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Glycinebetaine applied under drought improved the physiological efficiency of wheat (*Triticum aestivum* L.) plant

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Abstract

Study to find out the response of wheat (Triticum aestivum L.) cultivars to exogenous application of 100 mM glycinebetaine (GB) at different growth stages (vegetative, flowering and grain filling) was carried out under water limited environment, at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad during 2008-09, to find out the best GB application stage for improvement in drought tolerance potential. The wire house experiment was laid out in completely randomized design. Data regarding various physiological and biochemical parameters of crop were recorded using standard procedures. The data so collected were analyzed statistically by using the Fisher's analysis of variance technique and LSD at 5% probability was used to compare the differences among treatment means. Drought stress at all three critical growth stages adversely affected plant's nutrient uptake and it also reduced the net photosynthesis rate (Pn) and transpiration rate (E) of wheat plant. Exogenous application of GB under drought at all three critical growth stages improved tolerance of wheat by reducing toxic nutrient's uptake however, grain filling stage was found more responsive.

Key word: Drought, wheat, glycinebetaine, photosynthesis, transpiration

Introduction

Of various abiotic factors, water scarcity adversely affects the crop productivity (Jones and Corlett, 1992). Generally, drought stress reduces growth (Levitt, 1980) and yield of various crops (Dhillon *et al*., 1995) by decreasing chlorophyll pigments, photosynthetic rate (Asada, 1999), stomatal conductance and transpiration rates (Lawlor, 1995). However, it is now well evident that drought stressed plants exhibit various physiological, biochemical and molecular changes to thrive under drought stress (Arora *et al*., 2002). Water stress reduces crop yield regardless of the growth stage at which it occurs in wheat. Arid and semi arid environments besides other factors may induce water stress during crop growth and development, resulting in a reduction in crop yield (Ashraf *et al*., 1995). Drought stress, becoming the most widespread, adversely affects the plant growth and yield of a crop (Ashraf, 1994; Farooq *et al*., 2008). Reduction in productivity and impaired crop growth are caused when plants suffer from drought stress (Farooq *et al*., 2008). Plants are more tolerant to water deficit which have greater ability to accumulate GB (Monyo *et al.,* 1992). Plants treated with 100 mM glycinebetaine had a higher net photosynthetic rate during drought stress than non-GB treated plants. Glycinebetaine -treated plants also maintain higher anti-oxidative enzyme activities and face low oxidative stress (Ma *et al*., 2006).

Glycinebetaine applied exogenously can improve the resistance of numerous plant species to various types of abiotic stress, and it also enhanced subsequent growth and yield. Each level of GB has different intensity of its effect on the plants. Leaves of plants (old or younger) have higher GB accumulation under stressed environment (Agboma *et al.*, 1997).

As explained by Yang and Lu (2005), CO_2 assimilation rate increased in stressed plants under low GB concentration (from 2 to 20 mM) and maize plants grew normally. Increased stomatal conductance (due to high concentration of GB), decreased the CO_2 assimilation and growth also decreased. Observed by Sulian *et al*. (2007) in cotton plants, GB is also engaged in the osmotic adjustment. Studies of Agboma *et al*. (1997) showed that GB improved the tolerance of *Nicotiana tabacum*, *Zea mays*, and *Glycine max* under water stress. So, the present work was designed to observe the possible role of GB spray on wheat under drought at different growth stages in ameliorating the adverse effects of drought; in terms of photosynthesis and transpiration rate and nutrients' uptake.

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Materials and Methods

The experiment was carried out during 2008-09 in pots (wire house) at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan (latitude = 31° N, longitude = 73° E, and an altitude of 184.4 meters above the sea level). Physicochemical analysis of the experimental soil showed that it contained; sand 22%, silt 13%, clay 65%, organic matter 0.83%, nitrogen 0.33 (mg kg^{-1} dry soil), phosphorous 4.9 (mg kg^{-1} dry soil), potassium 128 (mg kg^{-1} dry soil), calcium 101 (mg kg^{-1} dry soil) and soil pH was 7.7.

Glycinebetaine was applied by creating drought at three critical growth stages; Zadoks GS 22, GS 60 and GS 73, representing tillering, flower initiation and milking, respectively on two wheat cultivars; Lasani-2008 (drought resistant) and Auqab-2000 (drought sensitive) selected from the screening experiment to find out the most resistant and sensitive variety. Screening of different crop plants to abiotic stresses is used to find out most resistant variety (Zafar-ul-Hye *et al.*, 2007), the germination test of seed may be useful, but these genetic differences may not be related to subsequent growth of seedling and seed yield (Ibrahim *et al.*, 2007). The experiment consisted of two wheat cultivars (Lasani-2008 and Auqab-2000) and seven GB application/drought induction schedules viz., T_0 (no drought and no GB spray), T_1 (drought at tillering stage without GB spray), T_2 (drought at tillering stage with GB spray), T_3 (drought at flower initiation stage without GB spray), T_4 (drought at flower initiation stage with GB spray), T_5 (drought at milking stage without GB spray) and T_6 (drought at milking stage with GB spray). Ten seeds were sown per pot, each containing 7 kg dry soil. After 14 days of germination, plants were thinned to four plants per pot. Drought stress was created by withholding irrigation at different growth stages (as per treatment) and then GB at 100 mM was sprayed with carboxymethyl cellulose (5% solution) as a sticking agent, whereas Tween-20 (0.1% solution) was used as a surfactant for foliar spray. Soil was used as a growth medium and soil sample was collected before filling the pots. The experiment was laid out in completely randomized design (CRD) with factorial arrangement and replicated thrice.

Gas exchange was measured on the flag leaves of fully-grown stressed and unstressed of the main tiller of three plants per pot using a portable IRGA (Infra Red Gas Analyzers). The traits reported here are net photosynthetic rate (Pn) and transpiration rate (E). Measurements were performed during day time (between 08:00 and 10:00). After harvesting, at maturity, the plant's shoot material with leaves was dried at 70 °C till constant weight in an oven and ground in a Wiley micro mill, so to pass through 2 mm sieve. The dried ground material (0.5 g) was digested in sulphuric acid and hydrogen peroxide (Wolf, 1982). The digested samples were run on flame photometer. A graded series of standards (ranging from 10-100 ppm) of Na, K and Ca was prepared and standard curves were drawn. The values of Na, K and Ca from flame photometer were compared separately for standard curve and total quantities were computed. Nitrogen was estimated by micro–Kjeldhal's method (Bremner, 1965). The phosphorus (P) was analyzed by spectrophotometer. The collected data were analyzed by using Fisher's analysis of variance technique and LSD test at 5% probability was used to compare the differences among treatments' means (Steel *et al.*, 1997).

Results

Contrasts (drought vs no drought) made it clear that drought significantly affected all physiological and biochemical parameters of wheat (Table 1) and glycinebetaine application under drought improved most of these parameters. The analyzed data regarding nitrogen, phosphorous, potassium, calcium and sodium uptake (Table 1) and photosynthesis and transpiration rate (Figure 1) indicate that both the varieties Lasani-2008 (drought resistant) and Auqab-2000 (drought sensitive) showed similar behavior to the GB application. The foliar application of GB on wheat cultivars improved the drought tolerance in the plants. Among drought treatments, maximum improvement in all the recorded growth, yield and gas exchange parameters was achieved when GB was applied at grain filling stage (T_6) than other critical stages. Although crop gained maximum value for all the parameters in control treatment (no drought), however it was at par with the treatment where crop faced drought at grain filling stage but GB was applied at these stages. Nutrient uptake is major factor contributing to the final yield of the crop. Well watered plants (T_0) produced highest phosphorous and calcium uptake while produced lowest nitrogen, potassium and sodium uptake (Table 1). Drought created at any stage (T_1, T_3 and T_5) significantly reduced P and Ca; whilst increased the N, K and Na uptake. However, comparison of T_1 vs T_2, T_3 vs T_4 and T_5 vs T_6 indicated that GB at any critical crop growth stage significantly increased wheat P and Ca uptake and reduced N, K and Na uptake. Comparing the efficiency of GB spray at different growth stages (T_2 vs T_4 vs T_6) indicated that nutrient uptake was affected maximum when GB was applied under stress at grain filling stage (T_6).

Discussion

Drought stress significantly affected crop growth and development by affecting physiological and biochemical

Table 1: Effect of drought and glycinebetaine spray on nutrient uptake in wheat

Treatment	N uptake	P uptake	K uptake	Ca uptake	Na uptake
	mg g^{-1}				
V_1 (Lasani-2008)	0.038	1.00	6.16 b	2.30 b	8.98
V_2 (Auqab-2000)	0.037	0.98	7.19 a	2.65 a	9.60
LSD (5%)	-	-	0.82	0.27	-
T_0 = Control (No drought and no GB spray)	0.023 e	1.83 a	5.43 b	3.00 a	7.49 c
T_1 = Drought at tillering without GB spray	0.046 c	0.83 cd	5.85 b	1.87 d	8.84 b
T_2 = Drought at tillering with GB spray	0.030 d	0.93 bc	7.46 a	2.32 bc	8.76 b
T_3 = Drought at flower initiation without GB spray	0.050 b	0.61 e	5.98 b	2.56 b	10.65 a
T_4 = Drought at flower initiation with GB spray	0.026 de	0.92 c	7.55 a	2.85 a	10.30 a
T_5 = Drought at milking stage without GB spray	0.059 a	0.74 de	6.18 b	2.20 c	10.88 a
T_6 = Drought at milking stage with GB spray	0.029 d	1.08 b	8.29 a	29.00 a	8.08 bc
LSD (5%)	0.003	0.14	1.23	0.36	1.31
Drought vs. no drought	*	*	*	*	*
GB vs. no GB	NS	NS	NS	*	*

Means not sharing the same letters within a column differ significantly at 5% probability.
* = Significant NS = Non-significant

parameters causing decrease in the final yield of wheat. Exogenous application of GB to wheat under water deficit condition on either growth stage (vegetative, flowering and grain filling) significantly affected biochemical parameters and also photosynthesis and transpiration rate of wheat cultivars.

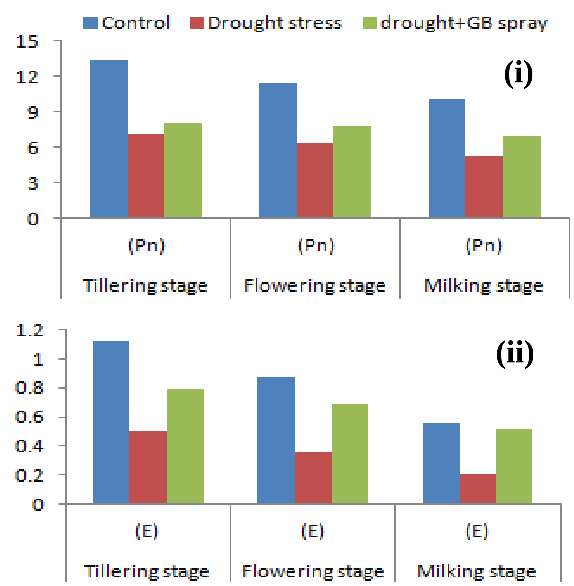

Figure1: Effect of glycinebetaine (GB) application on (i) photosynthesis rate (Pn= μ mol m^{-2} s^{-1}) and (ii) transpiration rate (E=μ mol m^{-2} s^{-1}) of wheat under drought at (a) tillering, (b) flowering and (c) milking stage

The negative effect of water deficit may be reduced by increasing the availability of water to the plant due to reduction in transpiration by partial closure of stomata and/or increased penetration of the roots (Blum *et al.*, 1980). The same findings were reported by Alfredo and Setter (2000) and Hoad *et al.* (2001). Taiz and Zeiger (1991) reported that reduced number of spikelets per ear may be due to limited photosynthetic activity (Figure 1) before spike emergence because spikelets per spike are determined before spike emergence. Reduction in 1000-grain weight and grains per spike due to water stress can also be related with decreased photosynthesis. Drought stress reduced photosynthates production and its translocation to reproductive organs (grains) (Asch *et al.*, 2005). Condon *et al.* (2002) recorded that net photosynthesis and transpiration rate was severely affected under drought. The *Pn* decrease could be explained by reduction in stomatal conductance, which reduced CO_2 diffusion into the leaves however, the internal CO_2 concentration remained stable under water deficit condition and it was similar to that observed in well-watered condition. Thus, reduced stomatal conductance was not supposed to be a major cause for the reduced Pn so that the effect of water deficit on photosynthesis needs further investigation, one possible reason is enzyme inactivation because of high leaf temperature and low leaf water potential (non-stomatal limitation). Glycinebetaine enhanced the photosynthetic capability of the plant by guarding the photosynthetic machinery from reactive oxygen species (ROS) generated during water shortage and by enhancing RuBP content under limited water condition (Ma *et al*. 2006).

Drought stress also affected the uptake of nutrients (N, P, K, Ca and Na) in the plant. The nitrogen accumulation in plants increased under drought and this increase was found maximum (61.01%) where crop faced drought at grain filling stage. The plants under drought stress have high N level due to the accumulation of free amino acids that are not synthesized into protein because under drought stress nitrate reductase is affected adversely, which in the sequence of reactions is the first enzyme responsible for assimilation of nitrate into amino acid and in cell N compounds (Sinha and Nicholas, 1981). Thus the growth of cell and plant particularly leaves accompanied by nitrate accumulation in plant tissue is inhibited under water deficit (Khondakar *et al*., 1983). Glycinebetaine application enhanced the tolerance of plant under stress. Exogenous spray of GB reduced the N accumulation to 50.84% at grain filling stage. Water deficit at any crop growth stage has strong damaging effect on phosphorous uptake; a marked reduction (66.66%) was observed with drought at flowering stage. In plants, the uptake of phosphorous was reduced under drought stress (Baligar *et al*., 2001). Phosphorous uptake decreased with decreasing soil moisture in wheat genotypes (Ashraf, 1998). In plants, phosphorous deficiency appeared in low to moderate level of drought stress (Alam, 1994). Kidambi *et al*. (1990) observed no effect of drought on phosphorous uptake, on the other hand, Khondakar *et al*. (1983) reported higher phosphorous uptake by wheat plants. The reduction in P-uptake under drought was ameliorated by GB spray and GB enhanced P-uptake under drought and this increase was maximum at flowering stage (33.69%).

Contrarily under increased drought stress, K contents increased; these findings are in accordance with those of Khondakar *et al*. (1983) and Ashraf (1998) who reported more potassium uptake under water deficit. In present study, more increase in K contents (12.13%) was observed when drought occurred at grain filling stage than other growth stages. Cuin and Shabala (2005) reported that exogenous spray of GB reduced the potassium efflux under drought stress in barley. During potassium deficiency, ion is transported from older leaves to the younger leaves and then to meristematic regions due to high mobility of K (Wignarajah, 1995), therefore wheat plants may have accumulated potassium contents in developing ears (new growing sinks) for osmotic adjustment. Exogenous application of GB reduced the increasing trend of K under drought and diluted (decreased) its concentration in plant on each growth stage; this reduction was recorded highest (25.45%) with GB sprayed at grain filling stage. In present study, Ca contents of wheat plant were reduced under drought stress and this reduction was recorded up to a maximum of 37.66% at vegetative stage. Exogenous GB spray enhanced plant's resistance to water deficit and maximum increase (19.39%) in Ca contents of wheat plant was recorded when it was sprayed at vegetative stage under drought stress. Na uptake was higher in the plants under drought. Maximum sodium uptake (31.15%) was observed in plants where crop faced drought at grain filling stage. Glycinebetaine mitigated the adverse effect of drought and reduced accumulation of Na. This reduction was maximum (25.73%) when GB was applied under drought at grain filling stage. However, Ashraf (1998) reported no effects of water deficit on sodium contents of plants. Different wheat varieties have differential response Ca concentration under water deficit it; some varieties have more Ca concentration under drought conditions while some have low Ca concentration. The varieties with decreased Ca concentration at critical growth stages yielded better than the varieties with high Ca concentration. It may be because of reduced transpiration under water stress that enhanced internal water contents of the plant and hence accelerated plant growth due to passive transport of Ca in transpirational stream (Wignarajah, 1995).

Conclusion

Water deficit at any critical crop growth stage severely affected the physiological and biochemical parameters of wheat. Exogenous application of GB on a drought stressed crop improved rate of transpiration and photosynthesis and uptake of P and Ca but reduced N, K and Na uptake.

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