American Journal of Agricultural and Biological Sciences 2 (3): 136-141, 2007
ISSN 1557-4989

Stomatal Resistance to Low Leaf Water Potential at Different Growth Stages Affects Plant Biomass in *Glycine max L.*

Adejare F. B. and Umebese C. E.
Department of Botany and Microbiology, University of Lagos, P. M. B. 1029 Unilag Post Office Akoka-Yaba, Lagos, Nigeria

Abstract: Two cultivars of *Glycine max* (L) Merril. (TGX536-02D and TGX 923-2E designated cv.A and cv.B respectively), were subjected to water stress for a period of 7 days at the vegetative (T2), flowering/fruiting (T3) and seed development (T4) stages of growth, with a control (T1). Stress was considered moderate at T2 stage (ψ_w : -1.53 to -1.57 MPa) and severe at T3and T4 stages (ψ_w : -2.23 to -2.67 MPa). Leaf water potential (ψ_w) and relative water content (RWC) were significantly reduced ($p<0.01$) by the stress treatment at all stages of growth and a strong positive correlation (r = 0.79 to 0.95) was found between them. Cv.A showed greater sensitivity to water stress by having lower RWC and leaf ψ_w values than cv.B with similar treatments. Cv.B had higher abaxial resistance (Rs) than cv.A at the T3 and T4 stages and higher adaxial Rs at the T2 and T3 stages of growth. The lower Rs of cv.A at the T3 and T4 stages resulted in lower tolerance to water stress than cv.B as shown by the significant reduction ($p<0.05$) in the biomass of T3 and T4 plants. Furthermore, T3 and T4 plants of cv.B showed leaf area adjustments. Hence, cv.B is better adapted to stress at the reproductive stage than cv.A,

Key words: Water stress, stomatal resistance, biomass, soybean

INTRODUCTION

One of the adaptations of plants that lead to the conservation and efficient use of acquired water during water stress is the reduction of water loss due to stomatal resistance[1]. When water status in a leaf falls below a threshold value, stomata respond by closing. Stomata close as a result of the production of abscisic acid at a critical water potential and this rapidly alters ion fluxes in guard cells[2]. The closing of stomata when water is limiting improves the leaf water potentials which help to buffer the effects of water stress[3].

The initial detection of water stress in leaves is related to its effects on photosynthesis. The closure of stomata normally cuts off access of the chloroplast to the atmospheric supply of carbon dioxide and low cellular water potential directly affects the structural integrity of the photosynthetic machinery[4]. Subsequently, there is a reduction in vegetative growth.

The degree of water stress effects on growth, yield and quality of plants depends on the timing of stress in relation to the stage of development of the plant and the duration of the stress. The stage at which water stress imposes drastic effects on the plant is referred to as the crucial/critical stage[5]. Leaf tissues of several sorghum varieties are less susceptible to desiccation injury at severe water stress levels (about -3.3 MPa) prior to anthesis than at the post anthesis stage[6]. Deficit at anthesis or seed fill stage also reduced yield in *Brassica*[7]. Water stress at the vegetative stage of growth has even been shown to induce a two-fold increase in growth and yield of *Spigelia anthelmia*[8].

This work was conducted to compare the changes in leaf water potential, the relative water content and stomatal resistance of two cultivars of *Glycine max* subjected to water stress at different growth stages. The resulting effect on plant biomass determines the drought hardiness of each cultivar.

MATERIALS AND METHODS

Plant material and growth experiment: Seeds of two cultivars of *Glycine max* (TGX536-02D and TGX 923-2E designated cv.A and cv.B respectively) were collected from the International Institute for Agriculture (IITA), Ibadan, Nigeria.

Plants were raised in a nursery bed for 2 weeks. Thereafter, plants of the same height were transplanted to plastic pots each filled with 4 kg garden soil. The two cultivars were planted together in each pot to ensure inter-mixing of the root system subjected to similar moisture availability. The first batch of pots was

Corresponding Author: Umebese C.E., Department of Botany and Microbiology, University of Lagos, P. M. B. 1029 Unilag Post Office, Akoka-Yaba, Lagos, Nigeria

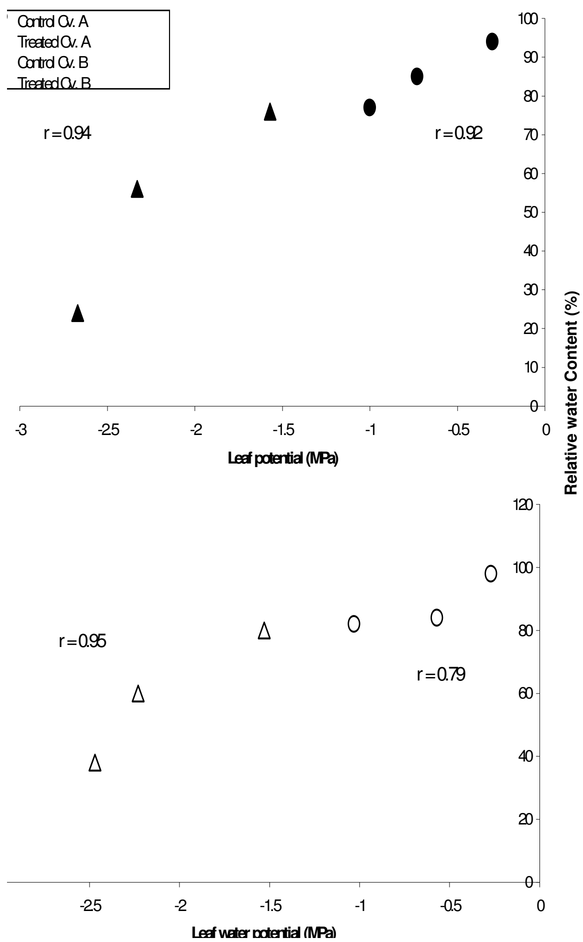

Fig. 1: Relationship between water content (%) and leaf water potential (MPa) of soybean cv. A and cv. B subjected to water stress at different growth stages

watered daily (control-T1). Three batches: T2, T3 and T4 were subjected to 7 days water stress at the vegetative (23 days after planting, DAP), flowering/fruit set (65 DAP) and seed development (75 DAP) stages respectively. The plants were arranged in a randomized complete block design with 5 replications. The pots were kept at the greenhouse of the Botanic garden of the University of Lagos with 12 h photoperiod and a relative humidity of 60% during the day and 68% at night. Temperature range was 29.3±0.3° C to 32.3±0.3° C during the day and 21.6±0.5° C to 23.4±0.5°C at night.

At two weeks intervals, samples of plants were taken in 5 replicates, for the measurement of plant biomass after seedlings were oven-dried at 80° C for 24h.

Measurement of leaf water potential: The degree of stress in the plant was measured by the determination of the leaf water potential (ψ_w), using a pressure bomb chamber (Model 600L, Chas. W. Cook and Sons, England) by the method of Boyer[9]. Using a sharp blade, a clean slanting cut was made from a twig from each plant. 2 cm bark was removed away from the cut end. The twig was inserted with the cut end protruding through the hole in the cover. Pressure was released from the cylinder of compressed nitrogen gas until sap was forced out through the cut end. A pressure as low as 0.3 MPa was regarded as low deficit (high ψ_w) while a pressure as high as 2.0 MPa was regarded as relatively high deficit (low ψ_w). Measurements were taken in three replicates at the end of each period of water stress, from 4-6 pm daily.

Measurement of relative water content: The relative water content (RWC) of leaf tissues at the end of the stress period was determined by the method of Weatherly[10]. Leaf samples were taken in triplicates from each stress treatment and cultivar. Using a cork borer (diameter of 2 cm) 10 discs were cut from each leaf, weighed fresh (FW) and floated in de-ionized water for 4h in a freezer at 0°C to minimize the rate of respiration. Then the discs were removed and dried between absorbent moist paper and the turgid weight (TW) was taken. Then leaf discs were dried at 80°C for 24h and weighed to give the dry weight (DW). RWC was expressed as:

$$RWC = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100$$

Measurement of stomatal resistance: Stomatal behaviour was measured by the determination of the stomatal resistance (Rs) to water vapour using an automatic diffusion porometer (mk II) manufactured by Delta-T devices and designed by Styles, Monteith and Bull. The cup of the porometer was clamped to the surface of the youngest fully mature leaf of the plant. An inbuilt sensor attached to the cup detects the rate of humidification of the closed system which is caused by transpiration of the leaf. The rate of humidification of the cup is inversely related to the Rs of the leaf. Adaxial and abaxial resistances were measured in the same leaf at midday.

RESULTS AND DISCUSSION

Two cultivars of *Glycine max* (TGX 536-02 and TGX 923-2E, cv.A and cv.B respectively) exposed to 7 days water stress at the vegetative (T2), flowering/fruiting (T3) and seed development (T4) stages showed differences in leaf water potential (ψ_w) and stomatal behaviour at each stage of growth, affecting subsequent biomass production. Stress was moderate at the vegetative stage (leaf $\psi_{w:}$ -1.53 to -1.57

Table 1: Effect of water stress on the leaf water potential of soybean cv. A and cv. B at different stages of growth

	Water Potential (MPa)			
	Cv. A		Cv. B	
Treatment Stage	Control (T1)	Treated	Control (T1)	Treated
Vegetative stage (T_2)	-0.30 ± 0.00	-1.57 ± 0.03*	-0.27 ± 0.03	-1.53 ± 0.03*
Flowering/fruiting stage (T_3)	-0.73 ± 0.03	-2.33 ± 0.03*	-0.57 ± 0.03	-2.23 ± 0.03*
Seed development stage (T_4)	-1.00 ± 0.06	-2.67 ± 0.03*	-1.03 ± 0.09	-2.47 ± 0.07*

*Difference between treated and control is significant at p <0.01.

Table 2: Effect of water stress at different stages of growth on the relative water content of soybean cv.A and cv.B

	Relative water content (%)			
	Cv. A		Cv. B	
Treatment Stage	Control (T1)	Treated	Control (T1)	Treated
Vegetative stage (T_2)	94 ± 3.00*	76 ± 2.00	98 ± 9.10*	80 ± 7.00
Flowering/fruiting stage (T_3)	85 ± 2.40*	55 ± 5.00	83 ± 7.20*	60 ± 2.00
Seed development stage (T_4)	77 ± 9.00*	23.3 ± 7.50	82 ± 1.50*	31.2 ± 9.00

*Difference between treated and control is significant at p<0.01.

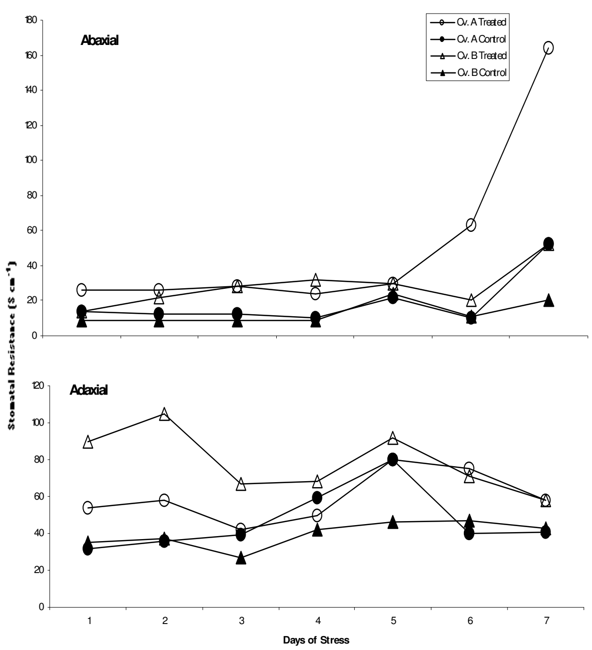

Fig. 2: Abaxial and Adaxial stomatal resistance of soybean Cv. A and Cv. B subjected to water stress at the early vegetative (T2) stage

MPa) and severe (leaf $\psi_{w:}$ -2.23 to-2.67 MPa) at the reproductive stages of both cultivars (Table 1). Leaf ψ_w of both cultivars was significantly reduced (p<0.01) by stress at all stages of growth and the older the plant the more severe was the impact of stress. Correspondingly, relative water content (RWC) was significantly reduced (p<0.01) by water stress at all stages of growth (Table 2). A strong positive correlation (r =0.79 to 0.95) was found between the RWC and the leaf ψ_w of both cultivars (Fig. 1). These parameters have been shown to respond both to age and environmental conditions[11]. Cv.B had slightly higher ψ_w values and RWC than cv.A under similar stress conditions. Furthermore, at T4 stage cv.A showed greater sensitivity to stress by having much lower RWC (23.3%) and lower ψ_w (-2.65 MPa) than cv.B. Drought tolerance in durum wheat was explained by the higher RWC of the ear during drought[12]. This infers that cv.B may be more tolerant to drought than cv.A.

Adaxial stomatal resistance (Rs) of both cultivars was twice or thrice as high in values as the abaxial Rs in both stressed and unstressed plants at all stages of growth. During the 7 days of stress at T2, cv.A showed a rapid and significant increase in abaxial Rs from the 5th-7th day of treatment while cv.B showed a fairly stable abaxial Rs. However, cv.B showed higher adaxial Rs than cv.A (Fig. 2). Both cultivars showed higher abaxial and adaxial Rs than the control. Stressed T3 plants had peak abaxial and adaxial Rs on the 5th day of treatment (Fig. 3) while stressed T4 plants had peak abaxial Rs on the 5th day and peak adaxial Rs on the 7th day of stress (Fig. 4). Cv.B had higher abaxial Rs than cv.A at the T3 and T4 stages and higher adaxial Rs at the T2 and T3 stages. Thus, cv.B showed higher total Rs than cv.A. Stomatal resistance increases and stomatal conductance decreases as a result of reduction in stomatal aperture[3].

Stomatal closure in response to water stress has been shown to maintain the water pressure in the leaf rachis xylem preventing extensive development of cavitation[13]. Cavitation renders xylem conduits non-

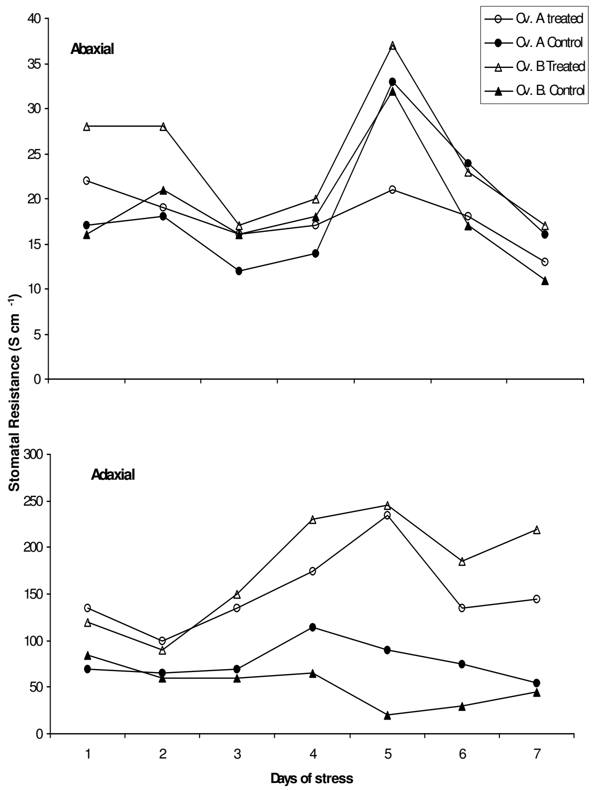

Fig. 3: Abaxial and adaxial stomatal resistance of soybean cv. A. and cv. B subjected to water stress at flowering/fruit set (T3) stage

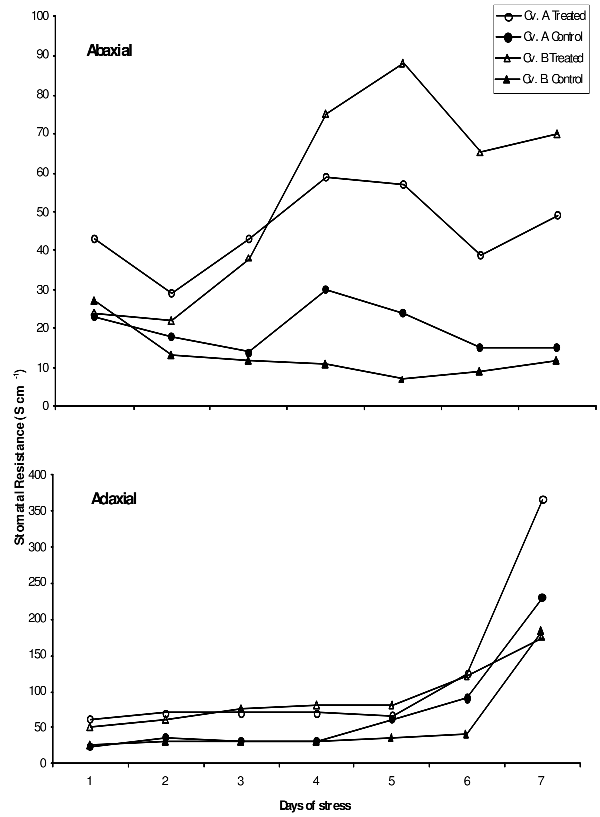

Fig. 4: Abaxial and adaxial stomatal resistance of soybean cv. A and cv. B subjected to water stress at the seed development T4 stage

conductive[14]. Cavitation avoidance is a physiological function associated with stomatal regulation during water stress[13]. A possible explanation of the reduction in stomatal aperture in cv.B leading to increased Rs, is the fact that cv.B shed its older leaves in response to water stress at the reproductive stages (at leaf ψ_w: -2.23 to -2.47 MPa) and produced new leaves that were much smaller in area than the shed leaves. This is referred to as leaf area adjustment; another mechanism for reducing leaf area and transpiration during limited water availability (Hopkins and Hüner, 2004). Reduced stomatal aperture as a result of water stress leads to high sensitivity to carbon-dioxide concentration. This induces the production of abscisic acid which subsequently closes the stomata, thus reducing water loss from the plant[15].

Water stress at T2 stage (leaf $\psi_{w:}$ -1.53 to -1.57 MPa) reduced plant biomass in both cultivars significantly ($p<0.05$) in the course of growth but at the 15th week of growth, stressed T2 plants of cv.A had similar biomass with the control. Cv.B regained similar biomass at the 17th week of growth. A single drought event at the juvenile or elongation stage had little effect on growth and seed yield in two genotypes of *Brassica* whereas water deficit at anthesis or seed fill stage reduced yield[7]. Water stress at T3 and T4 stages of growth (leaf $\psi_{w:}$ -2.23 to -2.67 MPa), caused a significant reduction ($p<0.05$) in biomass in both cultivars in the course of growth but the effect was more pronounced in cv.A. At the last week of growth cv.B had almost similar biomass as the control. This level of tolerance may be due to the shedding of its older leaves and the production of new leaves during stress at the reproductive stages. One of the early effects of water deficit is a reduction in vegetative growth. Growth was completely inhibited in maize plants when tissue water potential reached -1.00 to -1.40 MPa[4]. Though water stress reduced plant height in different cultivars of *Oryza*, only water stress at flowering stage had great yield reductions[16]. The disadvantage of stomatal closure for plants is that their carbon gain is lowered and growth is impaired[17]. Describing the transcriptional regulation of plant stress responses, the retarded germination and subsequent growth of transgenic *Arabidopsis* plants under drought

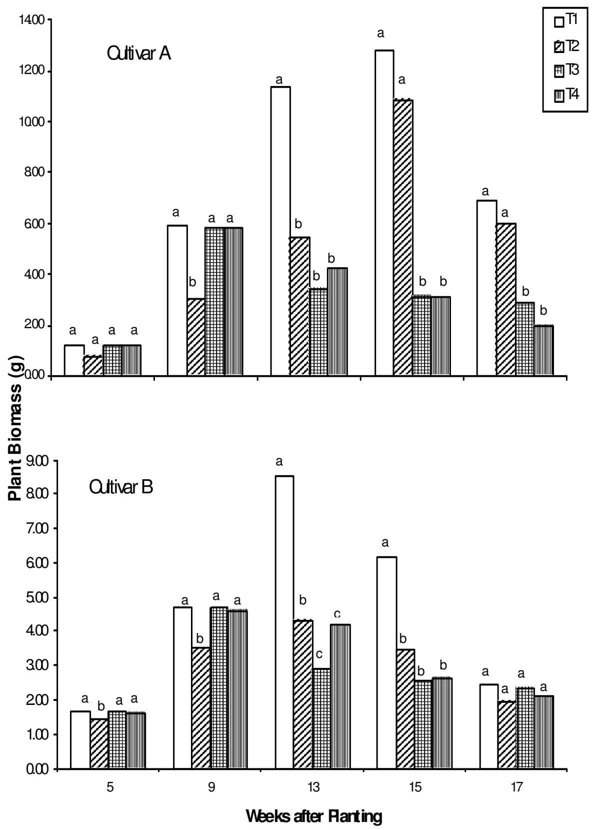

Fig. 5: Biomass of soybean cv. A and cv B subjected to water stress at the vegetative (T2), Flowering/fruiting (T3) and seed development (T4) stages and the control (T1) (Treatment means with similar letters on the vertical bars are not significantly different at $p<0.05$)

conditions was attributed to the over expression of high mobility group B (HMGB) proteins[18].

CONCLUSION

Under similar stress conditions at the reproductive stage (T4), cv.A showed greater sensitivity by having much lower RWC (23.3%) and lower leaf water potential (-2.65 MPa) than cv.B. Water stress at the vegetative stage had greater effect on the biomass of cv.B than cv.A. The reproductive stage is the crucial/critical stage of both cultivars since this was the stage at which water stress caused the most drastic effects on plant biomass. However, cv.B showed greater resistance to water stress than cv.A. Its adaptive features include the higher stomatal resistance, higher relative water content and higher water potential in response to water stress. Cv.B also showed a prompt response to loss of turgor by leaf area adjustments.

REFERENCES

1. Turner, N.C., 1979. Drought resistance and adaptation to water deficit in crop plants. In: Stress Physiology in Crop Plants. Edited by H. Mussel and R.C. Staples. Interscience, New York, pp: 343-372.
2. Leung, J. and J. Giraudat, 1998. Abscisic acid signal transduction. Ann. Rev. Plant Physiol. Plant. Mol. Biol., 49: 199-222.
3. Steudle, E., 2000. Water uptake by roots: effects of water deficit. J. Exp. Botany, 51: 1531-1542.
4. Hopkins, W G. and N.P.A. Hüner, 2004. Introduction to Plant Physiology. John Wiley and Sons, Inc. United States of America.
5. Forbes, J.C. and R.D. Watson, 1992. Plants in Agriculture. University Press, Cambridge.
6. Sullivan, C.Y., 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. In: Sorghum in the Seventies. Edited by N.G.P. Rao and L.R. House, Oxford and IBH Publishing Co. New Delhi, India, pp: 247-264.
7. Ma, Q., Nikman, S.R. and D.W. Turner, 2006. Responses of osmotic adjustment and seed yield of *Brassica napus* and *B. juncea* to soil water deficit at different growth stages. Aust. J. Agric. Res., 57: 221-226.
8. Umebese, C.E. and C.C. Iloba, 2001. Growth of *Spigelia anthelmia* L. ch. in response to water stress imposed at set stages of development. J. Res. Rev. Sci., 2: 209-212.
9. Boyer, J.S., 1967. Leaf water potential measured with a pressure chamber. Plant Physiol., 42: 13-137.
10. Weatherley, P.E. 1970. Some aspects of water relations. Adv. Bot., 3: 171-206.
11. Jones, M.M., N.C. Turner and B. Osmond, 1981. Mechanisms of drought resistance. In: Physiology and Biochemistry of Drought Resistance in Plants. Edited by L.G. Paleg and D. Aspinall. Academic Press, Australia, pp: 15-37.
12. Tambussi, E.A., S. Nogues and J.L. Araus, 2005. Ear of durum wheat under water stress: Water relations and photosynthetic metabolism. Planta (Berlin), 221: 446-458.
13. Cochard, H, L. Coll, Xavier Le Roux and T. Améglio, 2002. Unraveling the effects of plant hydraulics on stomatal closure during water stress in walnut. Plant Physiol., 128: 282-290.
14. Pickard, W.F., 1981. The ascent of sap in plants. Prog. Biophys Mol. Biol., 37: 181-229 (ISI).

15. Hsiao, T.C., 1973. Plant response to water stress. Ann. Rev. Plant Physiol., 24: 519-570.
16. Pirdashti, H., Z.T. Sarvestani, C. Nematzadeh and A. Ismail, 2004. Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L) cultivars. Proc. 4th Intl. Crop Sci. Cong., 26 Sep.-1 Oct., Brisbane, Australia.
17. Kramer, P.J. and J.S. Boyer, 1995. Water Relations of Plants and Soil. Academic Press, San Diego.
18. Kwak, K.J., J.Y. Kim, Y.O. Kim and H. Kang, 2007. Characterization of transgenic Arabidopsis plants overexpressing high mobility group B proteins under high salinity, drought or cold stress. Plant Cell Physiol., 48: 221-231.