

## Improving Photosynthetic Performance of Bread Wheat under Field Drought Stress by Foliar Applied Glycine Betaine

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### ABSTRACT

Nineteen bread wheat genotypes were selected to examine the effect of glycine betaine (GB, 100 mM) on various photosynthetic gas exchange parameters under drought stress and to study the relationship of these parameters with non-enzymatic antioxidants. Drought stress caused a significant decline in net CO<sub>2</sub> assimilation rate ( $P_n$ ), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ) and transpiration rate (E) among the studied wheat genotypes, with the tolerant genotypes characterized by higher net photosynthetic rate, lower drought susceptibility index (DSI), and higher maintenance of glutathione content (GSH) and ascorbic acid (AsA) levels than the sensitive ones. GB application significantly improved the photosynthetic characteristics, particularly  $P_n$  and  $g_s$ , of studied wheat genotypes which could be due to more utilization of glutathione and increased levels of ascorbic acid in flag leaves under drought stress. But this response was observed to be genotype specific. Positive correlation of AsA with DSI in GB treated plants, and of  $P_n$  with GSH under drought stress and GB applied conditions suggested the role of these non-enzymatic antioxidants in sustaining photosynthetic efficiency and yield stability under prolonged field drought stress conditions.

**Keywords:** Non-enzymatic antioxidants, Photosynthetic efficiency.

### INTRODUCTION

Drought is one of the dominant environmental constraints that limit growth, productivity, and grain yield of crop species including wheat (*Triticum aestivum* L.) (Kramer, 1980). Under favourable conditions, approximately 70-90% of wheat grain yield depends on the successful photosynthesis of flag leaves (Austin *et al.*, 1977; Bidinger *et al.*, 1977). Drought results in rapid decline in flag leaf photosynthesis during anthesis, limiting the contribution of current assimilates to grain and accelerates leaf and whole plant senescence (Yang *et al.*, 2001; 2003a), thus, cause severe reductions in photosynthetic efficiency of crop.

Accumulation of compatible solutes is a general response to overcome the negative consequences of water deficit in crop

production. It has long been proposed as an adaptive mechanism and selection criterion in traditional breeding programmes to improve grain yield in dry environments (Ludlow and Muchow, 1990; Zhang *et al.*, 1999). From among many compatible solutes in plants, glycine betaine (GB) occurs most abundantly in response to dehydration stress (Mohanty *et al.*, 2002; Yang *et al.*, 2003b). In addition to its role in osmotic adjustment, many studies suggest that GB plays a vital role in protection of thylakoid membranes and photosynthetic apparatus (Allakhverdiev *et al.*, 2003; Ma *et al.*, 2006; Zhao *et al.*, 2007; Wang *et al.*, 2010), stabilization of the complex proteins and membranes, protection of transcriptional and translational machineries and as a molecular chaperone in the refolding of enzymes such as Rubisco (Sakamoto and Murata, 2000; Chen and Murata, 2002).

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Effort has been devoted to genetically engineer plants for overproduction of this osmo-protectant using enzymes involved in GB biosynthesis such as betaine aldehyde dehydrogenase (BADH), choline dehydrogenase (CDH), choline monooxygenase (CMO) and choline oxidase (COD) (Mohanty *et al.*, 2002; Su *et al.*, 2006; Wang *et al.*, 2010). But, due to high energy costs, low availability of endogenous choline in recipient plant and decreased transport of choline across the chloroplast envelope, there has been little success in achieving the desired protective levels of GB in transgenics of tobacco, tomato, rice and wheat (Su *et al.*, 2006; Ma *et al.*, 2007; Park *et al.*, 2007; Wang *et al.*, 2010). Therefore, an alternative 'shot-gun' approach of exogenous application of GB to plants under stress conditions has gained some attention. But none of these studies are carried out under prolonged field drought stress conditions where multiple stresses and complexity in stress response occurs. Moreover, little information is available on exogenous applied GB affecting non enzymatic antioxidants levels in wheat. Keeping in view the above facts, the present study aimed to assess whether and how foliar applied GB alleviate reduction in photosynthetic efficiency of wheat genotypes under drought stress. Moreover, role of non-enzymatic antioxidants in protection of photosynthetic machinery under drought stress alone and with exogenous applied GB conditions was investigated.

## MATERIALS AND METHODS

Seeds of eight cultivars (C306, PBW175, PBW527, PBW343, PBW550, DBW17, PBW621 and HD2967) and eleven advanced lines (BW9022, BW9097, BW9183, BW9005, BW9016, BW8989, BW8366, BW9151, BW8362, BW9025 and BWL0089) of wheat were obtained from Department of Plant Breeding and Genetics, Punjab Agricultural University (PAU), Ludhiana. Among them, C306 (drought tolerant) and PBW343 (drought sensitive) played the key

role as check varieties. The field experiment was conducted during the growing seasons of 2010-2011 and 2011-2012 in experimental area of Department of Botany, PAU. The experimental site (30° 56'N, 75° 52'E; 247 m ASL) soil was loamy sand textured, low in organic carbon (3.8 g C kg<sup>-1</sup>) and slightly alkaline (pH 8.3). The experiment was conducted in a split plot design with three main plot treatments i.e. control or normal irrigation, drought stress, and drought stress with GB application) and 19 wheat varieties as subplot treatments. Each main plot treatment was tested by three replications with a subplot size of 2×3 m<sup>2</sup>. Wheat genotypes were sown in field according to standard agronomic practices at a seed rate of 40 kg acre<sup>-1</sup> and row to row spacing of 23 cm. Drought stress was imposed by withholding irrigation throughout the season, except a pre-sowing irrigation and life surviving irrigation at post anthesis (after estimations of biochemical parameters). Drought stressed subplots were covered with plastic sheets during precipitation or rains. In case of GB sprayed plots, aqueous 100 mM GB solution containing 0.1% TWEEN 20 (surface-active agent) was sprayed on the leaves until run off, twice a day as described by Ma *et al.* (2006) at maximum tillering and anthesis stages. Control plants were sprayed with water containing 0.1% TWEEN 20 and well watered with five irrigations under normal conditions (Bajwa, 2010). After one week of GB application, leaf samples were collected from each subplot (three replicates per treatment). Flag leaves were excised for biochemical estimations at anthesis stage, respectively.

## Photosynthetic Gas Exchange Measurements

The measurements of photosynthetic gas exchange parameters including net photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ), intercellular CO<sub>2</sub> concentration ( $C_i$ ), and transpiration rate ( $E$ ) were carried out with attached flag leaves of

wheat plants from respective treatments using a portable photosynthesis system with infrared gas analyzer (LI-6400, LICOR Inc., Lincoln, NE, USA). Photosynthetic photon flux density was fixed at  $800 \mu\text{mol m}^{-2} \text{s}^{-1}$  using a red blue LED light source built in the leaf cuvette, though other environmental factors such as air humidity and temperature were not controlled i.e. natural variation was permitted. Instantaneous water use efficiency (IWUE) was calculated from the ratio between net photosynthesis ( $P_n$ ) and transpiration rate (E) (Condon *et al.*, 2002). The measurements with flag leaf were made at least five times per plant; five plants per replica were used per cultivar for each treatment, but the mean results are presented in this study.

#### Determination of Photosynthetic Pigments

Total chlorophyll and total carotenoid contents were determined spectrophotometrically by non maceration method of Hiscox and Israelstam (1979) using dimethylsulphoxide (DMSO) as extracting solvent.

#### Assay of Non-enzymatic Antioxidants

Total glutathione was determined by DTNB method according to Smith (1985). The extraction was done by homogenizing fresh leaf tissues (0.1 g) in chilled 5% sulphosalicylic acid (2 mL) solution and centrifugation at  $10,000 \times g$  for 15 minutes. The extract (0.2 mL) was then mixed with 0.1 M potassium phosphate buffer (pH 7.5) containing 5 mM EDTA (3 mL), 2 mM 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (0.4 mL), 2 mM NADPH (0.2 mL) and glutathione reductase ( $2U \text{ mL}^{-1}$ ) and incubated at  $25^\circ\text{C}$  for 5 minutes. The reaction mixture was then placed in ice bath for termination, after which the optical density of solution was measured at 412 nm. Glutathione content was determined on fresh weight basis using standard curve.

The ascorbic acid content was determined as described by Law *et al.* (1983). Wheat flag leaves samples (0.1 g) from each replicate were homogenized in ice cold 5% metaphosphoric acid and centrifuged at  $10,000 \times g$  for 20 minutes. The reaction mixture for reduced ascorbate, consisting of supernatant (0.2 mL), 150 mM potassium phosphate buffer (pH 7.4) containing 5 mM EDTA (0.5 mL), 10% TCA (0.4 mL), 4% dipyrindyl in 70% alcohol (0.4 mL), 44% o-phosphoric acid (0.4 mL) and 3%  $\text{FeCl}_3$  (0.2 mL), was incubated at  $40^\circ\text{C}$  in a water bath for 40 minutes. The reaction was terminated at the room temperature and absorbance of solution was measured at 525 nm. The concentration of ascorbic acid was determined from a standard curve and calculated on fresh weight basis. All biochemical measurements were repeated at least three times.

#### Drought Susceptibility Index (DSI)

Drought susceptibility index was measured, as described by Fischer and Maurer (1978) AS follows:

$$DSI = \frac{1 - Y_d/Y_p}{D}$$

Where,  $Y_d$  = Mean grain yield of a genotype under drought,  $Y_p$  = Mean grain yield of a genotype under control/irrigated condition and  $D$  = Environmental stress intensity =  $1 - (\text{Mean grain yield of all genotypes under drought} / \text{Mean grain yield of all genotypes under control condition})$ . The observations on grain yield were recorded at the time of harvesting and the data were analyzed using standard method of ANOVA. The pooled data of the two years is mentioned in the present study.

## RESULTS

#### Photosynthetic Gas Exchange Parameters

Net photosynthetic rate ( $P_n$ ) was significantly reduced (48.2%) under drought



stress (Figure 1-a). This reduction was higher in wheat genotypes BW9151 (64.0%), BW9005 (60.4%) and BW9183 (58.1%). In other genotypes, the decrease in  $P_n$  values was observed to be 28-50%, with least reduction recorded in BW9025 (28.4%), PBW527 (33.6%) and PBW175 (38.7%). However, among the studied genotypes, C306, PBW175, PBW527, BW9025, BWL0089 and PBW621 possessed higher rate of net  $\text{CO}_2$  assimilation ( $P_n$ ) under drought stress conditions. In the case of stomatal conductance ( $g_s$ ), irrigated plots had significantly higher  $g_s$  values in comparison to drought stressed plots (Figure 1-b). A sharp decline in  $g_s$  under drought could be an adaptation mechanism to conserve water, but it reduced gaseous exchange on the other hand. When pooled across genotypes, reduction in  $g_s$  was recorded to be 45.2% with lesser decline in BW9151 (23.3%) and BW9022 (25%).

GB application significantly improved  $P_n$  (12.9%) and  $g_s$  (29.4%) across the genotypes under drought stress conditions. Furthermore, improved  $P_n$  in GB treated plots exhibited genotype specific response. In some genotypes such as C306, PBW175, PBW343, BW9022, BW9005, BW9016, BW9151, and BW8362 increased stomatal conductance ( $g_s$ ) in parallel resulted in  $P_n$  improvement under drought stress. While in others viz. PBW527, PBW550, DBW17, BW9097, BW9183, BW8989, BW8366, and PBW621, even after maintenance of higher  $g_s$ , either the percent increase in  $P_n$  was relatively small or decline in  $P_n$  rate occurred as compared to their drought stressed counterparts.

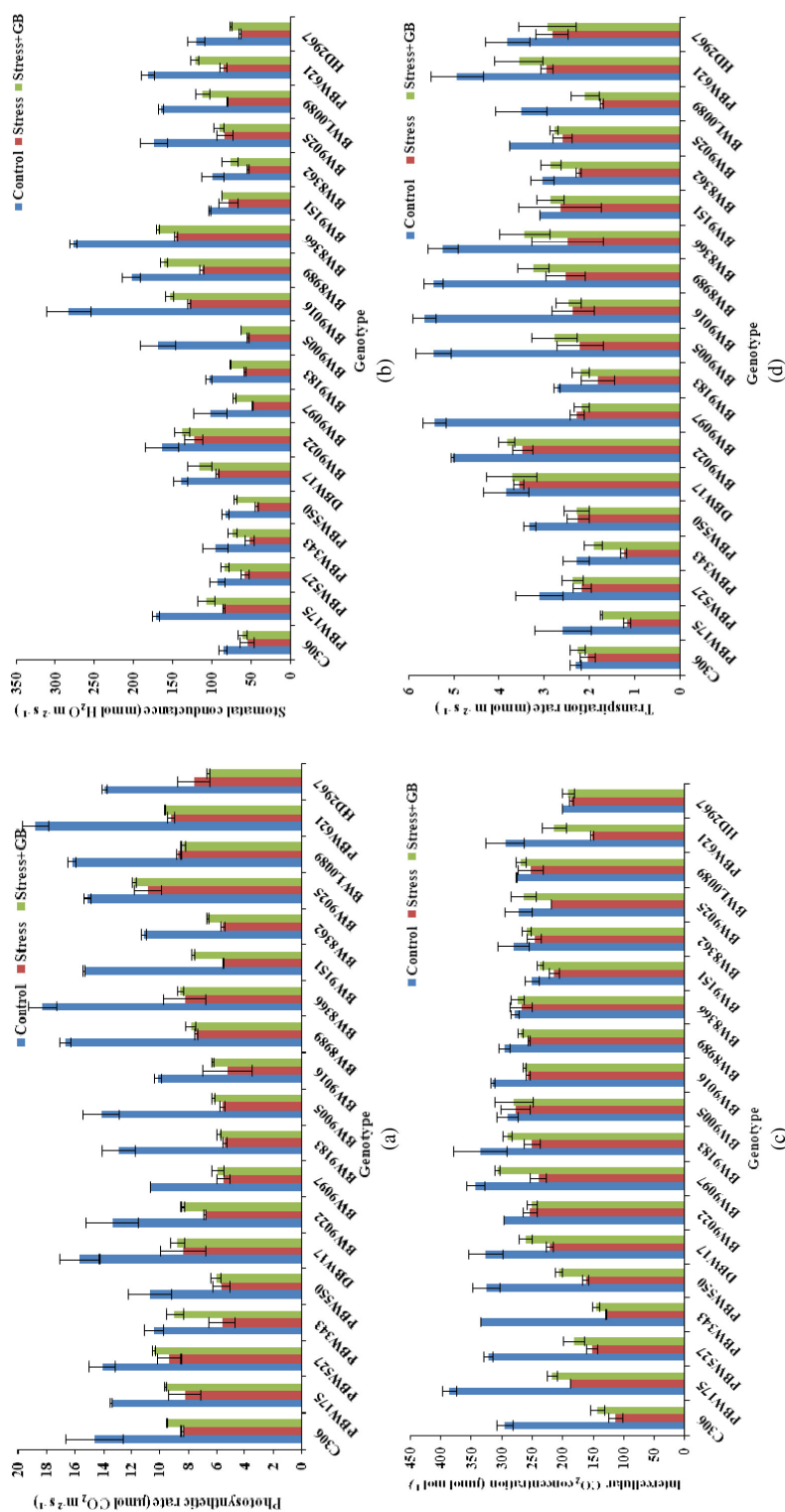
Drought stress caused significant reductions in intercellular  $\text{CO}_2$  concentration ( $C_i$ ) and transpiration rate ( $E$ ) of stressed plants as compared to their irrigated counterparts (Figure 1, c and d). A positive correlation between  $g_s$  and  $C_i$  ( $r_s = 0.469$ ) under drought stress suggested that this reduction in  $C_i$  could be due to decreased stomatal conductance (Table 2). But, in spite of greater percent reduction in  $g_s$  values of

genotypes BW9097, BW9005, BW9016, BW8989, BW8366, BW8362, BW9025, HD2967, and BWL0089, the decrease in  $C_i$  values were inconsistent. This decline in  $C_i$  was higher in C306 (61.6%), PBW343 (61.7%), PBW527 (52.7%), PBW175 (51.3%), PBW550 (50.1%) and PBW621 (48.6%). In contrast, GB treated plots possessed significantly increased  $C_i$  and  $E$  in comparison to stressed plots under drought stress conditions. When pooled across genotypes, the percent increase was recorded to be 13.7% for  $C_i$  and 17.9% for  $E$ . Furthermore, a positive correlation was observed between  $g_s$  and  $E$  with GB spray under drought stress ( $r_{s+gb} = 0.528$ ) and in irrigated conditions ( $r_i = 0.679$ ) (Tables 2 and 3).

The effect of field drought on instantaneous water use efficiency ( $IWUE$ ) of wheat genotypes was shown in Table 1. From among the genotypes selected presently, a significant increase in  $IWUE$  of PBW175, PBW527, BW9016, BW9025, and BWL0089 was observed under drought stressed conditions.  $IWUE$  was invariably affected at anthesis in wheat genotypes under GB applied conditions. Despite the decrease in  $IWUE$  under GB treatment due to enhanced  $E$  in genotypes such as PBW175, BW9183, BW9005, BW8989, BW8366, BW8362 and PBW621 still an increase in  $P_n$  was observed, except for HD2967 and BWL0089.  $IWUE$  was positively correlated to  $P_n$  observed (Tables 2 and 3) under drought stress ( $r_s = 0.495$ ) and GB applied conditions ( $r_{s+gb} = 0.651$ ).

### Levels of Photosynthetic Pigments

As shown in Table 1, drought stress caused a significant decrease in level of total chlorophyll in flag leaves of most wheat genotypes, except C306, PBW175, BW9016, BW9151, BWL0089, PBW621 and HD2967. Exogenous GB application increased chlorophyll content of GB treated plots but with non-significance. Moreover, a non significant increase in total carotenoid



**Figure 1.** Represent changes in photosynthetic characteristics of wheat genotypes with GB foliar spray under drought stress. (a) Net photosynthetic rate ( $G=1.259$ ;  $T=0.500$ ;  $G \times T=2.181$ ); (b) Stomatal conductance ( $G=19.116$ ;  $T=7.596$ ;  $G \times T=33.110$ ); (c) Intercellular  $\text{CO}_2$  concentration ( $G=23.575$ ;  $T=9.368$ ;  $G \times T=40.833$ ), and (d) Transpiration rate ( $G=0.582$ ;  $T=0.231$ ;  $G \times T=1.009$ ) where  $G$  is genotype;  $T$  is treatment and  $G \times T$  represent genotype and treatment interactions. The values of  $G$ ,  $T$  and  $G \times T$  are critical differences at 5% level of significance while vertical bars on graph represent standard errors.

**Table 1.** Effect of GB on drought susceptibility index (DSI), instantaneous water use efficiency (IWUE), total chlorophyll and total carotenoid contents of flag leaves of wheat genotypes subjected to prolonged field drought stress.

Genotypes	DSI			IWUE ( $\mu\text{mol mmol}^{-1}$ )			Chlorophyll content ( $\text{mg g}^{-1}$ FW)			Carotenoid content ( $\text{mg g}^{-1}$ FW)		
	Stress	Stress+GB	Control	Stress	Stress+GB	Control	Control	Stress	Stress+GB	Control	Stress	Stress+GB
C306	0.979	0.073	6.332	4.120	4.172	2.429	2.684	2.907	0.117	0.167	0.175	
PBW175	0.899	0.576	5.193	7.069	5.521	2.746	3.032	3.354	0.124	0.141	0.152	
PBW527	0.879	0.423	3.559	4.312	4.375	3.315	3.117	3.155	0.147	0.159	0.160	
PBW343	1.348	1.080	4.527	4.506	4.675	2.830	2.713	3.418	0.128	0.149	0.152	
PBW550	1.063	2.156	3.217	2.503	2.635	2.586	2.333	2.810	0.121	0.124	0.135	
DBW17	1.331	1.479	4.074	2.343	2.362	2.490	1.954	2.860	0.119	0.131	0.137	
BW9022	1.803	2.007	2.653	1.959	2.193	2.384	2.114	2.483	0.105	0.111	0.116	
BW9097	1.491	1.322	1.961	2.413	2.717	2.773	2.495	2.823	0.119	0.127	0.130	
BW9183	1.231	1.160	4.761	2.986	2.653	2.626	1.725	2.527	0.117	0.123	0.131	
BW9005	0.849	1.382	2.595	2.535	2.250	3.032	2.442	2.802	0.140	0.158	0.161	
BW9016	1.523	0.762	1.792	2.213	2.550	2.742	3.223	3.558	0.119	0.147	0.153	
BW8989	0.734	1.059	3.064	2.945	2.412	2.569	2.237	2.312	0.116	0.120	0.124	
BW8366	0.706	1.588	3.496	3.303	2.485	2.947	1.946	2.114	0.129	0.135	0.133	
BW9151	0.718	1.140	4.944	2.081	2.692	2.236	2.635	2.717	0.108	0.121	0.128	
BW8362	0.825	0.198	3.655	2.480	2.318	3.664	2.642	3.185	0.161	0.172	0.181	
BW 9025	0.385	0.321	4.019	4.154	4.256	2.054	1.900	2.203	0.102	0.119	0.120	
BWL0089	0.588	0.144	4.625	4.994	3.969	2.175	2.285	2.290	0.105	0.107	0.109	
PBW621	0.891	1.125	3.813	3.110	2.694	2.564	2.983	2.981	0.119	0.134	0.138	
HD2967	0.448	0.311	3.657	2.683	2.236	2.341	2.458	2.499	0.148	0.157	0.160	
CD <sup>a</sup> at 5%	G = 0.365; T= NS; G × T= 0.516	G = 2.260; T= 0.898; G×T= NS	G = 0.130; T= 0.518; G×T= 0.226	G = 0.130; T= 0.518; G×T= 0.226	G = 0.130; T= 0.518; G×T= 0.226	G = 0.130; T= 0.518; G×T= 0.226	G = 0.164; T= NS; G×T= NS	G = 0.164; T= NS; G×T= NS	G = 0.164; T= NS; G×T= NS	G = 0.164; T= NS; G×T= NS	G = 0.164; T= NS; G×T= NS	G = 0.164; T= NS; G×T= NS

<sup>a</sup> Critical Difference (CD) at 5% level of significance. Where G is genotype, T is treatment and G×T is the interaction between genotypes and treatments.

**Table 2.** Correlation analysis between photosynthetic parameters and non-enzymatic antioxidants under irrigated and drought stress conditions.

		Irrigated condition (Control)							
	$P_n^a$	$g_s^b$	$C_i^c$	$E^d$	$IWUE^e$	$GSH^f$	$AsA^g$	$DSI^h$	
$P_n$	1.000								
$g_s$	0.366	1.000							
$C_i$	-0.365	-0.063	1.000						
$E$	0.209	0.679*	-0.151	1.000					
$IWUE$	0.280	-0.397	0.006	-0.822*	1.000				
$GSH$	-0.134	-0.020	0.150	-0.070	-0.041	1.000			
$AsA$	-0.198	-0.310	-0.284	-0.150	-0.073	0.271	1.000		
$DSI$	-0.523*	-0.035	0.567*	0.182	-0.352	-0.252	-0.222	1.000	
		Drought stress condition							
	$P_n$	$g_s$	$C_i$	$E$	$IWUE$	$GSH$	$AsA$	$DSI$	
$P_n$	1.000								
$g_s$	0.227	1.000							
$C_i$	-0.269	0.469*	1.000						
$E$	0.180	0.408	-0.238	1.000					
$IWUE$	0.495*	-0.105	-0.376	-0.700*	1.000				
$GSH$	0.608*	-0.262	-0.496*	-0.239	0.645*	1.000			
$AsA$	-0.529*	0.136	0.357	0.270	-0.514*	-0.430	1.000		
$DSI$	-0.501*	0.098	0.054	0.129	-0.316	-0.512*	0.393	1.000	

<sup>a</sup> Net photosynthetic rate; <sup>b</sup> Stomatal conductance; <sup>c</sup> Intercellular CO<sub>2</sub> concentration; <sup>d</sup> Transpiration rate; <sup>e</sup> Instantaneous Water Use Efficiency; <sup>f</sup> Glutathione content; <sup>g</sup> Ascorbic Acid; <sup>h</sup> Drought Susceptibility Index. \* Significant at 5% level of significance.

contents of stressed plots and GB treated plots was recorded under drought stress.

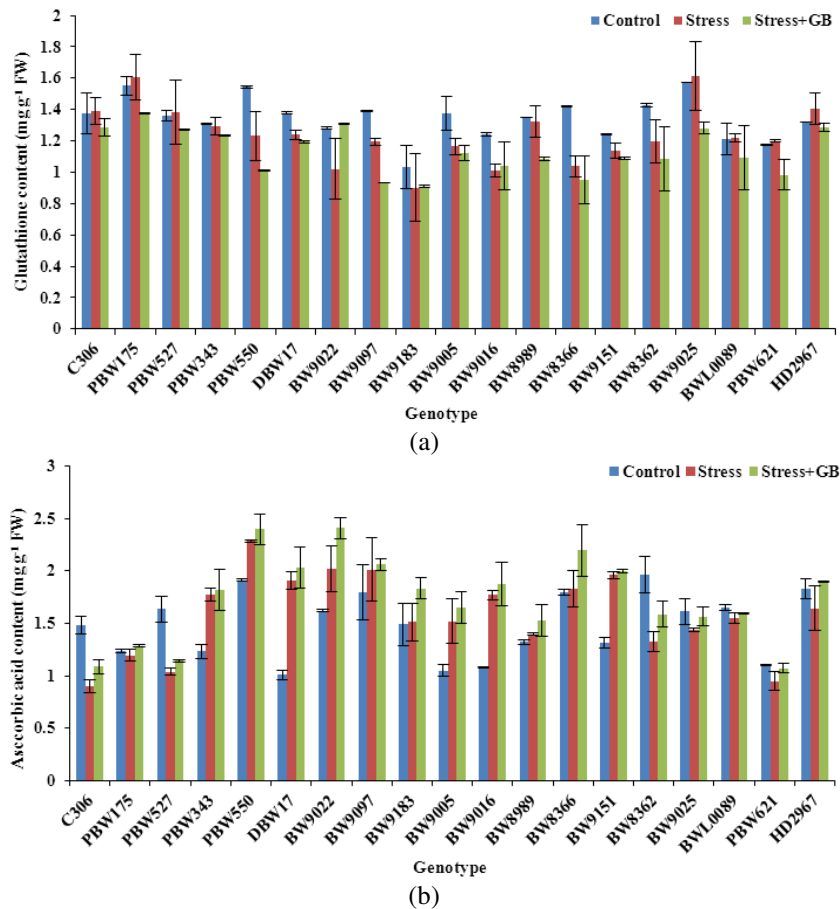
AsA content of flag leaf in most wheat genotypes.

### Non-Enzymatic Antioxidants

As depicted in Figure 2a, levels of total glutathione significantly decreased in most wheat genotypes of stressed plots, which could be due to prolonged field drought stress. A few genotypes (C306, PBW175, PBW527, BW9025, BWL0089, PBW621 and HD2967) were exceptions to the reduction in glutathione observed under drought stress. Genotypes×treatments interactions also differed non-significantly for glutathione content. GB treated plants had significantly lower GSH content than drought stressed plots. In case of ascorbic acid (AsA) content (Figure 2b), drought stress caused significant increase in their levels in selected wheat genotypes with exceptions C306, PBW175, PBW527, BW8362, BW9025, BWL0089, PBW621 and HD2967. GB application increased the

### Drought Susceptibility Index (DSI) and Correlation Analysis

For identification of genotypes with more stable performance under drought stress, drought susceptibility index (DSI) was determined in the present study (Table 1). Significant genotypic differences were observed for *DSI* under both drought stressed and GB sprayed conditions. GB application resulted in a genotype specific response for *DSI*. In some genotypes (C306, PBW175, PBW527, PBW343, BW9097, BW9183, BW9016, BW8362, BW 9025, BWL0089 and HD2967), GB application reduced *DSI* value. In others (PBW550, DBW17, BW9022, BW9005, BW8989, BW8366, BW9151 and PBW621), GB application resulted in increased *DSI* values. On the basis of photosynthetic and non enzymatic anti-oxidative performance and



**Figure 2.** Represent levels of non-enzymatic antioxidants in flag leaves of wheat genotypes as affected by GB foliar spray under drought stress. (a) Total Glutathione content ( $G = 0.154$ ;  $T = 0.611$ ;  $G \times T = \text{NS}$ ), and (b) Ascorbic acid content ( $G = 0.214$ ;  $T = \text{NS}$ ;  $G \times T = 0.371$ ) where  $G$  is genotype;  $T$  is treatment and  $G \times T$  represent genotype and treatment interactions. The values of  $G$ ,  $T$  and  $G \times T$  are critical differences at 5% level of significance while vertical bars on graph represent standard errors.

$DSI$  values, the selected wheat genotypes were divided into tolerant and sensitive categories. The genotypes HD2967, PBW621, BW9025, BWL0089, PBW527, PBW175 and C306 were considered as tolerant group, as characterized by lower  $DSI$ , higher rate of  $P_n$  and maintenance of GSH and AsA level under field drought stressed conditions. The sensitive genotypes, as characterized by higher  $DSI$  values and decreased  $P_n$ , were further divided into Group I including PBW343, PBW550, BW9022, BW9097, BW9183, BW9005 and BW9016, which were highly sensitive, and Group II including DBW17, BW9151, BW8989, BW8366 and BW8362 which

were moderately sensitive to drought stress. However,  $P_n$  and  $DSI$  were negatively correlated to each other under both irrigated ( $r_i = -0.523$ ) and drought stressed conditions ( $r_s = -0.501$ ). Correlation analysis of non-enzymatic antioxidants with gas exchange parameters (Tables 2 and 3) revealed a positive correlation of GSH levels to  $P_n$  ( $r_s = 0.608$ ;  $r_{s+gb} = 0.581$ ) and  $IWUE$  ( $r_s = 0.645$ ;  $r_{s+gb} = 0.548$ ) under both drought stressed and GB applied conditions. In contrast,  $C_i$  was negatively correlated to GSH levels under drought stress conditions ( $r_s = -0.496$ ;  $r_{s+gb} = -0.554$ ). A significant positive correlation of AsA levels with  $DSI$  was also found in GB treated plots ( $r_{s+gb} = 0.683$ ).



**Table 3** Effect of GB on correlation analysis between photosynthetic parameters and non-enzymatic antioxidants under drought stress conditions.

	Drought stress+Glycine betaine (100 mM) condition							
	$P_n^a$	$g_s^b$	$C_i^c$	$E^d$	IWUE <sup>e</sup>	GSH <sup>f</sup>	AsA <sup>g</sup>	DSI <sup>h</sup>
$P_n$	1.000							
$g_s$	0.156	1.000						
$C_i$	-0.385	0.334	1.000					
E	0.107	0.528*	0.256	1.000				
IWUE	0.651*	-0.229	-0.503*	-0.659*	1.000			
GSH	0.581*	-0.152	-0.554*	-0.064	0.548*	1.000		
AsA	-0.434	0.218	0.267	0.313	-0.524*	-0.195	1.000	
DSI	-0.338	0.228	0.270	0.383	-0.493*	-0.376	0.683*	1.000

<sup>a</sup> Net photosynthetic rate; <sup>b</sup> Stomatal conductance; <sup>c</sup> Intercellular CO<sub>2</sub> concentration; <sup>d</sup> Transpiration rate, <sup>e</sup> Instantaneous Water Use Efficiency; <sup>f</sup> Glutathione content, <sup>g</sup> Ascorbic Acid, <sup>h</sup> Drought Susceptibility Index. \* Significant at 5% level of significance.

## DISCUSSION

Photosynthesis of the flag leaf is the most important basis of wheat grain yields in rainfed/dryland agriculture because of extreme sensitivity of reproductive phase and early senescence during terminal drought stress. Net flag leaf photosynthesis in wheat contributes about 30 to 50% of the assimilates for grain filling (Sylvester-Bradley *et al.*, 1990). In the present study, we investigated the changes in levels of various photosynthetic gas exchange parameters and non-enzymatic antioxidants of wheat flag leaf under prolonged field drought stress and evaluated their response to exogenously applied GB (100 mM). Imposition of drought stress resulted in significant reductions in  $P_n$  and  $C_i$  of the studied wheat genotypes. This could be explained in terms of accelerated senescence and reduced stomatal conductance, which resulted in reduced CO<sub>2</sub> diffusion. Furthermore, positive correlation between  $g_s$  and  $C_i$  in drought stressed conditions supported contributions of stomatal limitations in  $C_i$  reductions, which in turn reduced  $P_n$  of unsprayed drought stressed plants. But, in spite of this, percent reductions in  $C_i$  of nine genotypes (BW9097, BW9005, BW9016, BW8989, BW8366, BW8362, BW9025, HD2967 and BWL0089) were of much lower magnitude in comparison to percent reductions observed in their  $g_s$  and  $P_n$  values under stress. This indicated that  $g_s$  could not be solely responsible for determining  $C_i$  or  $P_n$ . There

might be involvement of some non-stomatal factors in deciding  $P_n$  or CO<sub>2</sub> assimilation under drought stress. Non-stomatal limitations in photosynthesis could be attributed to impairments in photochemical/biochemical reactions such as damage to biomembrane structure, disorder of active oxygen metabolism, photochemistry of PSII, reduction in RuBP carboxylation efficiency, RuBP regeneration or the amount of ribulose-1,5-bisphosphate carboxylase/oxygenase (Mediavilla and Escudero, 2004; Demirevska *et al.*, 2008; Misson *et al.*, 2010) and dark respiration etc. Genotypic differences in gas exchange parameters suggested that genotype stress sensitivity and severity of stress might affect stomatal and non stomatal factors which in turn determine  $P_n$  and  $C_i$  of a genotype in an additive manner.

In the current study, exogenous application of GB was able to alleviate the disturbances in photosynthetic gaseous exchange and thus decreased the reduction in  $P_n$  under drought stress. This could be due to increased stomatal conductance as observed in GB sprayed plots. However, a genotype specific response was observed for  $P_n$  with GB spray under drought stress condition. A greater magnitude of  $g_s$  increase in comparison to  $C_i$  and  $P_n$  increase of the studied genotypes suggested that increased  $P_n$  was dependent on relative maintenance of non-stomatal factors with GB treatment in addition to  $g_s$ . This might be the reason for increased  $P_n$  even under the conditions of more transpiratory loss (E) of GB sprayed drought stressed plots. It



could be hypothesized that GB maintained  $g_s$  in such a way that water loss would be minimum so as to enhance water use efficiency. Roles of GB in protection of PSII complex from photodamage via acceleration of D1 protein turnover and maintenance of antioxidant enzyme activities (Allakhverdiev *et al.*, 2003; Ma *et al.*, 2006), improvement of lipid composition and function of thylakoid membranes (Zhao *et al.*, 2007) under different environmental stress conditions has been well established.

Significant decline in *IWUE* of wheat genotypes under severe drought stress occurred due to depression of photosynthesis, as confirmed by correlation analysis. But, a few exceptions (PBW175, PBW527, BW9016, BW9025 and BWL0089) suggested their inherent ability to reduce transpiratory loss more than decrease in  $P_n$ . Declined total chlorophyll content under prolonged drought stress revealed oxidative degradation of chlorophyll, which was suppressed by GB application to certain extent. This could also be the reason of increased  $P_n$  of GB treated plants as compared to their stressed analogues. Being a determining factor of yield stability under drought stress, significant genotypic differences for *DSI* existed in the studied wheat genotypes. GB application had genotypic effects on *DSI*. Out of 19 genotypes, eleven genotypes with GB spray possessed lower *DSI* values than their stressed counterparts (Table 1). This indicated enhancement in yield stability of these genotypes with GB spray under drought stress. But, increase in *DSI* of the rest nine genotypes with GB suggested that GB application might affect stability of yield (*DSI*) in a genotype specific manner. The positive correlation of *DSI* with ascorbic acid (AsA) content in GB applied conditions reflected that increased stability of yield characteristics by GB could be due to up-regulation of antioxidant defence system under drought stress.

Consideration of non-enzymatic antioxidants in the present study revealed significant reduction in glutathione (total, GSH) levels and increased AsA content of flag leaf under drought stress in most wheat genotypes. These molecules not only themselves act as antioxidants but are also substrates for various antioxidant enzymes. Seven genotypes exhibited contrasting behaviour in relation to their GSH and AsA levels under drought stress. However, GB application showed

a much higher magnitude of reduction in GSH levels of wheat genotypes than their stressed counterparts. Increased AsA content of GB treated plants indicated this reduction in GSH levels could be due to its role in AsA regeneration via ascorbate–glutathione cycle, although from correlation coefficients, no direct relation between their levels was observed in the current study. A positive correlation of  $P_n$  with GSH content could be explained by dependence of photosynthetic carbon assimilation on thiol regulated enzymes (i.e. fructose 1,6-bisphosphatase and NADP<sup>+</sup>-dependent glyceraldehyde-3-phosphate dehydrogenase) in C<sub>3</sub> plants whose sulfhydryl groups were protected against H<sub>2</sub>O<sub>2</sub> oxidation by GSH (Takeda *et al.*, 1995; Loggini *et al.*, 1999) as well as detoxification of active oxygen species by GSH dependent antioxidant enzymes such as glutathione reductase, glutathione peroxidase, dehydroascorbate reductase. In the present study, drought tolerant group consisted of genotypes HD2967, PBW621, BW9025, BWL0089, PBW527, PBW175 and C306, as characterized by their lower *DSI* values, higher  $P_n$  and maintenance of GSH and AsA levels under field drought stressed conditions. While exogenous application of GB led to significant improvements in photosynthetic characteristics and yield stability of wheat, yet this response to GB spray exhibited genotype specificity. Our results suggested that it might be the maintenance of cellular redox status by these non-enzymatic antioxidants and various other metabolites (osmoprotectants, enzymatic and other antioxidants, sugars, etc) which could be responsible for regulation of CO<sub>2</sub> assimilation and yield stability and ultimately enhanced tolerance under GB applied and drought stressed environments. Genotype specific response to GB application needs further precise research on alleviation of various abiotic stresses by exogenous application of such compatible solutes.

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## بهبود عمل فتوستت در گندم نان تحت تنش خشکی در مزرعه با پاشیدن گلايسين بتائين روی برگ

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### چکیده

در این پژوهش، اثر گلايسين بتائين (GB)، به مقدار 100mM روی پارامترهای مختلف فتوستت و تبادل گازی ۱۹ ژنوتیپ گندم نان و نیز رابطه این پارامترها با آنتی اکسیدان های غیر آنزیمی تحت تنش خشکی بررسی شد. تنش خشکی در بین ژنوتیپ های مزبور موجب کاهش معنی دار نرخ خالص جذب  $(P_n)CO_2$ ، هدایت روزنه ای ( $g_s$ )، غلظت بین سلولی  $(C_i)CO_2$  و نرخ تعرق (E) شد در حالی که ژنوتیپ های مقاوم به خشکی در مقایسه با ژنوتیپ های حساس، دارای نرخ فتوستت بیشتر و شاخص آسیب پذیری از تنش (DSI) کمتری بودند و مقدار بیشتری گلو تایون (GSH) و اسکریبک اسید (ASA) داشتند. افزایش GB به طور معنی داری پارامترهای فتوستت، به ویژه مقدار  $P_n$  و  $g_s$ ، را در ژنوتیپ های مطالعه شده بهبود بخشید. این نتیجه ممکن است به علت استفاده بیشتر از گلو تایون و افزایش سطح اسکریبک اسید در برگ پرچم تحت شرایط تنش باشد. اما مشاهده شد که این عکس العمل در ژنوتیپ های مختلف تفاوت داشت. نیز، رابطه مثبت ASA با DSI در گیاهانی که با GB تیمار شده بودند و همچنین رابطه  $P_n$  و GSH در شرایط تنش و افزودن GB، به نقش این آنتی اکسیدان های غیر آنزیمی در حفظ کارایی فتوستت و پایداری عملکرد در شرایط تنش خشکی طولانی در مزرعه اشاره دارد.