Bernousi, I., Hassani, A. and Sivritepe, N. (2012). Influence of water deficit on iron and zinc uptake by *Matricaria chamomilla* L. Chilean Journal of Agricultural Research 72:232-236.

Phutela, A., Jain, V., Dhawan, K. and Nainawatee, H. S. (2000). Prolinemetoblism under water stress in the leaves and roots of Brassica junce cultivars differing in drought tolerance. Journal of Plant Biochemistry and Biotechnology 9:35-39.

Pospisilova, J., Vagner, M., Malbeck, J., Travnickova, A. and Batkova, P. (2005). Interactions between abscisic acid and cytokinins during water stress and subsequent rehydration. Biologia Plantarum 49:533-540.

Quach, T. N., Tran, L. P., Valliyodan, B., Nguyen, H. T. M., Kumar, R., Neelakandan, A. K., Guttikonda, S. K., Sharp, R. E. and Nguyen, H. T. (2014). Functional analysis of water stress- responsive soybean GmNAC003 and GmNAC004 transcription factors in lateral root development in *Arabidopsis*. Plos One 9:1-12.

Rahbarian, R., Nejad, R. K., Ganjeali, A., Bagheri, A. and Najafi, F. (2011). Drought stress effects on photosynthesis, chlorophyll fluorescence and water relations in tolerant and

susceptible chickpea (*Cicer arietinum* L.) genotypes. Acta Biologica Cracoviensia Series Botanica 53:47-56.

Razi, S. S. and Sen, S. P. (1996). Amelioration of water stress effects on wetland rice by urea-N, plant growth regulations and foliar spray of a diazotrophic bacterium *klebsiella* sp. Biology and Fertility of Soils 23:454-458.

Renault, S., Croser, C., Franklin, J. A., Zwiazek, J. J. and Mackinnon, M. (2001). Effect of consolidated tailings water on red- osier dogwood (*Cornus stolonifera* Michx) seedlings. Environmental Pollution 113:27-33.

Russel, P. and Jimmy, K. A. (1980). Invertase in oat seedlings, separation, properties and changes in activities in segments. Plant Physiology 65:136-142.

Saeedipour, S. (2011). Activities of sucrose- metabolizing enzymes in grains of two wheat (*Triticum aestivum* L.) cultivars subjected to water stress during grain filling. Journal of Plant Breeding and Crop Science 3:106-113.

Said, A. and Naguib, M. I. (1964). Sucrose determination as a mean of estimation of the draw back tax of HalawaTehinia. Bulletin Faculty Science, Cairo University 39:207.

Sales, R. M. P., Fries, D. D., Pires, A. J. V., Bonomo, P., Santos, I. S., Campos, C. N., Brito, P. H. R. and Brito, M. S. (2013). Chlorophyll and carbohydrates in Arachispintoi plants under of water regimes and nitrogen fertilization. Revista Brasileira de Zootecnia 42:388-394.

Sanchez-Blanco, J., Fernandez, T., Morales, A., Morte, A., and Alarcon, J. J. (2006). Variation in water stress, gas exchange, and growth in *Rosmarinus officinalis* plants infected with *Glomus deserticola* under drought conditions. Journal of Plant Physiology 161:675-682.

Shindy, W. W. and Smith, O. (1975). Identification of plant hormones from cotton ovules. Plant Physiology 55:550.

Taiz, L. and Zeiger, E. (2006). Plant Physiology 4th edition. Massachusetts: Sinauer Associates Inc. Publishers.

Turkan, I., Bor, M., Ozdemir, F. and Koca, H. (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought tolerant *P. acutifolius* Gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. Plant Science 168:223-231.

Turner, N. C. and Kramer, P. J. (1980). Adaptation of Plant to Water and High Temperature Stress. John Wiley and Sons Inc.

Umbrient, W. W., Burris, R. H., Stauffer, J. F., Cohen, P. P., Johnse, W. J., Leepxgi, G. A., Patter, V. R. and Schneider, W. C. I. (1959). Monometric Techniques, a manual describing methods applicable to the study of tissue metabolism. Burgess Publishing Company.

Vajdehfar, T. S., Ardakani, M. R., Paknejad, F., Boojar, M. M. A. and Mafakheri, S. (2011). Phytohormonal responses of sunflower (*Helianthus annuus* L.) to magnetized water and seed under water deficit conditions. Middle-East Journal of Scientific Research 7:467-472.

Vardhini, B. V., Sujatha, E. and Rao, S. R. (2011). Brassinosteroids: Alleviation of water stress in certain enzymes of sorghum seedlings. Journal of Phytology 3:38-43.

Wasfy, W., Shindy, L. R. and Orrin, E. S. (1974). Identification of plant hormones from cotton ovules. Plant Physiology 55:550-550.

Wu, Q. and Xia, R. (2006). Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. Journal of Phytology 163:417-425.

Wu, Q., Xia, R. and Zou, Y. (2005). Reactive oxygen metabolism in mycorrhizal and non-mycorrhizalcitrus (*Poncirus trifoliate*) seedlings subjected to water stress. Journal of Plant Physiology 163:1101-1110.

Yamaguchi, M., Valliyodan, B., Zhang, J., Lenoble, M. E., Yu, O. Rogers, E. E., Nguyen, H. T. and Sharp, R. E. (2010). Regulation of growth response to water stress in the soybean primary root. I. Proteomic analysis reveals region-specific regulation of phenylpropanoid metabolism and control of free iron in the elongation zone. Plant, Cell and Environment 33:223-243.

Yang, J., Zhang, J., Wang, Z., Zhu, Q. and Liu, L. (2002). Abscisicacid and cytokinins in the root exudates and leaves and their relationship to senescence and remobilization of carbon reserves in rice subjected to water stress during grain filling. Planta 215:645-652.

Zeid, I. M. and Shedeed, Z. A. (2006). Response of alfalfa to putrescine treatment under drought stress. Biologia Plantarum 50:635-640.

Zhang, M., Duan, L., Tian, X., He, Z., Li, J., Wang, B. and Li, Z. (2007). Unicanazole-induced tolerance of soybean to water deficit stress in relation to changes in photosynthesis, hormones and antioxidant system. Journal of Plant Physiology 164:709-717.

(Received: 30 September 2014, accepted: 3 December 2014)