ORIGINAL ARTICLE

Glycine Betaine and Salicylic Acid Induced Modification in Water Relations and Productivity of Drought Wheat Plants

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A study of parameters associated with adjustments in internal water balance, namely: diurnal variation in transpiration rate, stomatal opening area, relative water content, water use efficiency, hormonal level of wheat flag leaves in relation to grain yield is presented. Drought induced marked decreases in diurnal and mean daily values of transpiration rate, stomatal pore areas (on upper and lower sides), relative water content, water use efficiency, indole-3-acetic acid (IAA), gibberellic acid (GA_3), cytokinins (CK_s) and grain yield but led to a significant increase in the abscisic acid (ABA) concentration in flag leaves of the wheat cultivars. Grain presoaking in salicylic acid or foliar application with glycine betaine alleviated the stress by keeping water within leaves and consequently recover the turgidity of stressed plants by restricting the transpiration rate, stomatal closure, decreasing the ABA level and enhancing the growth promoters particularly (IAA, GA_3 & CK_s) particularly with the sensitive cultivar. Furthermore, the effect was more pronounced with glycine betaine + salicylic acid treatment. The grain yield appeared to be positively correlated with IAA, GA_3, CK, RWC, WUE_G and WUE_B but negatively correlated with ABA, SWD, transpiration rate and stomatal areas on both wheat cultivars.

Key words: Drought, glycine betaine, salicylic acid, relative water content, transpiration, water use efficiency

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Great emphasis is still layed on the importance of the internal water balance in plant water relations because of the concrete relationship between this balance, and turgidity, to the rate of physiological processes that control the quality and quantity of plant growth and its productivity (Aldesuquy *et al.*, 1998, 2012). The internal water balance is not independent but is controlled by the

relative rates of water absorption and transpiration (Kramer, 1969).

Water-limited crop production depends on the intensity and the pattern of drought, which vary from year to year (Sankar *et al*., 2008). In some temperate environments, however, there is a high probability that crop water deficit increase in severity as the season progresses, due to lack of rainfall and to the high evaporative demand (Soriano *et al*., 2004). An efficient use of limited water resources and better growth under limited water supply are desirable traits for crops in drought environments (Sankar *et al*., 2008). Crop production and sustainable development are severely constrained by water limitations during the growing season (Jaleel *et al*., 2008). The decline of transpiration rate in response to water deficit is widely reported in literature (Xu and Bland, 1993) and the effect is more pronounced in the susceptible genotypes than the tolerant genotypes (Wood and Goldsbrough, 1997).

Relative water content (RWC) was proposed as a good indicator of water status, through its relation to cell volume, may closely reflect the balance between water supply to the leaf and transpiration rate (Sinclair and Ludlow, 1985). In recent years, many studies about the effects of supplemental irrigation on yield performance and water use efficiency (WUE) have shown that proper supplement of irrigation can increase crop yield by improving soil water conditions and their WUE significantly (Zhang and Cheng, 2004). Improving the efficiency of water use in agriculture is associated with increasing the fraction of the available water resources that is transpired, because of the unavoidable association between yield and water use (Shao *et al*., 2005, Shao *et al*., 2008a).

Plant endogenous hormones regulate various biological activities in plants (Tian and Shao, 2007). When plants are subject to abiotic stress, some of the plant endogenous hormones have been found to be the key elements involved in signal transduction and in the regulation of gene expression in plants in response to the stress (Xiong *et al.,* 2002; Zhu 2002). ABA plays a key role in the response of plants to drought. Under conditions of water stress, a rapid accumulation of ABA results in stomata1 closure, synthesis of specific proteins and mRNAs, regulation of physiological processes, and improved ability to adapt to water stress (Chinnusamy *et al*., 2004; Verslues and Zhu 2005). Among all the stresses to plant growth and development, drought is a major stress to agricultural production (Shao *et al*., 2005, 2008a). Plants synthesize ABA in response to drought, triggering a signaling cascade in guard cells that results in stomatal closure, thus reducing water loss, which may influence WUE in plants (Song *et al*., 2008).

Growth reduction under drought stress is mainly caused by a decrease in the concentration of IAA (Saugy and River 1988). Nevertheless, there are reports that there were no significant changes in IAA under drought stress (Li *et al.,* 2000) and that changes in IAA caused by drought have no significant regulatory function in the process of adaptation of a plant to drought.

In many crop plants the natural accumulation of GB is lower than sufficient to ameliorate the adverse effects of dehydration caused by various environmental stresses (Subbarao *et al*., 2001). Exogenous application of GB to low-accumulating or non-accumulating plants may help reduce adverse effects of environmental stresses (Yang and Lu, 2005). Externally-applied GB can rapidly

penetrate through leaves and be transported to other organs, where it would contribute to improved stress tolerance (Ashraf and Foolad, 2007).

Exogenous application of SA may influence a range of developmental and physiological processes, e.g., seed germination and fruit yield (Cutt and Klessing, 1992), transpiration rate (Larque, 1979), stomatal closure (Rai et al., 1986), membrane permeability (Barkosky and Einhellig, 1993), growth and photosynthesis (Khodary, 2004, Diğdem et al., 2007).

The present work was undertaken to investigate the effect of foliar application of glycine betaine and grain presoaking in salicylic acid and their interaction on induction of stomatal closure, reduction of transpiration, improvement of leaf turgidity, hormonal regulation in relation to grain yield of droughted wheat (Triticum aestivum L.) cultivars.

MATERIALS AND METHODS

Plant material and growth conditions

Two wheat cultivars (Triticum aestivum L.) Sakha 94 (sensitive var.) and Sakha 93 (resistant var.), which are common in Egypt, were used in this study. The variety Sakha 93 is known to be more drought resistant than Sakha 94. A homogenous lot of wheat grains (either sensitive or resistant var.) was separately surface sterilized by soaking in 0.01 % $HgCl_2$ for 3 minutes, followed by thoroughly rinsing in sterile water. The sterilized grains from each variety were divided into two sets (≈ 500 g per set for each var.). Grains of the 1st and 2nd sets were separately soaked in distilled water or salicylic acid (0.05 M), respectively. In 20 November 2005, grains of each set were planted in plastic pots (fifteen grains per pot; 25cm width X 30cm height) filled

with 6 kg mixture of soil (clay and sand = 2:1, v/v). The pots were kept in a greenhouse, and the plants were subjected to natural day/ night conditions (minimum /maximum air temperature and relative humidity were; 29.2/33.2 °C and 63/68 %, respectively). Irrigation to field capacity was carried out when soil water content had fallen to 60% of its initial value. Twenty days after planting, the plants were thinned to five uniform seedlings per pot.

On the day 65 (at heading) after planting the pots of the 1st set was allocated to four groups (20 pots per each group) as follow: control (cont.), water stress (WS), glycine betaine control (GB), glycine betaine + water stress (GB + WS). The 2nd set group was allocated as follow: salicylic acid control (SA), salicylic acid + water stress (SA+WS), control glycine betaine + salicylic acid (GB + SA) and glycine betaine + salicylic acid + water stress (GB+SA+WS). For glycine betaine (10 mM) treatment, the plants were sprayed by glycine betaine 48 hrs before starting the stress period and weekly during the stress period.

Water deficit was imposed by withholding water at the reproductive stage for 30 days within two periods: on the day 65 from planting (heading stage) and the day 80 from planting (anthesis stage). Each droughted pot received 500 ml water at the end of 1st stress period out. At the end of stress periods, re-watering to the field capacity was carried out. The undroughted (control) plants were irrigated to the field capacity during the stress period, and all plants were left to grow until grain maturation under normal irrigation with tap water. Samples were taken for measurements of transpiration, stomatal area, realative water content and saturation water deficit as well as growth bioregulators at the end of the 2nd stress period before re-watering (i.e after 95 days from

planting).

At the bud stage, 21 days from planting, (i.e. tillering stage) and before heading (i.e., at ear emergence) the plants received 35 kg N ha^{-1} as urea and 35 kg P ha^{-1} as potassium dihydrogen phosphate as fertilizers.

Monitoring the water status of the soil

Soil water content (SWC) at the end of the stress period was estimated by the destructive method as recommended by Ritchie et al. (1990).

Estimation of water use efficiency

Water use efficiency was calculated by dividing the grain yield (t ha^{-1}) or the biomass yield (t ha^{-1}) by the amount of water added by (gallons). Therefore water use efficiency for grain yield (WUE$_G$) was calculated from the grain yield and water use efficiency for biomass yield (WUE$_B$) was estimated from the biomass yield (Stanhill, 1987).

(WUE$_G$) = Grain yield (t) / Total water used (gallon)

(WUE$_B$) = Biomass yield (t) / Total water used (gallon)

Measurements of transpiration

Before starting transpiration measurement, the soil surface in the pots were covered by the projecting margins of the plastic bags lining the pots and wrapping then around the base of stems. This act prevents water loss by direct evaporation from the soil and any measurable loss, hence, represents loss by transpiration. Each pot was weighed periodically during daytime. This was carried out at 3 hours intervals, namely at 7 a.m., 10 a.m., 1 p.m., 4 p.m. and 7 p.m for one day and the plants were then harvested by cutting just above the soil surface. Fresh weight of leaves was recorded for each pot. Transpiration was expressed in "mg water/g leaf fresh weight /hour". Transpiration rate was calculated for the four daily periods. Aspiration

Assman Psychrometer (Slatyer and Mcilroy, 1961). Actual and saturation vapour pressures (mmHg) at the ambient temperatures were obtained from hygrometric tables and vapour pressure deficit (V.P.D.) was calculated.

Measurements of stomatal opening

Stomatal behavior in experimental plant was tested during the chosen for transpiration measurements. This would give an indication of its relation to other parameters such as the soil water potential and prevailing climatic variables. For measuring stomatal aperture, the epidermis was removed from the leaf by making a shallow incision on the upper or the lower surface of the leaf at a right angles to the veins using a sharp razor blade. Using this technique, the epidermis could be removed from the leaf (Rodriguez and Davies, 1982). After fixation of the epidermis in alcohol, the stomatal apertures were determined under the microscope by measuring the length and the width of its aperture using an occular micrometer.

Measurements of relative water content

Relative water content (RWC) of leaves was tested at the same time chosen for transpiration measurements. This would give an important measure of the internal water status in the plant studies and its response to other parameters. In measuring relative water content, the method of Weatherly (1950) and its modification by Weatherly and Barrs (1962) was adopted.

Estimation of saturation water deficit (SWD)

Saturation water deficit (SWD) was calculated from the following equation:

SWD = 100- RWC

Extraction, purification and determination of growth hormones

The extraction and purification procedure for

abscisic acid, indole acetic acid, gibberellic acid and cytokinins was carried out according to the method that originally described by Shindy and Smith (1975) while the estimation was by using two-dimensional HPLC according to Dobrev *et al.* (2005).

Statistical analysis

The main effect of factors (watering regime, both used chemicals, growth stages and wheat type) and interaction (watering regime, both used chemicals, growth stages and wheat type) were evaluated by general linear model (two way ANOVA)using SPSS program. Tests for significant differences between means at P =0.05 were given by LSD test. The correlation coefficient between the economic yield and all evaluated criteria was also evaluated.

RESULTS

Changes in soil moisture content

Soil moisture content was markedly reduced at the end of the stress period, and it was found to be about 23.3 and 4.6 % (% of oven soil dry weight) for control and droughted pots respectively.

Changes in diurnal values of transpiration and average daily amounts of transpiration

Transpiration rate and concurrent effective climatic parameters are shown in Figure 1. Transpiration generally followed both temperature and V.P.D. of air in their fluctuation.Transpiration rate in wheat plants of the two varieties and concurrent effective climate parameters were measured on one day (Figure 1 and Table 1). In general, the maximum transpiration rate was attained shortly after mid-day in control and differently treated plants, (at 10-1 p.m.). Water stress caused marked reduction (P≤ 0.05) in diurnal values of transpiration allover day time. The sensitive cultivar had higher transpiration rates

than the resistant one.

The applied chemicals (glycine betaine, salicylic acid or their interaction) induced additional and highly significant decreases (P≤ 0.05) in transpiration rates in stressed plants. Furthermore, the magnitude of reduction in transpiration rate depends mainly on the day time and on the chemical used. It was found that grain presoaking in salicylic acid was more effective than the foliar application of glycine betaine in the reduction of diurnal values of transpiration rate during the day. In addition GB + SA treatment decreased the transpiration rates more than the other treatments of both cultivars.

Diurnal changes in the stomatal area

Stomatal pores areas on the upper and the lower surface of the experimental plant leaves were measured during the same periods of measurements of transpiration (Table 2). There is a tendency among control plants to a progressive increase in stomatal areas on both upper and lower surfaces during morning until 1 p.m. when maximum opening takes place. A sharp decline in stomatal area is quite observed in both wheat cultivars. Water stress induced a noticeable decrease (P≤ 0.05) in stomatal pores areas allover the day time on both upper and lower surfaces of flag leaves of the two wheat cultivars. The sensitive cultivar had higher stomatal areas than the resistant one.

As compared with control and water stressed plants, the interaction between water stress and GB, SA or their interaction induced additional reduction (P≤ 0.05) in diurnal changes in stomatal area on both upper and lower surfaces of leaves. In general, grain presoaking with SA was more effective than GB in inducing stomatal closure

during the day time on both upper and lower surfaces in leaves of the two wheat cultivars. Moreover, GB and SA treatment decreased the stomatal areas more than the other treatments under controlled and stress conditions of both cultivars.

Diurnal changes in relative water content and saturation water deficit

There is a tendency among control and droughted plants to a progressive decrease in turgidity all over the day time. Reduction in turgidity was more pronounced at 1 p.m. in both wheat cultivars. As compared with control plants, drought caused additional reduction (P≤ 0.05) in the relative water content of both wheat cultivars. There was a progressive decline in the main values of turgidity and the magnitude of reduction was more pronounced with the sensitive wheat cultivar. Application by GB, SA or their interaction completely alleviated the stress induced by drought on the two wheat cultivars by regain the turgidity of the flag leaves during the day time. This effect was more obvious in GB+SA treatment in both wheat cultivars (Table 3).

The data in table 4 showed that, saturation water deficit (SWD) appeared to increase in flag leaves of control and droughted wheat plants in all the designated periods. The maximum increase (at 1 p.m.) in SWD in flag leaves of wheat cultivars. Furthermore, the sensitive cultivar had higher SWD levels than the resistant one. In the majority of cases, treatments with GB, SA or their interaction caused marked reduction (P≤ 0.05) in the SWD of both stressed and non-stressed plants. This effect was more pronounced with SA and GB+SA treatments in both wheat cultivars.

Changes in water use efficiency (WUE)

It is clear from the results in figure 2a that the values of WUE_G and WUE_B in the stressed plants were significantly lower than that of the control of both wheat cultivars. Application of GB, SA or their interaction clearly improved WUE_G and WUE_B values in stressed of both wheat cultivars. In addition, treatments with GB+SA had higher WUE_G and WUE_B values than the other treatments.

Changes in growth inhibitor (ABA)

The results indicated that drought caused a massive increase (P≤ 0.05) in the ABA levels in the flag leaves in both wheat cultivars. The sensitive cultivar had higher ABA levels than the resistant one. As compared with the stressed plants, treatment with GB, SA or their interaction caused a drastic reduction in the ABA levels. The reduction was more pronounced with pretreatment GB+SA in both wheat cultivars (Table 1).

Our result showed that ABA appeared to be correlated with the stomatal areas as well as the main daily values on upper and lower surfaces of the two droughted wheat cultivars. These correlation was significant at afternoon.

Changes in growth promoters (IAA, GA₃ and CK$_s$)

As compared to the control, drought induced a marked decrease (P≤ 0.05) in the IAA, GA₃ and CK$_s$ (zeatin-riboside, kinetin and benzyl adinine) as well as total cytokinins level in the flag leaves of both wheat cultivars. Foliar application of GB, grain presoaking with SA or their interaction, in general, caused more accumulation in growth promoters in flag leaves of both wheat cultivars.

Changes in Grain Yield

Water stress reduced (P≤0.05) the grain yield of both wheat cultivars. This effect was more pronounced with sensitive plants. The used

chemicals improved the grain yield of both cultivars. Glycine betaine + salicylic acid treatments appeared to mitigate the effect of water stress on wheat plants more than the other treatments under control and stress conditions (Fig. 2b).

The grain yield appeared to be positively correlated with IAA (0.95, 0.96), GA$_3$ (0.75, 0.96),

zeatin-riboside (0.88, 0.95), kinetin (0.87, 0.91), benzyl adinine (0.78, 0.86), total cytokinins (0.85, 0.91), RWC (0.79, 0.84), WUE$_G$ (0.85, 0.22) and WUE$_B$ (0.80, 0.46) but negatively correlated with ABA (-0.99, -0.95), SWD (-0.79, -0.84), transpiration rate (-0.12, 0.14) and stomatal area (-0.05, 0.03) for the sensitive and resistant wheat cultivars respectively.
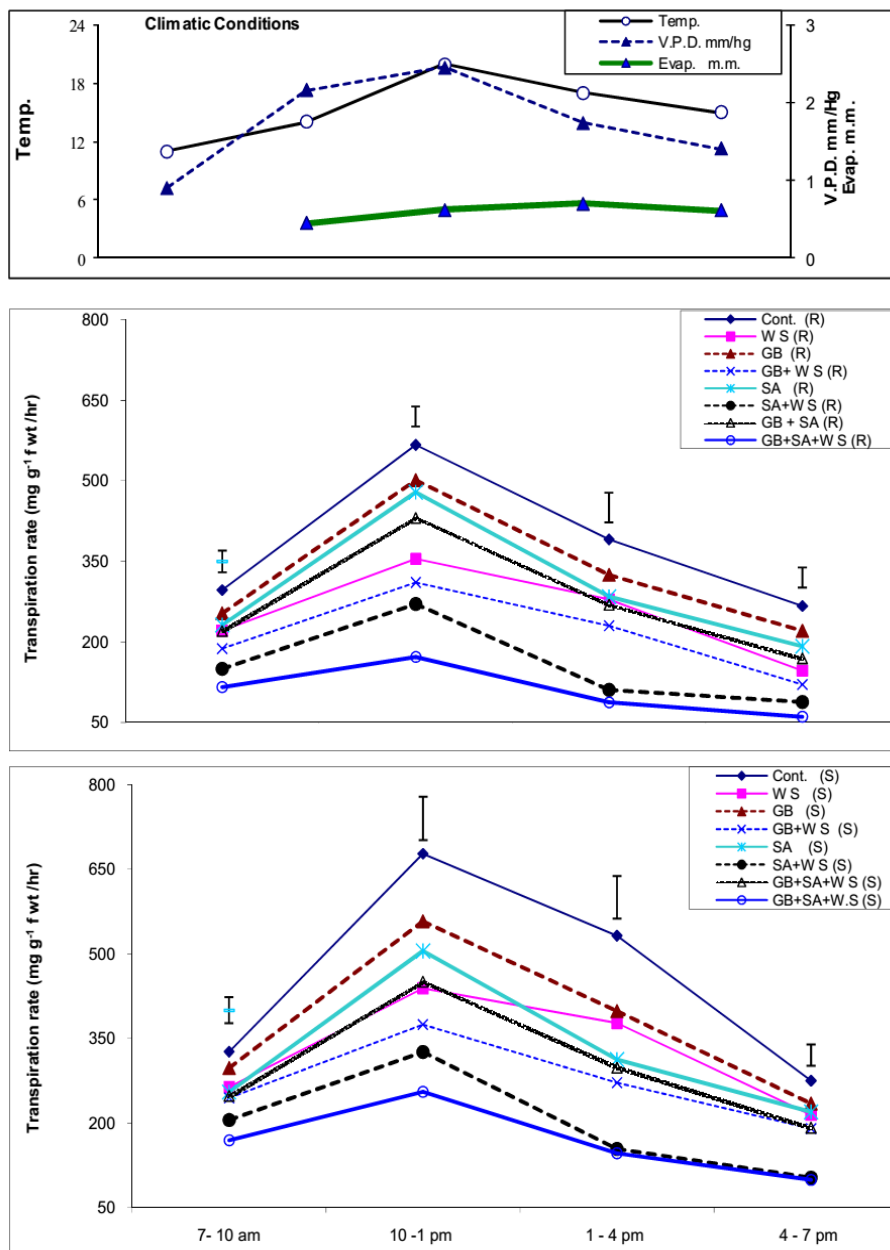
Figure 1. Effect of glycine betaine, salicylic acid and their interaction on the diurnal changes of transpiration rate (mg g^{-1} f wt /hr) of droughted wheat cultivars at anthesis stage. Vertical bars represent LSD at 0.05.
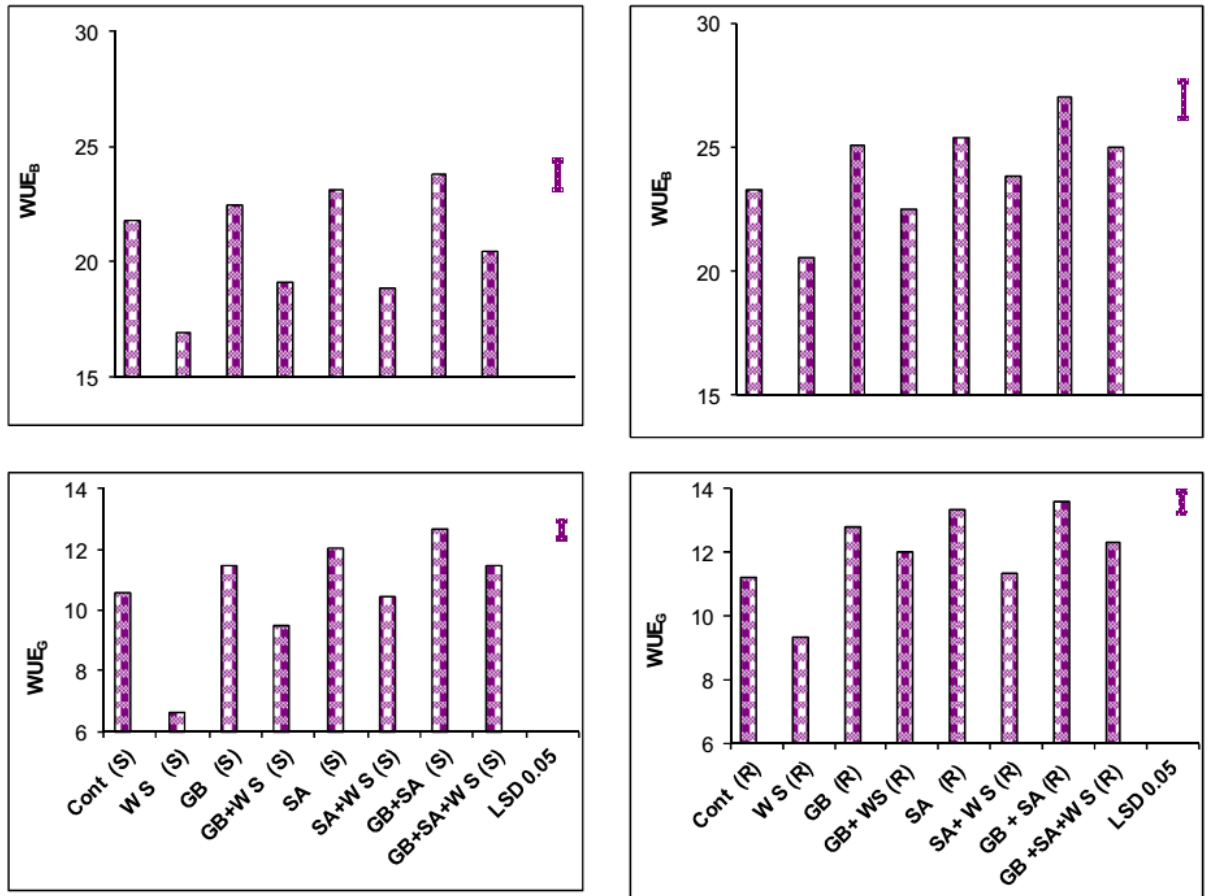
Figure 2a. Effect of glycine betaine, salicylic acid and their interaction on WUE_G and WUE_B of drought-stressed wheat cultivars. Vertical bars represent LSD values at P≤ 0.05.
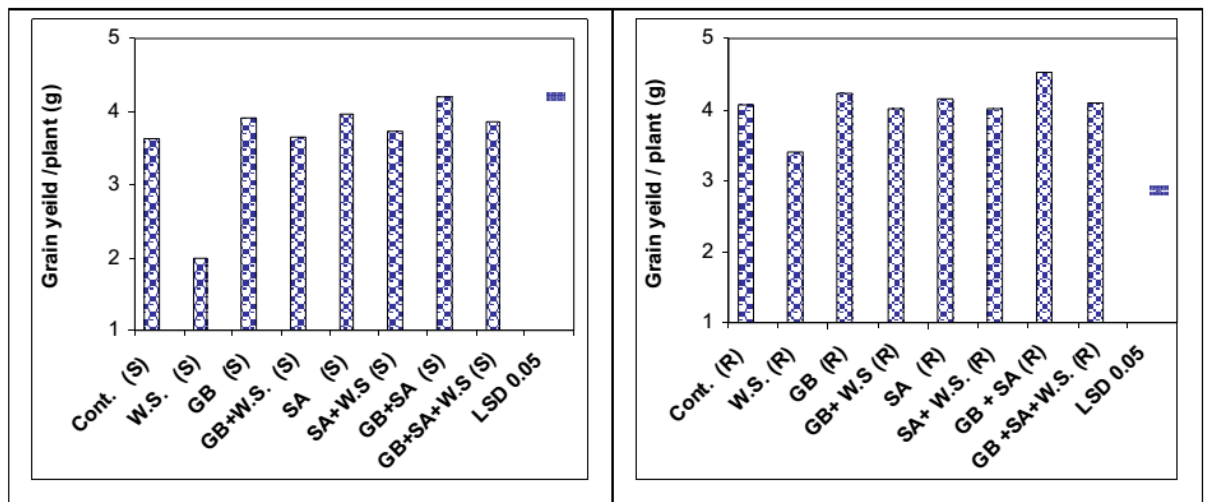
Figure 2b. Effect of glycine betaine, salicylic acid and their interaction on grain yield of wheat cultivars grown under water stress condition. Vertical bars represent LSD values at P≤ 0.05.

Table 1. Effect of glycine betaine, salicylic acid and their interaction on mean daily values of transpiration rate(mg g^{-1} f wt /hr) of drought-stressed wheat cultivars at the anthesis stage.

Wheat variety	Parameters / Treatments	Transpiration rate (mg g^{-1} f wt /hr) Mean daily values
Sensitive	Cont	452.7
	W S	323.5
	GB	372.0
	GB+WS	270.0
	SA	323.0
	SA+WS	197.0
	GB+SA	297.1
	GB+SA+WS	167.4
	LSD at p ≤ 0.05	**55.3**
Resistant	Cont.	380.4
	WS	250.2
	GB	324.9
	GB+ WS	212.2
	SA	296.6
	SA+ WS	154.8
	GB + SA	272.5
	GB +SA+WS	109.0
	LSD at p ≤ 0.05	**61.5**

Table 2. Effect of glycine betaine, salicylic acid and their interaction on stomatal area (μ^2) of flag leaf of drought-stressed wheat cultivars at anthesis stage.

Wheat variety	Parameters / Treatments	Stomatal area (μ2) Upper Epidermis						Lower Epidermis					
		7am	10am	1pm	4pm	7pm	Mean daily values	7am	10am	1pm	4pm	7pm	Mean daily values
Sensitive	Cont	559.9	622.6	647.3	602.3	513.5	589.1	525.7	547.8	575.3	532.8	463.9	529.1
	WS	441.6	484.7	502.9	467.2	404.6	460.2	397.7	446.7	477.8	426.7	368.7	423.5
	GB	504.2	542.8	569.4	528.4	456.6	520.3	442.5	491.6	528.2	480.9	436.2	475.9
	GB+WS	409.4	435.6	487.2	426.4	380.8	427.9	365.4	397.8	420.7	355.3	324.2	372.7
	SA	465.8	479.9	526.0	470.4	432.9	475.0	414.3	433.4	471.9	410.9	386.6	423.4
	SA+WS	379.0	420.3	455.6	382.0	329.5	393.3	350.5	362.1	389.4	336.1	316.1	350.8
	GB+SA	430.8	457.8	496.1	455.7	419.9	452.1	398.0	422.1	450.7	411.7	358.1	408.1
	GB+SA+WS	348.7	369.0	429.4	354.3	311.0	362.5	340.4	348.5	376.1	329.2	307.9	340.4
	LSD at p ≤ 0.05	**30.1**	**31.6**	**49.7**	**49.2**	**40.7**	**38.5**	**20.2**	**29.3**	**20.3**	**22.6**	**43.0**	**25.6**
Resistant	Cont.	515.1	605.9	631.9	540.6	485.1	555.7	483.3	597.6	615.9	552.3	465.0	542.8
	WS	418.3	454.0	488.1	418.3	383.6	432.4	401.2	438.7	469.1	419.1	343.9	414.4
	GB	483.6	517.1	546.2	477.9	430.9	491.1	471.7	479.8	507.0	458.8	421.7	467.8
	GB+ WS	372.6	431.4	459.0	358.7	322.3	388.8	333.2	378.3	445.6	343.3	304.1	360.9
	SA	449.3	470.2	518.4	450.0	433.7	464.3	406.7	424.8	448.7	421.0	355.4	411.3
	SA+ WS	348.6	396.3	451.8	365.3	324.8	377.4	335.1	358.2	376.2	341.1	296.1	341.3
	GB + SA	416.0	434.2	471.8	444.1	387.9	430.8	372.4	386.6	421.3	402.3	337.4	384.0
	GB +SA+WS	310.9	355.0	395.3	334.1	276.6	334.4	317.4	332.5	367.8	319.8	261.0	319.7
	LSD at p ≤ 0.05	**22.5**	**24.8**	**29.4**	**24.9**	**25.9**	**32.7**	**33.5**	**20.5**	**20.7**	**19.9**	**21.1**	**23.8**

Table 3. Effect of glycine betaine, salicylic acid and their interaction on the diurnal changes of relative water content (RWC %) of flag leaf of drought-stressed wheat cultivars at the anthesis stage.

Wheat variety	Parameters / Treatments	Relative water content %					
		7am	10am	1pm	4pm	7pm	Mean values
Sensitive	Cont	94.07	82.34	76.61	89.39	92.63	87.01
	W S	81.90	78.17	67.72	75.66	80.97	76.89
	GB	94.35	85.17	79.28	91.83	95.96	89.32
	GB+WS	85.00	80.20	75.99	81.28	85.27	81.55
	SA	96.07	87.14	81.97	92.38	95.58	90.63
	SA+WS	87.15	83.14	74.14	81.82	84.01	82.05
	GB+SA	97.21	88.22	82.51	93.81	96.17	91.58
	GB+SA+WS	88.25	83.13	77.22	82.12	85.54	83.25
	LSD at p≤ 0.05	1.43	2.66	2.40	2.35	2.61	2.31
Resistant	Cont.	96.05	85.35	78.74	91.20	95.60	89.39
	WS	85.32	80.90	74.79	78.37	83.49	80.57
	GB	96.94	86.24	80.35	91.70	97.11	90.47
	GB+ WS	89.28	83.77	77.17	81.56	87.73	83.90
	SA	98.23	87.85	83.94	93.04	96.41	91.89
	SA+ WS	88.16	84.58	77.68	83.46	84.47	83.67
	GB + SA	100.13	89.12	84.03	94.79	97.71	93.15
	GB +SA+WS	89.94	86.33	79.39	84.38	88.54	85.72
	LSD at p ≤ 0.05	1.24	1.73	1.60	1.79	1.92	1.01

Table 4. Effect of glycine betaine, salicylic acid and their interaction on the diurnal changes of saturation water deficit of flag leaf of droughted wheat cultivars at the anthesis stage.

Wheat variety	Parameters / Treatments	Saturation water deficit					
		7am	10am	1pm	4pm	7pm	Mean daily values
Sensitive	Cont	5.93	17.66	23.39	10.61	7.37	12.99
	W S	18.10	21.83	32.28	24.34	19.03	23.11
	GB	5.65	14.83	20.72	8.17	4.04	10.68
	GB+WS	15.00	19.80	24.01	18.72	14.73	18.45
	SA	3.93	12.86	18.03	7.62	4.42	9.37
	SA+WS	12.85	16.86	25.86	18.18	15.99	17.95
	GB+SA	2.79	11.78	17.49	6.19	3.83	8.42
	GB+SA+WS	11.75	16.87	22.78	17.88	14.46	16.75
	LSD at p ≤ 0.05	3.91	1.35	1.90	4.95	5.27	3.96
Resistant	Cont.	3.95	14.65	21.26	8.80	4.40	10.61
	WS	14.68	19.10	25.21	21.63	16.51	19.43
	GB	3.06	13.76	19.65	8.30	2.89	9.53
	GB+ WS	10.72	16.23	22.83	18.44	12.27	16.10
	SA	1.77	12.15	16.06	6.96	3.59	8.11
	SA+ WS	11.84	15.42	22.32	16.54	15.53	16.33
	GB + SA	1.00	10.88	15.97	5.21	2.29	7.07
	GB +SA+WS	10.06	13.67	20.61	15.62	11.46	14.28
	LSD at p ≤ 0.05	1.43	1.26	0.89	4.24	1.95	3.54

Table 5. Effect of glycine betaine, salicylic acid and their interaction on growth bioregulators ($\mu g \ g^{-1}$ f wt) of drought-stressed flag leaf of wheat cultivars at anthesis stage.

Wheat variety	Parameters / Treatments	Growth Inhibitor	Growth Promoters					
					Cytokinins			
		ABA	IAA	GA$_3$	Zeatin-riboside	Kinetin	Benzyl-adenine	Total
Sensitive	Cont	0.29	135.70	60.10	1.51	6.30	4.15	11.96
	WS	0.92	63.10	25.61	0.90	4.22	1.22	6.33
	GB	0.27	142.30	61.00	1.62	8.05	4.57	14.23
	GB+WS	0.49	82.80	48.62	1.23	6.34	2.17	9.74
	SA	0.21	169.50	60.40	1.54	7.13	4.18	12.84
	SA+WS	0.66	95.40	55.68	1.24	5.90	2.24	9.37
	GB+SA	0.18	182.60	73.53	1.70	8.64	5.21	15.54
	GB+SA+WS	0.50	127.40	60.31	1.49	6.87	4.13	12.49
	LSD at p ≤ 0.05	**0.07**	**4.81**	**2.07**	**0.10**	**0.43**	**0.16**	**0.47**
Resistant	Cont.	0.26	145.40	60.68	1.78	5.65	4.36	11.79
	WS	0.76	81.40	30.76	1.31	4.12	1.82	7.24
	GB	0.20	176.50	64.84	2.10	8.21	7.90	18.20
	GB+ WS	0.45	127.40	52.53	1.71	6.23	4.20	12.14
	SA	0.18	178.40	67.95	1.99	7.60	5.19	14.79
	SA+ WS	0.52	132.10	61.32	1.67	6.10	2.74	10.52
	GB + SA	0.14	223.50	75.61	2.48	8.36	9.06	19.89
	GB +SA+WS	0.43	142.30	64.35	1.82	6.33	4.38	12.54
	LSD at p ≤ 0.05	**0.04**	**6.78**	**2.27**	**0.17**	**0.38**	**0.76**	**0.74**

DISCUSSION

The results of this investigation clearly elucidated that drought markedly reduced (P≤0.05) the diurnal values of transpiration allover day time of both wheat cultivars. Consequently, there was also marked depletion in the mean daily values of transpiration. The reduction in transpiration rate appeared mainly to be due to firstly, transpiration rate could be reduced through the creation by leaf rolling (which minimized the leaf area subjected to the suns´ rays) occurred obviously in the sensitive cultivar than the resistant one and secondly, the obvious reduction in stomatal area on both upper and lower surfaces. Moreover, these results were in conformity with those obtained by El-Sharkawi and Salama (1975) who found reduced soil potential induced by either moisture deficiency or increased salinity reduced both transpiration rate and lay turgidity in wheat and barely plants during the day time and the reduction was furthered by increased water stress. Furthermore, Bieloria and Mendel (1969) conducted simultaneous measurements of apparent photosynthesis and transpiration rates and they found that these parameters decreased with decreased soil moisture.

Glycine betaine, salicylic acid or their interaction caused an additional decrease in the transpiration rates of both wheat cultivars. According to Ibrahim and Aldesuquy (2003), water stress remarkably reduced sorghum transpiration rate and total leaf conductance at both lower and upper leaf side. Application with GB, shikimic acid or their interaction caused additional decreases in the transpiration rates and total leaf conductance.

The reduction in stomatal aperture induced by water stress was mainly due to increasing in the

biosynthesis of ABA in wheat flag leaves. This hormone increases the turgor pressure of the whole plant by inducing stomatal closure. These results were in accordance with those obtained by Schroeder *et al.* (2001); Dorothea and Ramanjulu (2005). In addition, these data are consistent with the finding of Tardieu and Davies (1992) who observed diurnal variations in the sensitivity of maize stomata to ABA. They provide strong modifies sensitivity, with an increased stomatal sensitivity to ABA during the afternoon hours when leaf water potentials are low.

Treatments with GB, SA or their interaction caused an additional decrease in the transpiration rates and stomatal areas of both wheat cultivars. The effect was more pronounced with SA and GB+SA treatments. These results were in good agreement with those obtained by Agboma *et al.* (1997) who found that exogenous application of GB to soybean reduced transpiration rate, under all conditions, to 85% of untreated plants. In this respect, Aldesuquy *et al.* (1998) studied the effect of antitranspirants (sodium salicylate or ABA) and salinity on some water relations of wheat plants. They found that sodium salicylate or ABA appeared to regain the leaf turgidity of saline-treated plants by inducing additional decreases in transpiration rates through more reduction in stomatal pores on both upper and lower surfaces of leaf and therefore improves the water use efficiency.

The pattern of changes in leaf turgidity resulted from water stress condition appeared also to go in close parallelism with the changes in transpiration rates and stomatal areas. This indicates that there is a concrete relationship between the extent of stomatal opening, transpiration rate, and the leaf turgidity in wheat plants. The decrease in the leaf turgidity of droughted wheat plants may be due to

insufficient root system to compensate for the water lost by transpiration and/or unavailability of water in the soil. These results were in agreement with those obtained by (Lawlor and Cornic, 2002).

Application of GB, SA improves the RWC of the wheat plants grown under water stress conditions. These were the same result obtained by Aldesuquy *et al.* (1998) with wheat plants under the application of salinity and antitranspirants (sodium salicylate or ABA).

The WUE_G and WUE_B of the two wheat cultivars were greatly reduced by water stress and this was clear with the sensitive cultivars more than the resistant one. This was in agreement with Anyia and Herzog (2004) with cowpea subjected to water stress; Aldesuquy and Ibrahim (2001) with wheat plants under salinity. However, treatments with GB, SA or their interaction mitigated the effect of drought on WUE_G and WUE_B. In fact, WUE is given much attention as a physiological trait related to plant drought resistance. Especially, the molecular research regarding the enhancement of WUE playing a crucial part in the selection and cultivation of drought-resistant or drought-tolerant crop varieties. When breeding for drought tolerance, biomass productivity and WUE are considered as fundamental agronomic characteristics (Blum, 1993).

In fact, unfavorable environmental factors lead to sharp changes in the balance of growth hormones associated with not only the accumulation of ABA, but also with a decline in the level of the growth activating hormones IAA GA_3 and cytokinins (Jackson, 1997). These changes would result in a new endogenous hormone balance that would be favorable to the plant's response to drought. In the present investigation the water stress caused marked increases in the

ABA levels in the flag leaves of two wheat cultivars and this was pronounced with the sensitive one. In addition, such accumulation of ABA may be the reason of stomatal closure on both upper and lower leaves of the flag leaves. These results were in agreement with those obtained by Assmann *et al.* (2000) and Raphael *et al*. (2003). Whether, the stomatal closure system in plant leaves is abscisic acid (ABA)–dependent or –independent is unknown (Rao *et al*., 2006) but, stomatal closure in many plants is incomplete even after application of high concentrations of ABA (Mustilli *et al*., 2002). However, field-grown plants, woody plants and wild watermelon plants show almost complete stomatal closure and transpiration rates of almost zero during severe drought stress (Yokota *et al*., 2002).

During vegetative growth, ABA-mediated adaptive responses are critical to plant survival during drought, salt and cold stress (Dorothea and Ramanjulu, 2005). These stressors serve as a trigger for the accumulation of ABA, which in turn activates various stress-associated genes that are thought to function in the accumulation of osmoprotectants, (LEA) proteins, and signaling, transcriptional regulation.

Growth reduction under drought stress is mainly caused by a decrease in the concentration of IAA (Saugy and River 1988). Nevertheless, there are reports that there were no significant changes in IAA under drought stress (Li *et al.,* 2000) and that changes in IAA caused by drought have no significant regulatory function in the process of adaptation of a plant to drought. However, relatively little is known about the changes in plant endogenous GA_3 under water stress condition (Tian and Shao 2007). In this study, water stress showed some inhibitory effect on endogenous GA_3 in wheat flag leaves. It is consistent with the results obtained

in corn (Shi *et al.* 1994) and in apple leaves (Tian and Shao, 2007).

Water stress caused significant reduction in the cytokinins levels of the two wheat cultivars. The protective role of cytokinins is due to their regulatory effects on the renewal of disrupted cellular structures, the condition of the stomata, and de novo synthesis and activation of proteins that are required for increasing plant resistance to water stress (Chernyadèv, 2005). This is may probably be due to water stress restricts the transpiration rate by inducing stomatal closure and thus inhibit the rate of translocation of CK_s from root toward shoot. Furthermore, water stress decrease the rate of biosynthesis of CK_s within the root system of wheat plants (Blackman and Davies, 1985). In addition, many studies have investigated the role played by cytokinin in the communication process among root, stem, and leaf under water stress (Kaur *et al.*, 2000).

Our study showed that under water stress, ABA, IAA, GA_3, zeatin, kinetin, benzyl adenine concentration in flag leaves was significantly correlated with RWC, SWD, stomatal area, transpiration rate, WUE_G, WUE_B, and consequently grain yield indicating that growth hormones were involved in the plant water relationships and its productivity.

Exogenous spray with GB pre-sowing treatment with SA or their interaction may increase the drought tolerance of sensitive cultivar by acceleration of growth promoters (IAA, GA_3 and cytokinins) and at the same time the reduced accumulation of inhibitor represented by ABA in droughted plants. These result were in accord with those obtained by Sakhabutdinova *et al*. (2003) and Shakirova, *et al.* (2003) who stated that, SA prevented the drought-induced decline in

concentration of IAA and cytokinins in seedlings of wheat plants under sanity and reduced the accumulation of ABA. Maintaining a high level of ABA under stress conditions in plants pretreated with SA is important as ABA could play a regulating role in SA-induced unspecific plant resistance.

The present data showed that grain yield was reduced by water stress condition. In fact yield is a result of the integration reactions in the plants, consequently any factor that influences this metabolic activity at any period of plant growth can affect the yield (Ibrahim *et al.*, 1999). These were the same results obtained by Ali *et al*. (2007) with wheat plants under water stress. In this respect, Shao *et al.* (2008b) state that, seed yield and yield components are severely affected by water deficit. Water stress reduced the head diameter, 100-achene weight and yield per plant in sun flower (Blumwald *et al*., 2004). These authors also observed significant but negative correlation of head diameter with fresh root and shoot weight under water stress. A positive and significant relation was recorded between dry shoot weight and achene yield per plant.

The applied chemicals appeared to mitigate the effect of water stress on wheat yield and the effect was more pronounced with (GB+SA) treatment. This improvement would result from the beneficial effect of the provided chemicals on growth and metabolism of wheat plants under water deficit conditions. These results were in agreement with the results obtained by Iqbal *et al.* (2008). In addition, Arfan *et al*. (2007) investigated the improvement in growth and grain yield of wheat salt-tolerance due to SA application. Shakirova *et al*. (2003) reported that, the treatment of wheat plants with 0.05 mM salicylic acid (SA) increased the level of cell division within the apical meristem of

seedling roots causing an increase in plant growth and elevated wheat productivity.

In conclusion, application of GB (osmoregulator), SA (growth bioregulator and antitranspirant) or GB+SA improve the turgidity in flag leaf by reducing the transpiration rate of wheat plants grown under stress conditions through more reduction in stomatal pores (controlled by ABA) on upper and lower surfaces of flag leaves and thus decreasing the SWD, increase the RWC and consequently water use efficiency which control crop yield. Moreover, The grain yield appeared to be positively correlated with IAA, GA_3, CK, RWC, WUE_G and WUE_B but negatively correlated with ABA, SWD, transpiration rate and stomatal areas on both wheat cultivars.

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