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ORIGINAL ARTICLE

Does Glycine Betaine and Salicylic Acid Ameliorate the Negative Effect of Drought on Wheat by Regulating Osmotic Adjustment through Solutes Accumulation?

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A pot experiment was conducted to evaluate the beneficial effect of foliar application of glycine betaine (10mM), grain presoaking in salicylic acid (0.05 M) and their interaction on drought tolerance of two wheat (*Triticum aestivum* L.) cultivars (sensitive, Sakha 94 and resistant, Sakha 93). Osmotic pressure, some osmolytes concentration and grain yield were determined. Water stress caused an increase in osmotic pressure, proline, total soluble nitrogen, total soluble sugars, organic acids, ions (Na^+, K^+, Ca^{+2}, Mg^{+2} and Cl^-) content as well as Na^+/K^+ ratio in cell sap flag leaves of both wheat cultivars. The resistant variety had higher values of osmotic pressure, proline, organic acids and ions content than the sensitive one. On the other hand, water stress induced marked decrease ($P<0.05$) in grain yield. The applied chemicals mitigated the effect of water stress on the used wheat cultivars. The effect was more pronounced with glycine betaine + salicylic acid treatment. The applied chemicals increased the osmotic pressure, the osmolytes concentrations as well as the grain yield. Furthermore, the osmotic pressure of flag leaf sap appeared to depend on proline, TSN, TSS, organic acids and the ions content. The economic yield (grain yield) was positively correlated with proline, keto-acids and osmotic pressure but negatively correlated with TSN, TSS and citric acid.

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Key words: (Triticum aestivum L.)/drought/ glycine betaine/ osmolytes/salicylic acid/ yield

List of abbreviations: Glycine betaine = GB, Salicylic acid = SA, Water stress = WS

Osmotic adjustment is the main component of physiological machinery, by which plants respond to soil water deficits (Chaves *et al*., 2003). Plants with higher osmotic regulators can absorb water from soil water deficit condition. In wheat, many reports showed that wheat cultivars with higher K^+, proline, soluble sugar at different growth stages performed better drought resistance (Dhanda *et al*., 2004). Furthermore, osmotic adjustment results in an active accumulation of solutes within the plants in response to a lowering of soil water potential (WP) under water deficit (Turner and Jones, 1980). In general, OA is achieved by absorbing ions (e.g., K^+, Na^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^- , SO_4^- and HPO_4^-) or by accumulating organic solutes (e.g., free amino acids, sugar alcohols, quaternary ammonium compounds, and sugars) (Moinuddin *et al*., 2005). Waste water caused marked increases in OP, osmolytes [proline, organic acids, chloride and heavy metals (Cd^{++}, Pb^{++}, Cu^{++}, Ni^{++} & Zn^{++})] content in flag leaves of wheat plants at heading and anthesis stages. On the other hand, waste water stress induced marked decreases in total soluble nitrogen (TSN), total soluble sugars (TSS) and ions (Na^+, K^+, & Ca^{++}) as well as grain yield. The osmotic pressure appeared to depend mainly on proline, organic acids, chloride and heavy metals content, where there is positive correlations between OP and proline, organic acids, and heavy metals. The economic yield (grain yield) was positively correlated with TSN, TSS and ion contents but negatively correlated with proline, organic acids, chloride, heavy metals and OP (Aldesuquy *et al*., 2011).

Compatible osmolytes reported to be affected by drought stress include: proline, have improved plant tolerance to drought and salinity in a number of crops (Zhu *et al*., 2005). Carbohydrates, metallic ions, nitrogen and organic acids have been intensively studied in plants under drought conditions as osmolytes (Ashraf and Foolad, 2007; Lee *et al*., 2008). In addition, a positive relationship between osmotic adjustment OA and grain yield under water deficit has been shown in grain sorghum (Ibrahim *et al*., 1999), wheat (Blum *et al*., 1999), pea (Rodríguez-Maribona *et al*., 1992), and pigeonpea (Subbarao *et al.,* 2000). A significant increase in seed yield of a group of genotypes with high OA over that with low OA has also been reported under water deficit condition in different crops (Moinuddin and Chopra, 2004).

Among wheat genotypes, significant differences regarding OA capacity exist and are associated with differences in crop growth and grain yield when water deficits in the soil and atmosphere are large enough to cause substantial reductions in plant WPs (Moinuddin *et al*., 2005).

Glycine betaine (N,N,N-trimethyl glycine, GB), which is found in plants, animals, and bacteria (Prasad and Pardha-Saradhi, 2004) and many studies indicate that GB might play an important role in enhancing plant tolerance to some abiotic stresses such as salt, drought, and extreme temperatures (Quan *et al.*, 2004). The accumulation of endogenous GB is induced under stress conditions, and the levels are correlated with the extent of increased tolerance (Park *et al.*, 2004). Significant advances have been made in alleviating the effects of environmental stresses by exogenously applied glycine btaine in different crops, such as wheat (Sayed *et al*., 2007), rice (Rahman *et al*., 2002), sorghum (Ibrahim and Aldesuquy 2003; Ibrahim, 2004) as well as sunflower (Iqbal *et al*., 2008).

Salicylic acid has received much attention due to its role in plant responses to abiotic stresses such as ozone (Koch *et al.,* 2000), UV-B (Surplus *et al.,* 1998), heat stress (Senaratna *et al*., 2000; Clark *et al*., 2004) drought (Nemeth *et al*., 2002; Singh and Usha, 2003), oxidative stress (Shim *et al*., 2003), salt and osmotic stress (Khodary, 2004; El-Tayeb, 2005; Arfan *et al.,* 2007; Sawada *et al*., 2007), as well as the injurious action of heavy metals on rice plants (Mishra and Choudhuri, 1999); in sunflower (El-Tayeb *et al*., 2006).

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 osmolytes in relation to osmotic adjustment and grain yield of droughted wheat (*Triticum aestivum L.*) cultivars by determining osmotic pressure, some organic solutes, ions and grain yield.

MATERIALS AND METHODS

Plant material and growth conditions

Two wheat cultivars (*Triticum aestivum* L.) Sakha 94 (sensitive var.) and Sakha 93 (resistant var.) 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 (i. e. 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 the beginning of 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. At the end of stress periods, rewatering 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 osmotic pressure and other osmolytes at the end of each stress period before re-watering (i.e after 80 and 95 days from planting).

At the bud stage, 20 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).

Measurement of osmotic pressure

The osmotic pressure of flag leaf sap was measured by the cryoscopic method (Walter, 1949) and described by El-Sharkawi and Abdel-Rahman (1974).

Determination of total soluble sugars

Total soluble sugars was extracted and determined by anthrone method of Riazi *et al*.(1985) as modified by Ibrahim (1999).

Determination of the total Soluble Nitrogen

The total soluble nitrogen was determined by the conventional semimicromodification of Kjeldahl method (Pine, 1955).

Estimation of proline

The method adopted for estimation of proline was essentially that described by Bates *et al* (1973).

Determination of keto acids

Keto acids was determined according to the method adopted by Friedemann and Haugen (1943).

Determination of citric acid

The method adapted for estimation of citric acid was essentially that described by Snell and Snell (1949).

Determination of some mineral ions

The extracts of the experimental plants were analyzed for the cations: Na^+, K^+ and Ca^{+2} Mg^{+2} measured by flam emission spectrophotometery (Williams and Twine 1960) and the anions Cl^- chlorides were determined by the $AgNO_3$ titration method as described by Hansen and Munns (1988).

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 osmotic pressure

Water stress induced a noticeable increase ($P<0.05$) in osmotic pressure, as compared to control values, of wheat flag leaf of both cultivars at heading and anthesis stages. The resistant plants showed higher values osmotic pressure than the susceptible ones. Treatments with GB or SA caused significant increases ($P<0.05$) in the osmotic pressure of both wheat cultivars at heading and anthesis except GB with susceptible cultivar and SA with resistant one at heading stage. Furthermore, GB and SA treatment increased osmotic pressure more than the other treatments under controlled and stress conditions at heading and anthesis stages of both cultivars (Fig. 1a). The osmotic pressure appeared to be positively correlated with the osmolytes (proline, TSN, TSS, keto-acid and citric acid) and ions of the two wheat cultivars at heading (r =0.73-0.98) and at anthesis (r =0.49-0.79).

Changes in proline

As compared to the control values, water stress caused non-significant increases in proline concentration in wheat flag leaf of both cultivars at heading and anthesis stages. The proline level of the resistant cultivar was higher than the sensitive one. In general, foliar application of GB or grain presoaking in SA caused significant increase ($P<0.05$) the proline concentrations in flag leaf of both wheat cultivars at heading and anthesis. Plants treated with GB+SA had higher proline concentrations than those treated with GB or SA only at heading and anthesis of the two wheat cultivars under controlled and water stress conditions (Fig.1b).

Changes in total soluble nitrogen (TSN)

In relation to control values, water stress induced a noticeable increase ($P<0.05$) in total soluble nitrogen of wheat flag leaf of both cultivars at heading and anthesis stages, but the susceptible plants accumulated more soluble nitrogen than the resistant plants. In the majority of cases, treatments either with GB or SA caused additional increases in the TSN in flag leaf of both wheat varieties at heading and anthesis. Furthermore, GB and SA treatments increased ($P<0.05$) TSN concentration more than the other treatments under controlled and stress conditions of the two wheat varieties at heading and anthesis stages (Fig. 1c).

Changes in total soluble sugar (TSS)

Drought induced marked increases ($P<0.05$) in total soluble sugar of wheat flag leaf of both cultivars at heading and anthesis stages as compared to control values. It is clear that the susceptible plants accumulated more sugars than the resistant ones. In general, the applied chemicals GB or SA, increased ($P<0.05$) the TSS in flag leaf of both wheat cultivars at heading and anthesis under control and water stress conditions. In comparison to all treatments, the effect of GB+SA on TSS of wheat flag leaf of both cultivars was more effective (Fig. 1d)

Changes in keto-acids

In relation to control values, keto-acids accumulated in response to drought stress in wheat flag leaf of both cultivars at heading and anthesis stages and the resistant plants accumulated more keto-acids than the susceptible one. In general, GB or SA induced a marked increases ($P<0.05$) in the keto-acids concentration in flag leaf of both wheat varieties at heading and anthesis under control and water stress conditions. The effect was more pronounced with GB+SA treatment (Fig. 2a).

Changes in citric acid

Water stress caused a marked increase ($P<0.05$) in the citric acid in wheat flag leaf of both cultivars at heading and anthesis stages and the resistant plants accumulated more citric acid than the susceptible ones. GB, SA or their interaction caused a noticeable increases ($P<0.05$) in the citric acid concentration under water stress of both cultivars at heading and anthesis stages (Fig. 2b).

Changes in ions content

In relation to control values, water stress induced significant increases ($P<0.05$) in ions content (Na^+, K^+, Ca^{+2}, Mg^{+2}, and Cl^-) as well as Na^+/K^+ ratio in wheat flag leaf of both cultivars at heading and anthesis stages. On the other hand, the resistant plants accumulated more ions than the susceptible ones. In general, GB or SA caused an additional increase ($P<0.05$) in the ions content under controlled and water stress conditions and this effect was more pronounced with GB and SA treatment (Fig. 2c, 2d, 3a, 3b, 3c and 3d).

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. 4).

The economic yield (grain yield) appeared to be positively correlated with proline, keto-acids and osmotic pressure (r= 0.48-0.77) for the sensitive wheat variety and (r = 0.36-0.7) for the resistant one. However, grain yield was negatively correlated with total soluble nitrogen, total soluble sugars and citric acid (r = -0.08-0.32) for the sensitive wheat variety and (r = -0.18 -0.39) for the resistant one.

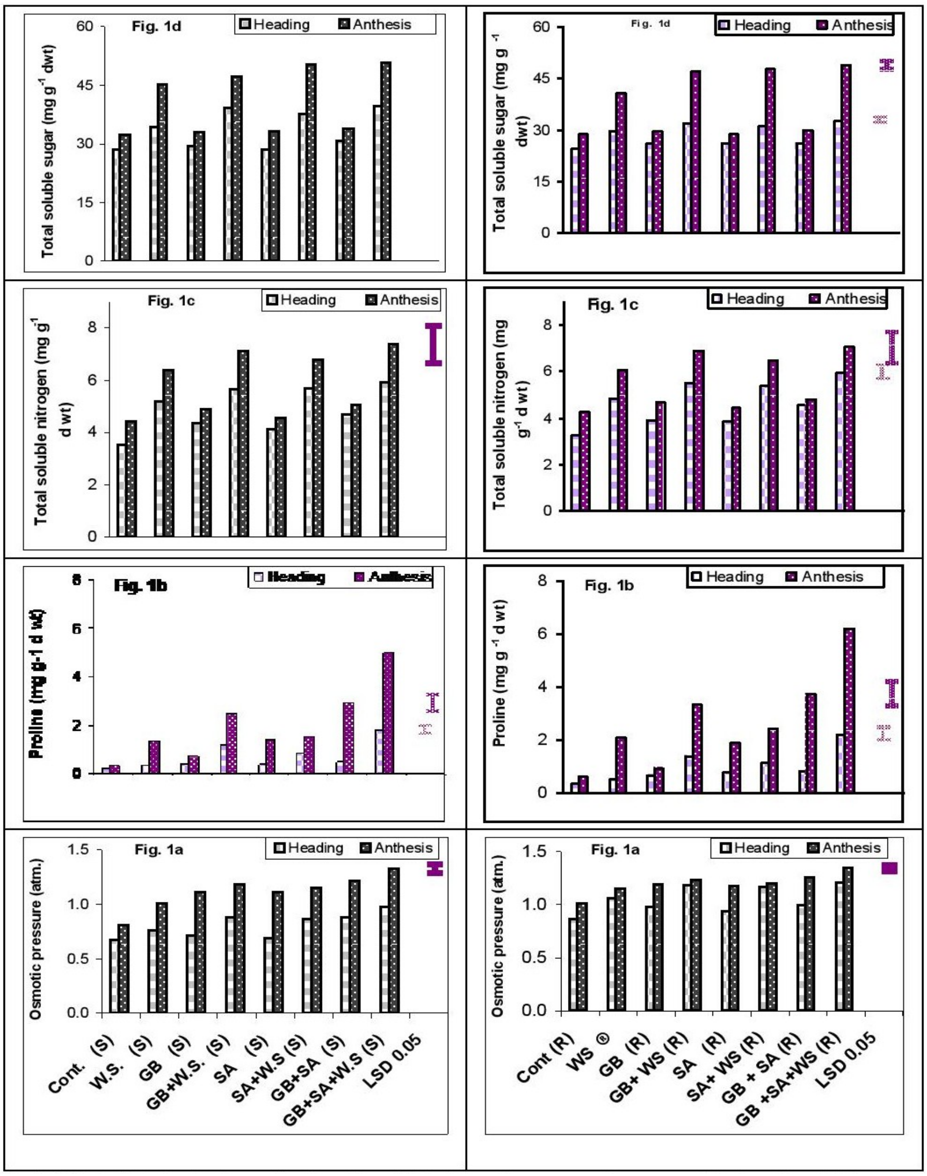

Figure 1. Effect of glycine betaine, salicylic acid and their interaction on osmotic pressure (atm.) proline, total soluble nitrogen and total soluble sugars (mg g^{-1} dwt) in flag leaf sap of stressed wheat cultivars at heading and anthesis. Vertical bars represent LSD values at P< 0.05.

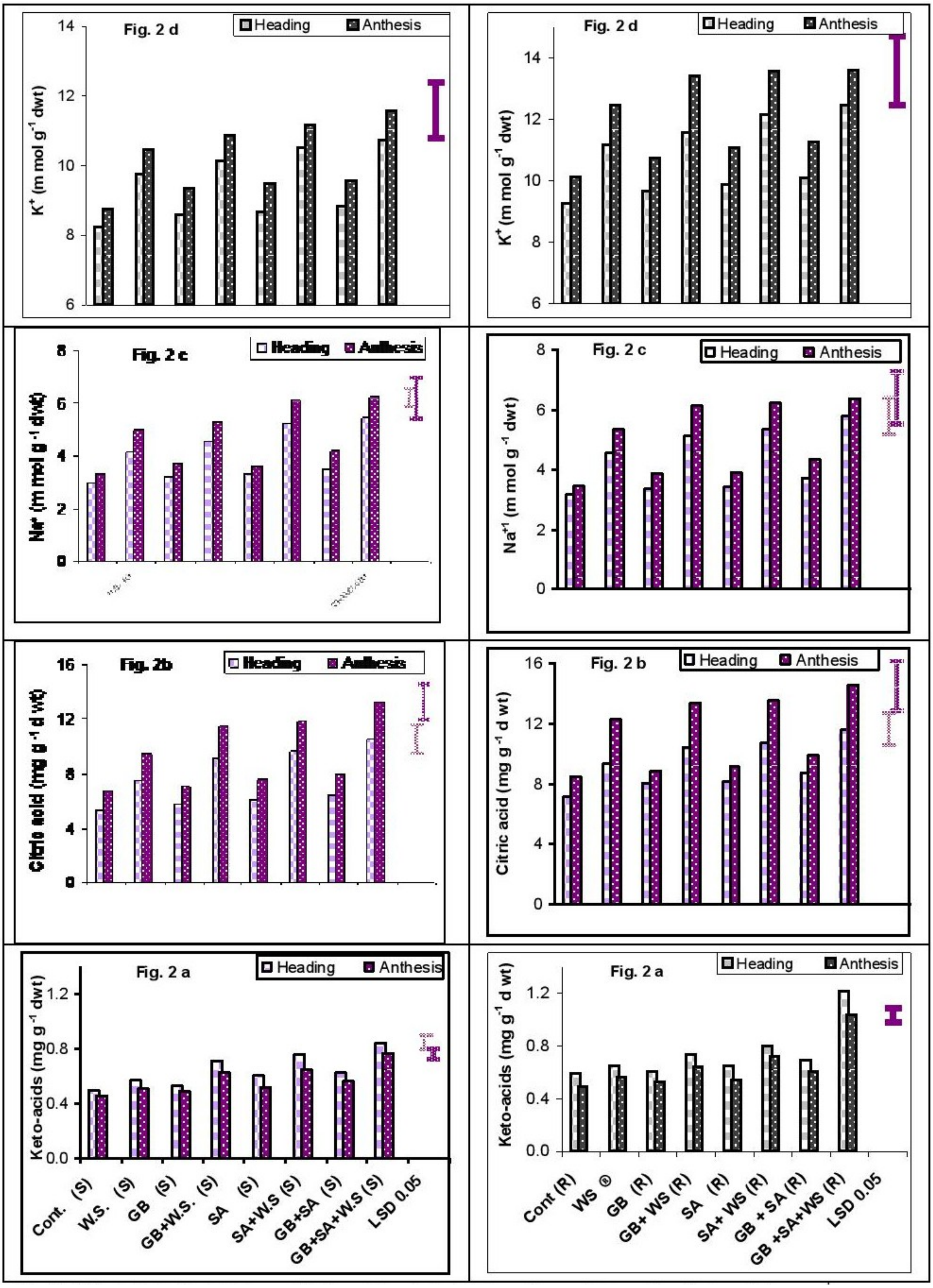

Figure 2. Effect of glycine betaine, salicylic acid and their interaction on keto-acids, citric acid (mg g^{-1} dwt), Na^{+} and K^{+} (mmol g^{-1} dwt) in flag leaf sap of water stressed wheat cultivars at heading and anthesis. Vertical bars represent LSD values at P< 0.05.

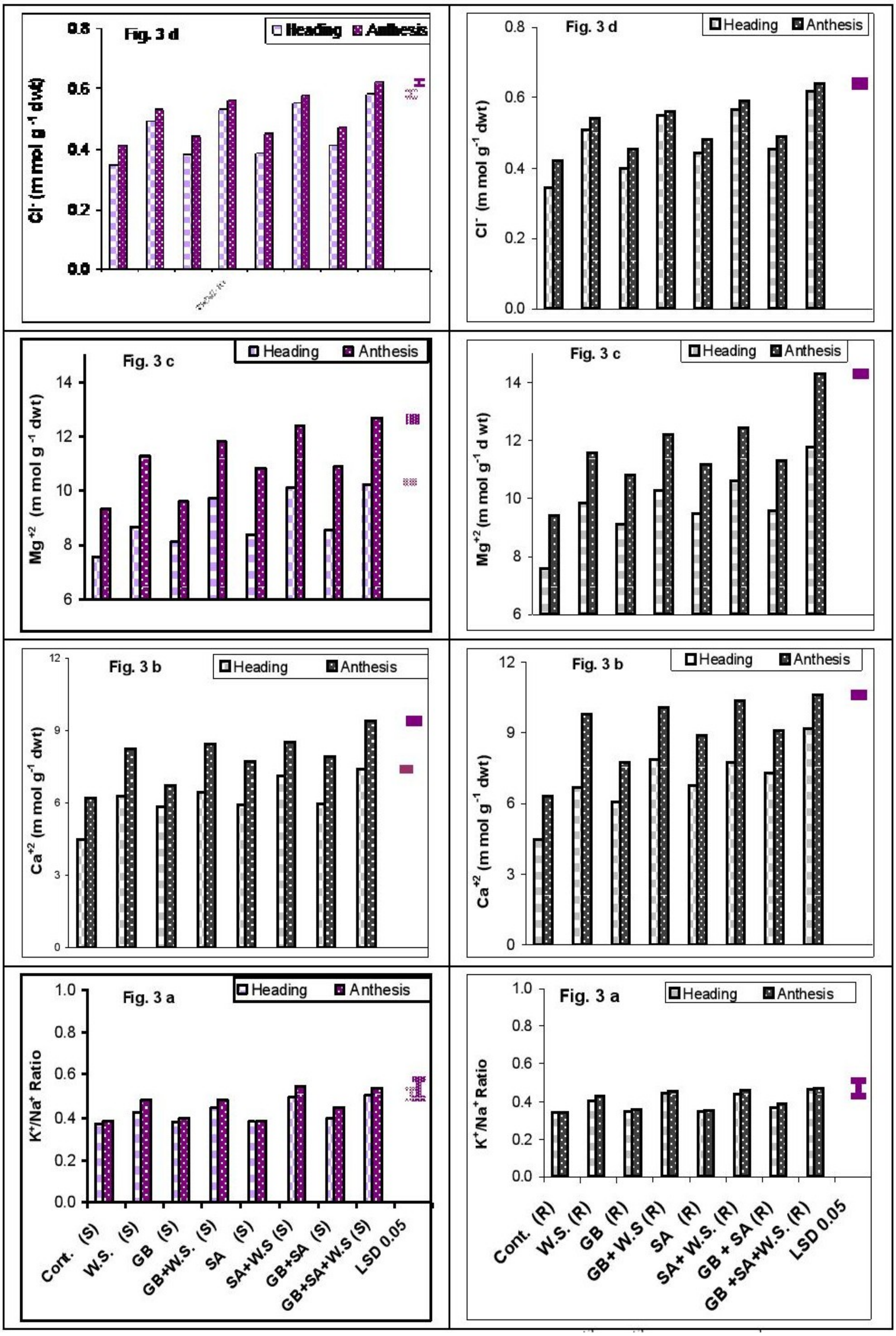

Figure 3. Effect of glycine betaine, salicylic acid and their interaction on Ca^{+2}, Mg^{+2}, Cl^{-} (m mol g^{-1}dwt) and Na^{+}/K^{+} in flag leaf sap of water stressed wheat cultivars at heading and anthesis. Vertical bars represent LSD values at $P< 0.05$.

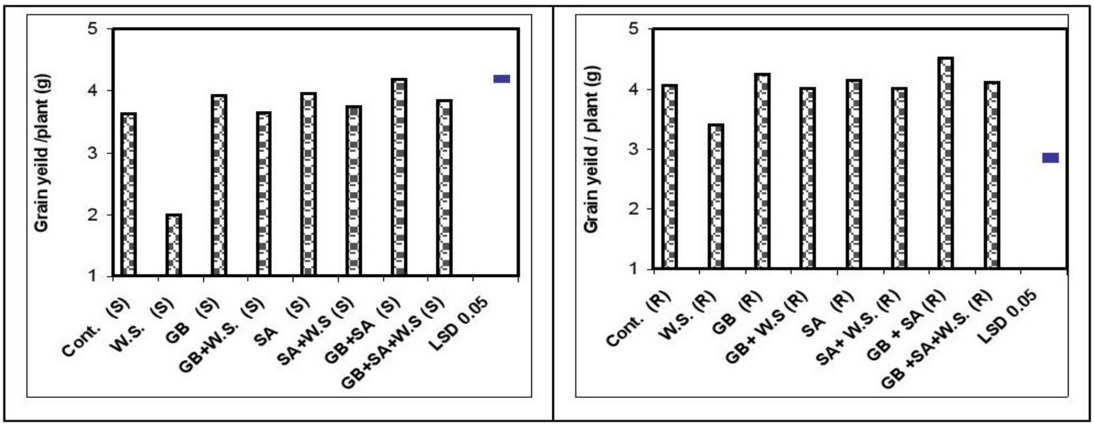

Figure 4. Effect of glycine betaine, salicylic acid and their interaction on grain yield of wheat cultivars grown under water stress condition. Vertical bars represent LSD values at P< 0.05.

DISCUSSION

In order to understand the physiological adaptation of *Triticum aestivum* to stress induced by water stress osmotic pressure, proline, TSN, TSS, organic acids and ions content in flag leaf sap were studied particularly after ear emergence (at heading) and at the beginning of the grain set (at anthesis). Thus, the results showed that water stress induced a marked increase in osmotic pressure. This is probably due to the increasing in proline, TSN, TSS, organic acids and ions content. Furthermore, osmotic pressure appeared to be positively correlated with organic osmolytes as well as inorganic acids. In accordance to these results, Bulm (1996) concluded that osmolytes accumulation (OA) in plant cells results in a decrease of the cell osmotic potential and thus in maintenance of water absorption and cell turgor pressure, which might contribute to sustain physiological processes, such as stomatal opening, photosynthesis, and growth expansion. Furthermore, occurrence of OA at sensitive crop reproductive stages has been reported to play a constructive role against floral abortion (Wright *et al*., 1983), which results in maintaining grain number under water deficit (Moinuddin and Chopra, 2004). Additionally, OA has also been claimed to facilitate a better translocation of preanthesis carbohydrate reserves to the grain during the grain-filling period (Subbarao *et al.,* 2000).

Solutes accumulation in plant cells creates an intracellular osmotic potential which in the presence of rigid cell wall generates turgor pressure. Maintaince of turgor is necessary for maintance of growth through cell elongation (Yancey *et al*., 1982). Moreover, The accumulation of these solutes may not be important for osmotic stress tolerance but the metabolic pathways may have adaptive value (Hasegawa *et al.*, 2000). A further hypothesis is that compatible solutes are also involved in scavenging reactive oxygen species (Chen and Murata, 2002).

Aldesuquy *et al*. (2012) reported that seawater concentrations (10, 25%) caused noticeable increase in osmotic adjustment, organic solutes (TSS, TSN, proline, organic acids and glycerol) and inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^-) in two wheat cultivars. On the other hand, clear reduction in K/ Na ratio in the flag leaves of both cultivars was observed. The capacity of osmotic adjustment was greater in younger leaves than in older ones particularly with higher concentration (25%) in both cultivars. Osmotic pressure of flag leaf sap appeared to depend mainly on proline, TSN, TSS, organic acids, glycerol and ions content, where there is a positive correlation between osmotic pressure and all of them.

The present results indicated that GB, SA or their interaction increased the measured osmotic pressure and osmolytes concentration (proline, total soluble nitrogen, total soluble sugars, ions content (Na^+, K^+, Mg^{+2}, Ca^{+2} and Cl^-) as well as Na^+/K^+ in wheat plants subjected to water deficit. In fact, GB added more increase to wheat osmotic pressure and these results are similar to those obtained by Ibrahim (2004) with sorghum plants grown under salinity stress. In this respect, Iqbal *et al*. (2008) found that water stress significantly decreased leaf water contents, osmotic and turgor potentials in two sunflower lines and foliar application of GB at the vegetative or reproductive growth stage increased leaf water and turgor potentials to some extent in both sunflower lines when grown under water stress. Moreover, Sulian *et al.* (2007) suggested that GB may not only protect the integrity of the cell membrane from drought stress damage, but also be involved in OA in transgenic cotton plants. Salicylic acid caused an additional increase in wheat osmotic pressure as well as the studied osmolytes and these are the same results obtained by Chinnusamy and Zhu (2003).

Proline content of the flag leaves increased at both stages of growth in both wheat cultivars under water stress condition. The accumulation of proline, primarily in the cytosol, often occurs in plants under stress with strong correlation between stress tolerance and proline accumulation, but the relationship is not universal and may be species dependent (Ashraf and Foolad, 2007). There is other roles were proposed for proline besides osmotic adjustment in stressed plants include acting as hydroxyl scavenger, stabilization of membrane and protein structure, as a sink for carbon and nitrogen for stress recovery, and buffering cellular redox potential under stress (Lee *et al*., 2008). Moreover, high levels of proline enabled the plant to maintain low water potentials (Sankar *et al.*, 2007). By lowering water potentials, the accumulation of compatible osmolytes, involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism (Kumar *et al*., 2003).

It is important to note that proline accumulated with GB application in the two wheat cultivars under water stress compared to drought-stressed plants alone suggests that proline accumulation together with GB in leaves presumably decreased the extent of drought-induced damage even more than drought-stressed group alone. The protective role of GB was more pronounced in the sensitive cv. Therefore, exogenous GB application might be a useful method to improve growth and productivity of higher plants and retard aging process under drought-stressed conditions.

An increase in proline concentration in SA-treated plants under normal and stress conditions was also observed. Proline can thus be considered as an important component in the spectra of SA-induced ABA-mediated protective reactions of wheat plants in response to drought, contributing to a reduction in the injurious effects of drought and an acceleration of the reparation processes following stress, evidencing the protective action of SA on wheat plants (Shakirova, 2003). .

In the present investigation the TSN accumulated in response to water stress conditions in both wheat cultivars. Such accumulation may be mainly resulted from a sharp increase in total free amino acids, total soluble proteins and glycine betaine (Ibrahim, 2004). This change in nitrogen content may be related to the inhibition of translocation from root to shoot, inhibition of protein synthesis or the increase in protease activity (Khalil and Mandurah, 1990). The increase in the soluble nitrogen compounds are of importance in plant osmoregulation in response to water deficit. The application of GB, SA or their interaction caused an accumulation in TSN in both wheat cultivars at heading and anthesis.

Our findings indicate that water stress increased TSS in both wheat cultivars. This may result from increased starch hydrolysis, synthesis by other pathways or decreased conversion to other products. Several studies have shown an increase in amylase activity in water-stressed leaves (e.g. Keller and Ludlow, 1993). Alternatively, increased translocation of carbohydrates into leaves or a decrease in translocation of carbohydrates from leaves could also contribute to the observed sugar accumulation. In this respect Xue *et al*. (2008) found that water deficit in wheat leaves caused a reduction in photosynthesis and high demands for osmolyte synthesis especially total soluble sugars.

The marked increase in the content of soluble sugars in flag leaf sap of stressed and nonstressed plants treated with GB, SA or their interaction agrees with the results obtained by El Tayeb (2005) who recorded an additional increase in Na, soluble proteins and soluble sugars in salt-stressed barley grains due to application of SA. SA treatments increased K^+/Na^+ ratio in the plant leaves under drought condition.

Organic acid content (citric acid and keto-acids) were increased in both wheat cultivars after withholding water. The increase in organic acids content may be a result of drought induced synthesis (Venekamp *et al.*, 1989) and it is important for plant osmotic adjustment under water stress (Morgan, 1984), and regulation of pH of plant cells (Venekamp *et al*., 1989). These results were in a good conformity with those obtained by many others who recorded the increase in organic acids in response to water stress (Ibrahim *et al*., 1999) and salinity (Shi and Sheng, 2005). Also, Hasaneen *et al*., (1990) found that α-ketoglutaric and some carboxylic acids of Krebs cycle increased in *Zea mays* seedlings and plants in response to salinity stress.

The application of GB, SA or their interaction caused an accumulation in organic acids in both wheat cultivars at heading and anthesis. The dramatic increase in the organic acids in response to GB, SA or their interaction may probably be due to the importance of organic acids in plants osmotic adjustment under water stress (Morgan,1984). Moreover, increased concentration of organic acids may involve in oxidative respiration originate from enhanced synthesis induced by dehydration and are directly linked to the proline synthesis pathway, a mechanism which control the cytoplasmic pH level (Venekamp *et al*., 1989).

An important results of the present study is that water stress, in the absence of salinity in the root zone, induced a conspicuous increase in the flag leaves ions (Na^+, K^+, Mg^{+2}, Ca^{+2} and Cl^-) as well as Na^+/K^+ concentrations. The effects of both stresses (water and salt stress) are not strictly additive in reducing plant performance and that tolerances to water and salt stress are linked through a common mechanism of Na uptake for osmotic adjustment (Glenn and Brown, 1998). A plant in drying soils is exposed to increasing levels of both water deficit and osmotic stress because the soil matrix potential decreases simultaneously with decreasing soil moisture. Even if osmotic adjustment occurs, a decrease in the hydraulic conductivity of root membrane is observed and it has been clearly demonstrated that the impact of Na^+ on water-channel function is not due to its osmotic effect (Carvajal *et al*., 2000).

Another role of Na in C_3 species such as wheat is related to its involvement in photosynthesis (Brownell and Bielig, 1996). An increased sodium concentration in plants experiencing water stress may be related to an increase in the metabolic requirement of sodium to sustain photosynthesis in these conditions. Sodium has been reported to be involved in the maintenance of mesophyll chloroplast structure, mainly in relation to granal stacking (Brownell and Bielig, 1996), and thus sodium deficient C_3 plants exhibit a wide range of chlorophyll a fluorescence perturbations (Grof *et al*., 1986). Sodium may also be involved in the regeneration of phosphoenolpyruvate in mesophyll chloroplasts (Murata *et al*. 1992) because a Na^+ gradient across the envelope could be an alternative energy source for the active transport of pyruvate (Ohnishi *et al*., 1990). An increase in sodium uptake could then be the consequence of a stress-induced decrease in the efficiency of the Na^+/pyruvate co transport-system. This confirms the previous findings of Martınez *et al*. (2003) who reported that drought-induced specific increase in Na^+ concentration in two populations of *Atriplex halimus* under water stress.

The application of GB, SA or their interaction caused an accumulation in Na^+ in both wheat cultivars at heading and anthesis. This is in agreement with Ibrahim (2004) who studied the efficacy of exogenous glycine betaine application on sorghum plants grown under salinity stress. Salinity reduced sorghum growth. Foliar application of glycine betaine at 75 mM mitigated the adverse effects of salinity. The measured osmotic pressure and solutes concentration (Na, K, Ca, Mg, total soluble sugar and betaine) increased while Na^+/K^+ ratio decreased in sorghum plants. Glycine betaine added more increase to sorghum osmotic pressure and increased the concentration of soluble sugars

Potassium content was increased to some extent in both wheat cultivars at heading and anthesis, higher K^+

concentrations, effectively stabilize native protein (Borowitzka, 1981).This had been observed by (Ibrahim, 1999). Moreover, the drought resistant cultivar had a higher K^+ concentrations in the face of drought stress more than the sensitive variety and this reflects the important role of this cation in wheat plants adaptation to water stress conditions.

The application of GB, SA or their interaction caused an accumulation in K^+ in both wheat cultivars at heading and anthesis. In this respect, Günes *et al.* (2005) demonstrated that SA treatments caused N accumulation in plants and increased P, K, Mg and Mn concentrations under stress conditions. On the other hand Hamada and Al-Hakimi (2001) observed positive effects of SA in the Na, K, Ca and Mg content of wheat plants grown under salinity. SA application inhibited Na accumulation in salinity condition. Thus seed pretreatment with SA induced a reduction in sodium absorption and toxicity, which was further reflected in low membranes injury, high water content and dry matter production.

Our data showed, that calcium ion accumulated in response to water stress. Ca^{2+} plays important roles in plant responses to drought resistance. Nayyar (2003) also found in wheat that Ca^{2+} appeared to reduce the devastating effects of stress by elevating the content of proline and glycine betaine, thus improving the water status and growth of seedlings and minimizing the injury to membranes. In fact, Calcium ion has unique properties and universal ability to transmit diverse signals that trigger primary physiological actions in cells in response to hormones, pathogens, light, gravity, and stress factors. Being a second messenger of paramount significance, calcium is required at almost all stages of plant growth and development, playing a fundamental role in regulating polar growth of cells and tissues and participating in plant adaptation to various stress factors. Many researches showed that calcium signals decoding elements are involved in ABA-induced stomatal closure and plant adaptation to drought, cold, salt and other abiotic stresses and some new studies show that Ca^{2+} is dissolved in water in the apoplast and transported primarily from root to shoot through the transpiration stream (Song *et al*., 2008).

Glycine betaine, salicylic acid or their interaction treatments added more increases in Ca^{+2} content in wheat cultivars. These results were in a good agreement with Sayed *et al*. (2007) who reported higher increases in GB, K^+, Ca^{+2} and Na^+/K^+ ratio in two cultivars of wheat (tolerance and salt sensitive) treated with GB under salt stress. High accumulation of GB and K^+ mainly contributed to osmotic adjustment, which is one of the factors known to be responsible for improving growth and yield under salt stress.

Magnesium content was increased to some extent in both wheat varieties at heading and anthesis, this increase was also demonstrated by Ibrahim (1999) who reported that drought stress favors the accumulation of Mg^{+2} in plants. On the other hand the applied chemicals added more increase in the Mg^{+2} of wheat cultivars. These are the same result obtained by Ibrahim (2004).

Perusal 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.* (2008) 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. Reddy *et al*. (1998) supported the view that water stress for more than 12 days at the grain-filling and flowering stage of sunflower (grown in sandy loam soil) was most damaging and reduced the achene yield.

In this respect, Morgan (1995) reported comparisons of wheat grain yield under drought for low and high osmotic-adjusting lines. Among the nine comparisons presented, only three pairs had a significantly greater yield in high osmotic adjustment lines, and these were limited to cases of severe water deficits and very low grain yield. Three other comparison pairs had small non-significant advantage for high osmotic-adjusting lines, and three pairs had lower although non-significant yield for the high osmotic-adjusting group. Furthermore, Moinuddin *et al*. (2005) investigated the substantial differences in OA among the bread wheat genotypes. A high positive correlation ($P<0.001$) between OA of greenhouse-stressed plants and the grain yield recorded in the field under water deficit indicates that improved bread wheat cultivars could be successfully screened for drought environments in the greenhouse on the basis of OA. Besides, a significant ($P<0.05$) correlation between OA and grain yield under simulated water deficit condition in the field further advocates that OA could be regarded as a selection criterion for screening improved bread wheat genotypes for water deficit environments. Moreover, this study also indicates that OA is a heritable character and plays an important role in maintaining yield stability under water deficit.

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 an elevated wheat productivity.

Finally, we can conclude that the interactive effect of glycine betaine + salicylic acid represent the most effective treatment in improvement the drought tolerance of sensitive wheat cultivars by increasing the osmolytes and consequently increase the osmotic adjustment and grain yield.

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