



Evaluation the water stress tolerance of ten durum wheat genotypes by some physiological parameters

Bouchareb Radia^{*1}, Boulaacel Mouad¹, Hazmoune Tahar¹, Guendouz Ali²

¹*Department of Vegetal Biology and Ecology, University Constantine 1, Algeria*

²*National Institute of Agricultural Research of Algeria (INRAA), Unit of Setif, Algeria*

Key words: Wheat, Glycine betaine, Protein, Chlorophyll, Malon Di Aldehyde (MDA)

<http://dx.doi.org/10.12692/ijb/10.5.250-256>

Article published on May 29, 2017

Abstract

Wheat is important food for more than 35% of the world's population and its cultivation is mainly limited to such areas with water scarcity. The current study involves studying changes in the accumulation of glycine betaine, membrane stability, chlorophyll, protein and Malon (MDA) Di Aldelyde of ten varieties of durum wheat under stresses. The results of this study showed a significant decrease in the accumulation of glycine betaine, relative water content, membrane stability, chlorophyll content, MDA. The study showed that varieties responded to water stress with different mechanisms in different proportions between varieties to maintain the vital functions of the durum varieties studied.

* **Corresponding Author:** Bouchareb Radia ✉ p.radia@hotmail.com

Introduction

Drought is a worldwide problem constraining global crop production serious (Dhanda *et al.*, 2004; Glombitza *et al.*, 2004). Drought is a complex physical-chemical process, in which many biological macro molecules and small molecules are involved, such as nucleic acids (DNA, RNA, micro RNA), proteins, carbohydrates, lipids, hormones, ions, free radicals, mineral elements (Apel *et al.*, 2004; Casati *et al.*, 2004). . Water availability mostly affects accumulation of some organic compatible solutes such as sugars, betaines and proline, which adjust the intercellular osmotic potential, is early reaction of plants to water stress. Sairam and Saxena (2000) reported that oxidative stress which caused metabolic damage in water stress, increases lipid per oxidation, resulting in greater membrane injury and pigment bleaching. Zlatev and Stoyanov (2005) suggested that the proline accumulation of plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism. Vendruscolo *et al.* (2007) found that Malon dialdehyde (MDA) is involved in tolerance mechanisms against oxidative stress and this was the main strategy of plants to avoid detrimental effects of water stress. The physiological and biochemical approaches have a great importance in order to understand the complex responses of plants to water deficiency and develop rapidly for the purpose of crop production, yield improvement and yield stability under water stress conditions, developing of drought tolerant varieties is the best option to select a new varieties (Siddique *et al.*, 2000) .

Materials and methods

Plant materials

Ten durum wheat genotypes (Vitron, GTA, Waha, Cirta, Bidi, Wahbi, OTB4, Ter1-3, F4, Bousselem) were provided by experimental fields, as cultivars weren't in a similar pedigree and were suitable in field of station. These experiments were carried during 2014-2015 in the experiment was conducted under green house (Bio pol, Chaabat Erssas), University of the Brothers Mentouri Constantine, Algeria, by using of split plot design that main factors contains 100% Fc and 50% Fc and sub factors contains ten Varieties of wheat in four replications.

Determination of glycine betaine content

The glycine betaine content was measured by the method of (Grieve et Grattan, 1983). The plant material (0.5g) was grinded with 20mL Distilled water for 48 h at 25 °C Leave in the refrigerator until the day of the heat treatment. 0.5 ml of H.sub.2 SO.sub.4 are added and left to the ice laying 1 hour Measured by the spectrophotometer at 365 nm.

Determination of content lipid peroxides

The level of lipid per oxidation was measured in terms of Malonyl Dialdehyde (MDA) content, a product of lipid peroxidation following method of Heath and Packer (1968).

The plant material (0.3g) was grinded with 3mL of 0.1% Trichloroacetic Acid (TCA). The homogenate was centrifuged at 1000g for 20 min. A 0.5mL aliquot of the supernatant was mixed with 1.5mL solution of 20% TCA containing 0.5% Thiobarbituric Acid (TBA).

The mixture was incubated in a boiling water bath for 30min then quickly cooled in an ice bath and then warming to room temperature. The extinction was measured at 532nm and 600nm.

Determination of chlorophyll

Drought stress treated and control leaf samples were extracted with 80% Acetone and absorbance of supernatants were measured spectrophotometrically. Chlorophyll A was determined at wavelength 663nm and A at 645nm, and total chlorophyll at 652nm.

Determination of protein

Determination of soluble protein contents were determined according to Bradford (1979) methods, used to measure the concentration of total protein in a sample. The principle of this assay is that the binding of protein molecules to Coomassie dye under acidic conditions results in a color change from brown to blue.

Statistical analysis

Statistical analysis was carried out with the SAS statistical computer package (SAS, Verison, 9). Experimental data were analyzed with the protected Duncan's test $p < 0.05$ level.

Results and discussion

Glycine betaine

The results of the present study showed positive effect of drought stress on glycine betaine accumulation, membrane stability.

Our results show that the variety Cirta has a maximum concentration (3%). The minimum value is marked by the Vitron and Bousselem varieties with 0.3%, -0.2% respectively, with an 0.85 (Fig. 1).

Table 1. The results of the different concentrations studied with descriptive statistics.

Géotypes	Polyphénols		Protéines		Glycine betaine		MDA	
	The control plants	Stressed plants						
Vitron	0.040	0.045	0.370	0.255	0	0.2	5.662	11.546
Gta dur	0.040	0.050	0.343	0.313	0.1	2	4.417	18.656
Waha	0.040	0.045	0.310	0.187	0	0.3	8.447	15.630
Cirta	0.040	0.050	0.320	0.257	0	3	5.192	17.545
B17	0.070	0.080	0.297	0.328	0	0.5	2.945	32.756
Wahbi	0.040	0.050	0.341	0.252	0	0.7	3.545	27.323
Otb4(3)	0.060	0.060	0.250	0.243	0	1	5.812	20.685
Ter(2-1)	0.070	0.080	0.258	0.249	0	0.3	2.980	18.471
F4/3	0.040	0.050	0.332	0.286	0	0.2	29.767	30.446
Bousselem	0.060	0.060	0.286	0.160	0	0.3	3.283	32.731
Minimum	0.040	0.040	0.250	0.154	0	0.2	2.077	11.625
Maximum	0.070	0.080	0.400	0.344	0.1	3	30.550	36.735
Moyenne	0.050	0.056	0.311	0.253	0.01	0.85	7.249	23.080
Ecart-type	0.0131	0.0134	0.043	0.050	0	0.93	8.153	7.220
Pr > F	NS	***	*	***	NS	***	***	***

These results are confirmed in the ANOVA test, which reveals no significant differences for this parameter. Our study shows that the local variety Cirta recorded a significant accumulation of glycine betaine, this accumulation of betaine could contribute to osmoregulation in natural storage batteries, and however osmoprotection seems to be responsible for the tolerance to abiotic stresses in transgenic plants Zlatev and Stoyanov (2005). Important work on glycine betaine suggested its varied roles in plants. New evidence suggests that the contribution of differential expression of endogenous genes in glycine betaine mediated stress tolerance in plants. Additional work to determine whether transcriptome modifications are direct targets of glycine betaine or are produced from metabolic adjustment in transgenic plants. Analysis of variance (ANOVA) showed a significance in the stressed varieties $P < 0.05$ (Table 1).

The figure 2 shows that the controls exhibit a fairly high stability percentage which varies from (86.60%) in the Cirta variety to a low percentage (46.57%) in the Wahbi variety with an average (62.77%).

On the other hand, in the stresses, the variety B17 registered a large percentage (419.43%) and the variety Bousselem showed the low percentage (51.49%) with an average (106.76%). The results show that the leaves retain an important structural integrity despite the presence of salt which causes a physiological dryness. This ability to maintain the integrity of its membranes seems to be associated with mechanisms of avoidance of saline stress.

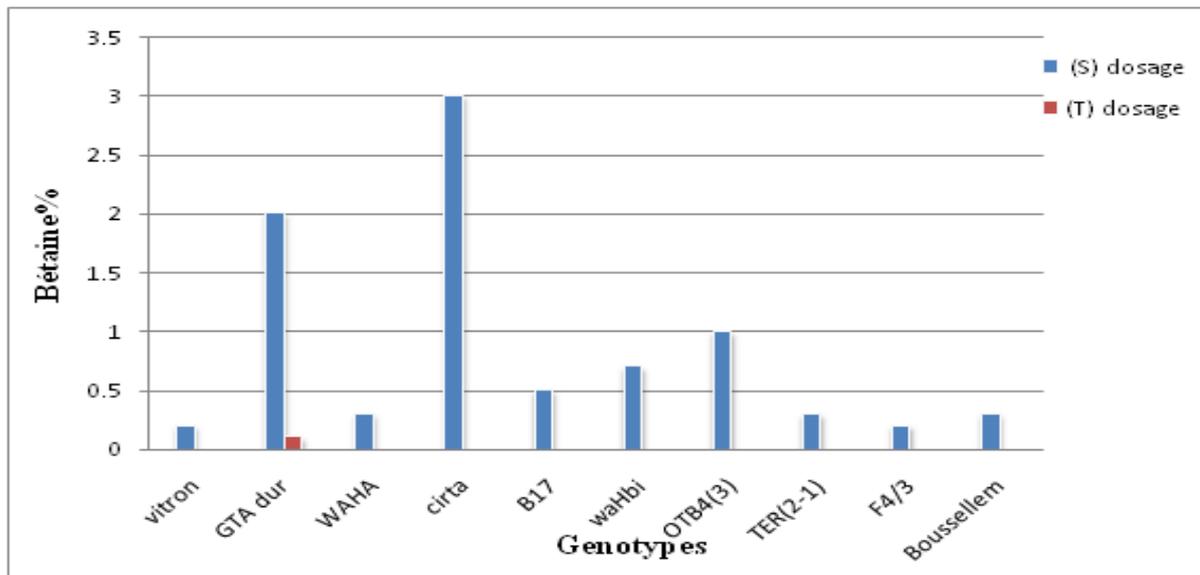


Fig. 1. The effect of drought on leaf glycine betaine.

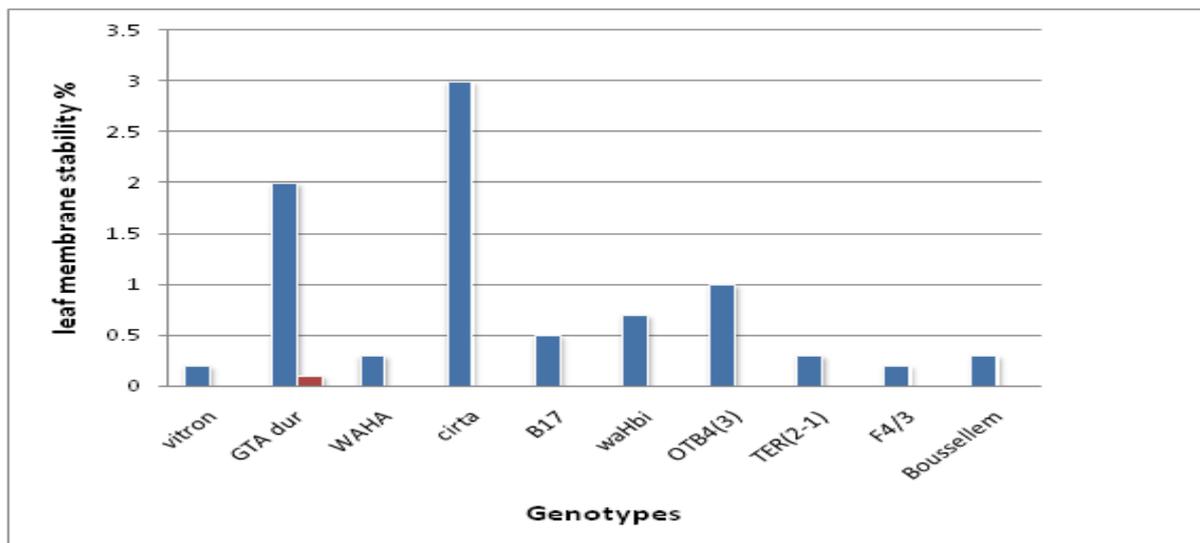


Fig. 2. The effect of drought on leaf membrane stability.

Indeed, in many plants, there has been a disorganization of the ultra-structure of the walls caused by stress (BLUM, 1981). These alterations may result from mechanical destruction by plasmolysis. Analysis of variance (ANOVA) showed a very high significance in the ten control and stressed varieties $P < 0.001$ (Table 1).

Chlorophyll variations are shown in the histograms of Fig. 3. These results show that the Ter (2-1) variety registered the large value (15.14%), but the Vitron variety marks the low value (9.2%) For the stresses, the F4/3 variety has a higher value (17.72%), whereas the inferior value (10.19%) is marked by the variety

Gat Hard for an average (12.76%). Chlorophyll is a plant pigment responsible for the green coloring of plants. This pigment, which is found in plant cells, is used with other pigments by plants to perform photosynthesis. This process allows the plant to use the energy of the sun to convert carbon dioxide (CO_2) and water into oxygen and organic matter.

The chlorophyll content is considered as a suitable parameter of abiotic stress tolerance (salinity, drought) in several species (Srivastava *et al.*, 1988). Analysis of variance (Anova) showed a high significant difference in ten genotypes and under controlled and stressed conditions $P < 0.01$ (Table 1).

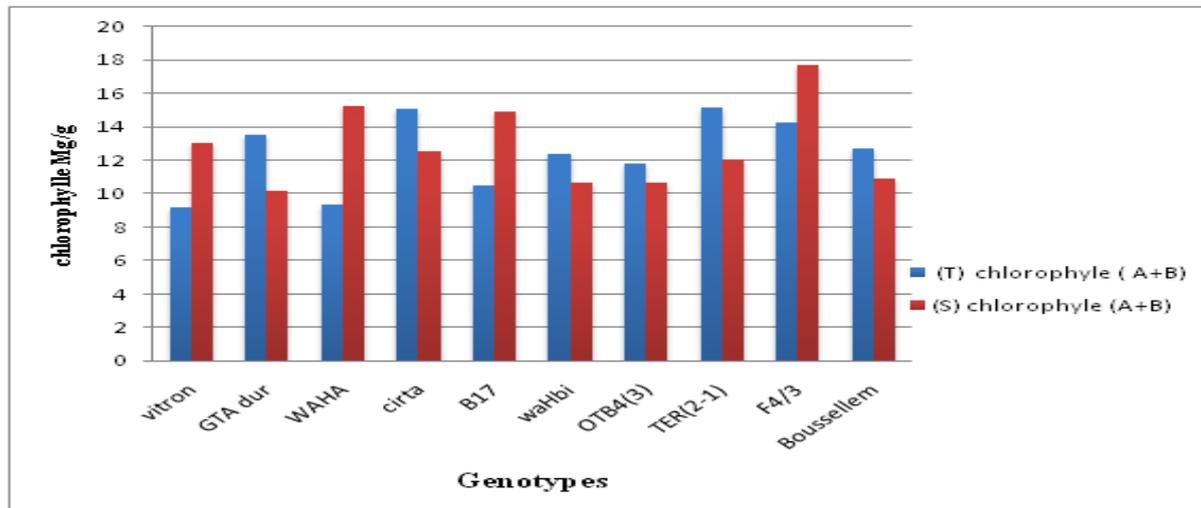


Fig. 3. The effect of drought on leaf chlorophyll.

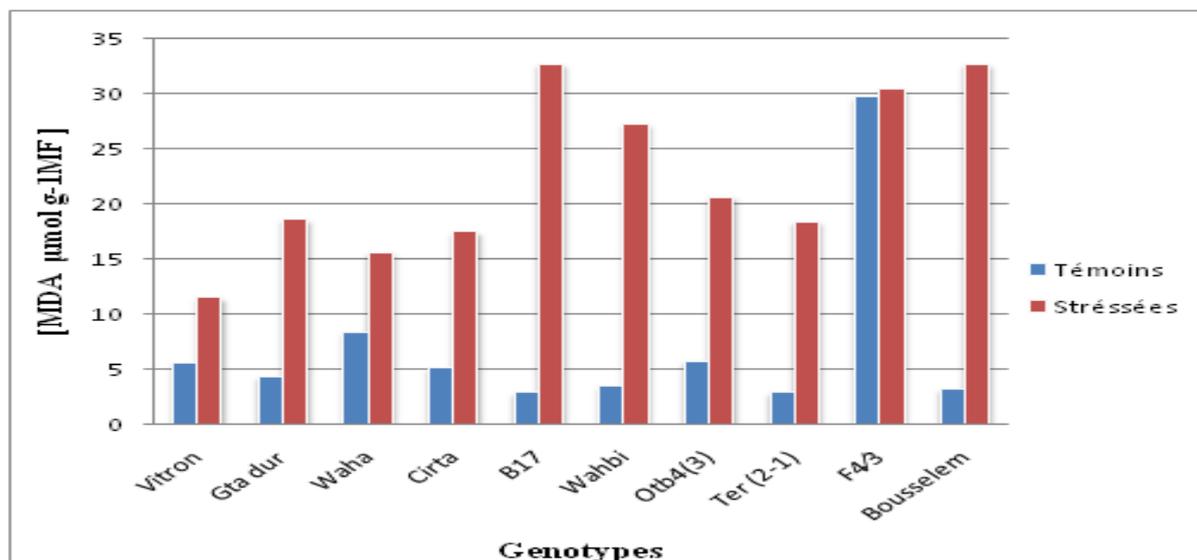


Fig. 4. The effect of drought on leaf MDA.

Stress increased the concentration of Malonaldehyde (MDA) in the genotypes studied (Fig. 4). The maximum value is noted in the stressed variety B17 of 32,756 $\mu\text{mol g}^{-1}\text{MF}$ by contribution to the controls. While the stressed Vitron variety has a minimum value of 11,54 $\mu\text{mol g}^{-1}\text{MF}$ as a contribution to the controls. The mean is 23,080 $\mu\text{mol g}^{-1}\text{MF}$ between the stratified varieties and 7,249 $\mu\text{mol g}^{-1}\text{MF}$ between the control varieties. MDA plays an abiotic stress indicator and therefore can be used as a bio marker for oxidative stress (Lakdhar-Chaabouni *et al.*, 2007, Funes *et al.*, 2005, Giguère *et al.*, 2003). Analysis of variance (ANOVA) showed a very high significant difference in the ten genotypes studied and under controlled and stressed conditions $P < 0.001$ (Table 1).

In this study stress decreased the concentration of proteins in the genotypes studied (Fig. 5). This decrease is marked in the Bousselem variety under stressed condition with a minimum value of 0.160 mg/ml per serving of the controls. Whereas the variety B17 in stressed condition marked, a maximum value of 0.328 mg/ml per serving of the controls. The mean was 0.253 mg/ml between the ten stressed varieties and 0.311 mg/ml between the ten control varieties. Analysis of variance (ANOVA) showed a very high significant difference in the ten genotypes studied and under controlled and stressed conditions $P < 0.001$.

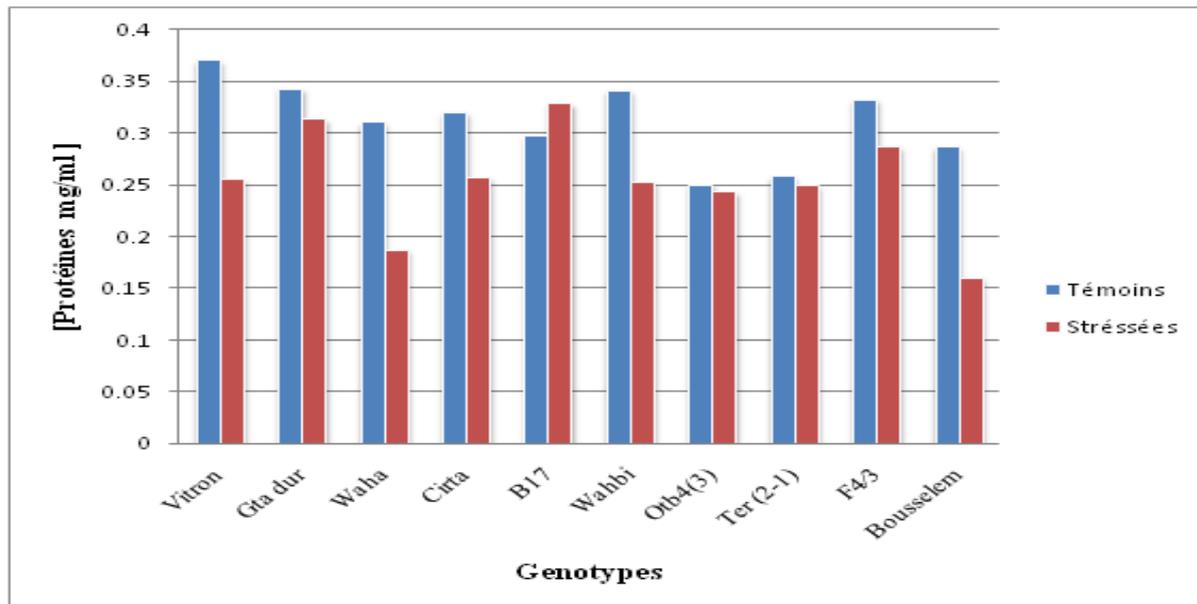


Fig. 5. The effect of drought on protein content.

Conclusion

Our study showed a difference response of the ten genotypes studied under controlled and stressed conditions for all parameters. The stressed condition reduced significantly relative water content for all genotypes. In addition, chlorophyll content and glycine betaine content are significantly decreasing under stressed conditions. The results of this study suggest that some osmoregulators is preferable in wheat if water supply is limiting.

References

- Anand A, Trick HN, Gill BS.** 2003. Stable transgene expression and random gene silencing in wheat, *Plant Biotechnology Journal*, **1(4)**, 241-251.
- Apel K, Hirt H.** 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. *Plant Biologie*. **55**, 373-399.
- Bradford M.** 1976. Analyse. *Biochimie* **72**, 248-250.
- Casati P, Walbot V.** 2004. Rapid transcriptome responses of maize (*Zea mays*) to UV-B irradiated and shielded tissues, *Genome Biologie*. **5**, R16.
- Charest C, Phan CT.** 1990. Cold acclimation of Wheat (*Triticum aestivum* L.): Properties of enzymes involved in proline metabolism. *Physiologie Plant*, **80(2)**, 159-168.

- Chaves MM, Maroco J, Pereira J.** 2003. Understanding plant responses to drought from genes to the whole plant. *Funct, Plant Biologie*. **30**, 239-264.

- Chen Z, Gallie DR.** 2004. The ascorbic acid redox state controls guard cell signaling and stomatal movement, *Plant Cell* **16**, 1143-1162.

- Dhanda SS, Sethi GS, Behl RK.** 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth, *Journal. Agronomie. Crop Science*. **190(1)**, 6-12.

- Ekanayake IJ, De Datta SK, Steponkus PL.** 1993. *Annatomie Botanic*. **72**, 73-80.

- European Commission.** 2025. Plants for the future: a European Vision for Plant Genomics and Biotechnology towards 2004. www.europabio.org/

- Glombitza C, Dubuis PH, Thulke O.** 2004. Crosstalk and differential response to abiotic and biotic stressors reflected at the transcriptional level of effector genes from secondary metabolism, *Plant Molecular. Biologie*. **51**, 1-19 (uncorrected proof).

- Grieve CM, Grattan SR.** *Plant Soil*. 1983. **70**, 303. <http://dx.doi.org/10.1007/BF02374789>.

- Hsiao TC.** 1973. Plant responses to water stress, *Ann. Rev. Plant Physiol.* **24**, 519-570.
- Lochtenthaler HK, Method Enzymol.** 1987, **148**, 350-382.
- Munns R.** 2002. Comparative physiology of salt and water stress, *Plant Cell Environ.* **25 (2)**, 239-252.
- Pathnik D, Khurana P.** 2001. Wheat biotechnology: a mini review, *Electron. Journal. Biotechnol.* **4(2)**, 74-102.
- Poustini K, Siosemardeh A, Ranjbar M.** 2007. Proline accumulation as a response to salt stressing 30 wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. *Genet Resour. Crop Evol.*, **54(5)**, 925-934.
- Saba J, Moghaddam M, Ghassemi K.** 2001. Genetic properties of drought resistance indices, *Journal. Agriculture, Science. Technologie* **3**, 43-49.
- Sairam RK, Saxena DC.** 2000. Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. *Journal. Agronomie. Crop Sci.* **184(1)**, 55-61.
- Siddique MRB, Hamid A, Islam MS.** 2000. Drought stress effects on water relation of wheat *Botanic. Bull. Acad. Sin.* **41(1)**, 35-39.
- Tian XR, Lei YB.** 2007. Physiological responses of wheat seedlings to drought and UV-B radiation, effect of exogenous sodium nitroprusside application. *Plant Physiologie* **54(5)**, 676-682.
- Vasl IK.** 2003. The science and politics of plant biotechnology **68**.
- Vendruscolo ACG, Schuster I, Pileggi M, Scapim CA, Molinari HBC, Marur CJ, Vieira LGC.** 2007. Stress induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant Physiol.* **164(10)**, 1367-1376.
- Wang WX, Vinocur B, Altman A.** 2003. Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance, *Planta* **218(1)**, 1-14.
- Yu SW, Tang, KX.** 2004. MAP kinase cascades responding to environmental stress in plants, *Acta Botanica. Sin.* **46(2)**, 127-136.
- Zhu JK.** 2002. Salt and drought stress signal transduction on plants, *Annu. Rev. Plant Biologie* **53**, 247- 273.
- Zhu JK.** 2003. Regulation of ion homeostasis under salt stress, *Curr. Opin Plant. Biologie.* **6(5)**, 441-445.
- Zlatev Z, Stoyanov Z.** 2005. Effect of water stress on leaf water relations of young bean plans. *J. Central Europeen. Agriculture.* **6(1)**, 5-14.