

## COMBINED APPLICATION OF GLYCINEBETAINE AND POTASSIUM ON THE NUTRIENT UPTAKE PERFORMANCE OF WHEAT UNDER DROUGHT STRESS

Muhammad Aown Sammar Raza<sup>\*1</sup>, Muhammad Farrukh Saleem<sup>2</sup> and Imran Haider Khan<sup>2</sup>

<sup>1</sup>Department of Agronomy, University College of Agriculture & Environmental Sciences, The Islamia University of Bahawalpur, Pakistan; <sup>2</sup>Department of Agronomy, University of Agriculture, Faisalabad, Pakistan

<sup>\*</sup>Corresponding author's e-mail: aown\_samar@yahoo.com

Global warming is posing threat to agriculture through increasing incidence of abiotic stresses particularly drought stress. Water stress at grain filling stage of wheat crop is detrimental to crop mineral nutrition because it reduces soil moisture and inhibits the optimal crop growth and development by decreasing the availability of nutrients uptake. Glycine betaine and potassium production is accelerated in plants particularly under drought stress conditions for regulation of cell homeostasis (protective mechanism). For the reason, a pot and two field trials were conducted to evaluate the impact of foliar application of glycine betaine and potassium alone and in combination, when water was withheld at grain filling stage. Different doses of glycine betaine (0, 50, 100 and 150 mM) and potassium (0, 0.5, 1.0 and 1.5%) and all possible combinations were used. After harvesting, plant mineral content (N, P, K, Na, and Ca) was assessed. The results indicated that the application of K at 1.5% in combination with glycine betaine at 100 mM was most beneficial in diluting the impact of water stress at grain filling stage. Further it was suggested that the responses of wheat crop to these osmolytes should be studied at molecular level under drought to identify the mechanisms which are tangled for variable plant responses.

**Keywords:** Glycine betaine, potassium, drought, wheat, grain filling stage, mineral nutrition

### INTRODUCTION

Plants living in different agro-climatic conditions have to face extreme situations like drought which affects their growth and productivity (Ashraf, 2010). For the reason, they evolved with certain mechanisms to abide by the harsh environments and make them competent inhabitants. Drought stress is most prevalent in arid and semi-arid regions of the world (Deng *et al.*, 2004) and water deficit reduces the availability and transport of the mineral nutrients in the rhizosphere which ultimately cause wilting symptoms in plants. Therefore, plants nutritional status can be referred as an indicator of water stress (Raza *et al.*, 2012). To evade the internal damage from water deficit stress, plants regulate their defense mechanism *i.e.* osmoregulation, hormonal balance, and reactive oxygen species scavenging factors (Yang *et al.*, 2007; Ashraf, 2010; Hossain and Fujita, 2010). Osmotic adjustment has been considered most important phenomenon for mitigating the adverse effects of drought stress in plants (Ashraf, 2010). For that certain organic (proline, soluble sugars, glycine betaine, proline betaine) and inorganic (potassium ions) compatible solutes are accumulated in the plant cells (Taiz and Zeiger, 2002; Zhang *et al.*, 2009; Baysal Furtana *et al.*, 2013). Among these compatible solutes glycine betaine and potassium are very effective in the regulation of plants homeostasis under drought stress. Glycine betaine production is accelerated in plants particularly under drought stress conditions (Hessine

*et al.*, 2009). However, its production and accumulation depends on plant type, species, organelles and specific stress condition (Zhu *et al.*, 2003; Moghaieb *et al.*, 2004; Hassine *et al.*, 2008; Zhang *et al.*, 2008). Glycine betaine protects cell membranes, quaternary structure of proteins, stabilizes the membrane structure of photosystem II complex, maintains enzyme activity and boosts the antioxidant defense under drought/ osmotic stress (Ma *et al.*, 2006; Yang *et al.*, 2007; Hossain and Fujita, 2010). Similarly, potassium uptake and accumulation is increased during drought and it plays major role in stomata opening and closing, transpiration, photosynthesis, protective protein synthesis, and osmoregulation of plant cells (Cakmak, 2005; Milford and Johnston, 2007). Deficiency of potassium cause retarded growth under stress (Hermans *et al.*, 2006). Because potassium is vital for enhancing plants resistance or immunity against stress, regulation of physiological processes, translocation of cations, activation of enzymes and regulation of turgor pressure (Mengel and Kirkby, 2001). Therefore, nutrient status of plant is very important and has major role in the adaptation of crop plants to stress (IPIQUAT-IPNI Intern Symposium, 2009). Different plants have varied capacity to synthesis glycine betaine at a considerably lower level than the required to protect them from stress (Subbarao *et al.*, 2001). Similarly, reduced availability of potassium under stress makes crop plants more susceptible to damage from drought. However, exogenous application of glycine betaine or potassium has

been reported to improve plants vigor, growth, productivity and mineral nutrition under drought (Athar *et al.*, 2009; Cha-Um and Kirdmanee, 2010; Wang *et al.*, 2010a,b; Raza *et al.*, 2012, 2013). The effectiveness of exogenously applied glycine betaine or potassium varies with the level of application, number of applications, plant species and plant developmental stage (Ashraf *et al.*, 2008; Raza *et al.*, 2012, 2013). In wheat, grain filling stage has been observed most sensitive to drought stress and highly responsive to exogenous application of potassium or glycine betaine (Raza *et al.*, 2012, 2013). Hence the present study was designed to investigate the optimal dose of potassium and glycine betaine whether alone or in combination for ameliorating the impact of drought at grain filling stage of wheat in terms of mineral nutrition of plants.

## MATERIALS AND METHODS

**Experimental arrangements:** An experiment was conducted in pots (2008-09) and repeated in fields at National Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan (2009-10) and University of Agriculture Faisalabad (UAF), Faisalabad, Pakistan (2010-11). Wheat cultivars Lasani-2008 (drought tolerant) and Auqab-2000 (drought sensitive) were chosen for this study. Potted trial was managed in a wire house to avoid damage from insects and birds. Interruption from rain during drought treatment was eluded using plastic sheet on top of the wire house. Recommended doses of NPK (90:60:60 kg ha<sup>-1</sup>) were applied in pots (containing 7 kg soil) and field plots (area = 4 m × 1.2 m) using urea, diammonium phosphate and sulphate of potash fertilizers. Ten seeds per pot were sown and after two weeks of germination thinned to 4 plants per pot. Five lines per plot were maintained in each plot in the field. Drought was applied in pots and fields by withholding irrigation water at Zadoks GS 73 grain filling stage of the crop (Zadok et al., 1974). During drought, different doses of glycine betaine (GB at 0, 50, 100, 150 mM) and potassium (K at 0, 0.5, 1.0, 1.5%) were foliar sprayed in all possible combinations. Tween 20 solution (0.1%) was used as solvent for foliar spray. Soil used in pot and field trial in NIAB had sandy clay loam texture; OM, 0.83%; N, 0.33 mg kg<sup>-1</sup>; P, 4.9 mg kg<sup>-1</sup>; K, 128 mg kg<sup>-1</sup>; Ca, 101 mg kg<sup>-1</sup> and pH, 7.7. Soil at the research farm of UAF was also sandy clay loam in texture; OM, 0.74%; N, 0.18 mg kg<sup>-1</sup>; P, 7.2 mg kg<sup>-1</sup>; K, 172 mg kg<sup>-1</sup>; Ca, 120 mg kg<sup>-1</sup> and pH, 7.9. All the recommended agronomic and plant protection measures were adopted. At maturity crop was harvested and plant samples were dried.

**Nutrient analysis:** Ground dried (0.5 g) samples were wet digested using concentrated H<sub>2</sub>SO<sub>4</sub> and H<sub>2</sub>O<sub>2</sub> following Wolf (1982). The digested extract was used for the measurement of K, Na and Ca using flame photometer and the concentrations were calculated with the help of standard curves ranging 10-100 mg L<sup>-1</sup> for each nutrient (Na, K, and

Ca). Micro-Kjeldhals' method was followed to measure N contents (Bremner, 1965) and P contents were analyzed and calculated using spectrophotometer.

**Data analysis:** Data collected were subjected to analysis of variance (ANOVA) following completely randomized design (CRD) for pot trial and randomized complete block design (RCBD) for field trials in factorial arrangement. Treatment means were compared using least significance difference (LSD) test at  $p \leq 0.05$  (Steel *et al.*, 1997).

## RESULTS

Drought at grain filling stage of wheat was detrimental to crop growth and yield. Therefore, varied potential in two wheat cultivars was recorded for the uptake of N, P, K and Ca.

**Table 1. Interactive effect of GB and K spray under drought at grain filling stage on nitrogen uptake (percent) of wheat**

Varieties (V)	Pots trial	2009-10	2010-11
V <sub>1</sub> =Lasani-2008	0.35b	0.41b	0.41b
V <sub>2</sub> =Auqab-2000	0.36a	0.43a	0.45a
<b>GB application rate (T)</b>			
T <sub>0</sub> =0 mM	0.41a	0.49a	0.52a
T <sub>1</sub> =50 mM	0.34c	0.39c	0.40c
T <sub>2</sub> =100 mM	0.33d	0.37d	0.38c
T <sub>3</sub> =150 mM	0.36b	0.43b	0.44b
<b>K application rate (K)</b>			
K <sub>0</sub> =0%	0.32d	0.38c	0.38d
K <sub>1</sub> =0.5%	0.33c	0.39c	0.41c
K <sub>2</sub> =1.0%	0.36b	0.41b	0.45b
K <sub>3</sub> =1.5%	0.41a	0.49a	0.49a
<b>T x K</b>			
T <sub>0</sub> K <sub>0</sub> =0 mM GB+0% K	0.33fgh	0.40efg	0.41efg
T <sub>0</sub> K <sub>1</sub> =0 mM GB+0.5% K	0.34efg	0.42def	0.48c
T <sub>0</sub> K <sub>2</sub> =0 mM GB+1% K	0.41b	0.46b	0.56b
T <sub>0</sub> K <sub>3</sub> =0 mM GB+1.5% K	0.55a	0.69a	0.63a
T <sub>1</sub> K <sub>0</sub> =50 mM GB+0% K	0.31hi	0.36hij	0.36hi
T <sub>1</sub> K <sub>1</sub> =50 mM GB+0.5% K	0.32ghi	0.38ghi	0.38f-i
T <sub>1</sub> K <sub>2</sub> =50 mM GB+1% K	0.35def	0.39fgh	0.41efg
T <sub>1</sub> K <sub>3</sub> =50 mM GB+1.5% K	0.38c	0.43cde	0.43de
T <sub>2</sub> K <sub>0</sub> =100 mM GB+0% K	0.30i	0.34j	0.35i
T <sub>2</sub> K <sub>1</sub> =100 mM GB+0.5% K	0.31hi	0.35ij	0.37ghi
T <sub>2</sub> K <sub>2</sub> =100 mM GB+1% K	0.34efg	0.37hij	0.39e-h
T <sub>2</sub> K <sub>3</sub> =100 mM GB+1.5% K	0.35def	0.41def	0.42de
T <sub>3</sub> K <sub>0</sub> =150 mM GB+0% K	0.33fgh	0.41def	0.39e-h
T <sub>3</sub> K <sub>1</sub> =150 mM GB+0.5% K	0.36cde	0.42def	0.42def
T <sub>3</sub> K <sub>2</sub> =150 mM GB+1% K	0.36cd	0.44bcd	0.44d
T <sub>3</sub> K <sub>3</sub> =150 mM GB+1.5% K	0.37c	0.45bc	0.48c
V x T, V x K, V x T x K	Non significant		

Means not sharing the same letters within a column differ significantly at 5% probability.

Auqab-2000 (drought sensitive) cultivar was highly efficient to uptake N and K (Tables 1, 3) whereas significantly high amounts of P and Ca (Tables 2, 4) were taken up by Lasani-2008 (drought tolerant) cultivar in pot and field conditions under drought at grain filling stage of the crop. However, significantly lower amount of Na was taken up by Lasani-2008 under pot conditions but in field conditions non-significant results were obtained (Table 5).

**Table 2. Interactive effect of GB and K spray under drought at grain filling stage on phosphorus uptake (mg/g) of wheat**

Varieties (V)	Pots trial	2009-10	2010-11
V <sub>1</sub> =Lasani-2008	0.91a	1.01a	0.96a
V <sub>2</sub> =Auqab-2000	0.86b	0.97b	0.91b
<b>GB application rate (T)</b>			
T <sub>0</sub> =0 mM	0.80c	0.90c	0.83c
T <sub>1</sub> =50 mM	0.89b	0.99b	0.94b
T <sub>2</sub> =100 mM	0.99a	1.10a	1.04a
T <sub>3</sub> =150 mM	0.87b	0.97b	0.93b
<b>K application rate (K)</b>			
K <sub>0</sub> =0%	0.77c	0.87c	0.85d
K <sub>1</sub> =0.5%	0.82b	0.92b	0.90c
K <sub>2</sub> =1.0%	0.97a	1.07a	0.96b
K <sub>3</sub> =1.5%	0.98a	1.09a	1.02a
<b>T x K</b>			
T <sub>0</sub> K <sub>0</sub> =0 mM GB+0% K	0.70h	0.80h	0.73h
T <sub>0</sub> K <sub>1</sub> =0 mM GB+0.5% K	0.79fg	0.89fg	0.83g
T <sub>0</sub> K <sub>2</sub> =0 mM GB+1% K	0.84ef	0.94ef	0.87fg
T <sub>0</sub> K <sub>3</sub> =0 mM GB+1.5% K	0.85def	0.95def	0.89d-g
T <sub>1</sub> K <sub>0</sub> =50 mM GB+0% K	0.80fg	0.90fg	0.88efg
T <sub>1</sub> K <sub>1</sub> =50 mM GB+0.5% K	0.80fg	0.90fg	0.90d-g
T <sub>1</sub> K <sub>2</sub> =50 mM GB+1% K	0.97bc	1.07bc	0.96cd
T <sub>1</sub> K <sub>3</sub> =50 mM GB+1.5% K	1.00b	1.09b	1.03bc
T <sub>2</sub> K <sub>0</sub> =100 mM GB+0% K	0.83efg	0.93efg	0.93def
T <sub>2</sub> K <sub>1</sub> =100 mM GB+0.5% K	0.87de	0.97de	0.96cd
T <sub>2</sub> K <sub>2</sub> =100 mM GB+1% K	1.13a	1.23a	1.07b
T <sub>2</sub> K <sub>3</sub> =100 mM GB+1.5% K	1.14a	1.27a	1.20a
T <sub>3</sub> K <sub>0</sub> =150 mM GB+0% K	0.78g	0.88g	0.88fg
T <sub>3</sub> K <sub>1</sub> =150 mM GB+0.5% K	0.83efg	0.93efg	0.91def
T <sub>3</sub> K <sub>2</sub> =150 mM GB+1% K	0.92cd	1.02cd	0.95cde
T <sub>3</sub> K <sub>3</sub> =150 mM GB+1.5% K	0.94bc	1.05bc	0.96cd
V x T, V x K, V x T x K	Non significant		

Means not sharing the same letters within a column differ significantly at 5% probability.

Maximum N uptake was recorded in all three trials without the application of GB whereas potassium application at the rate of 1.5% significantly improved the N uptake than lower K application rates and control (Table 1). In the interactive treatments where K and GB were applied in different combinations, maximum increase in N uptake was observed

in plants where only K at 1.5% and no GB was applied (Table 1).

When phosphorus uptake in plants under water stress at grain filling stage was calculated, plants had significantly high P uptake when GB was applied at 100 mM (Table 2). In the same way, K applied at 1.5% showed significantly more P uptake in all three trials followed by K at 1% which showed statistically similar results with K at 1.5% for P uptake in pot trial and field trial during 2009-10 (Table 2). However, the combined application of GB (100 mM) and K (1 and 1.5%), showed significantly high P uptake among the treatments in pot and field trial during 2009-10. But in field trial during 2010-2011, P uptake was significantly high among the treatments due to the combination of 100 mM GB and 1.5% K.

**Table 3. Interactive effect of GB and K spray under drought at grain filling stage on potassium uptake (mg/g) of wheat**

Varieties (V)	Pots trial	2009-10	2010-11
V <sub>1</sub> =Lasani-2008	5.98b	6.35b	6.25
V <sub>2</sub> =Auqab-2000	6.06a	6.40a	6.37
<b>GB application rate (T)</b>			
T <sub>0</sub> =0 mM	5.85c	6.06d	6.15b
T <sub>1</sub> =50 mM	6.12a	6.21b	6.21b
T <sub>2</sub> =100 mM	6.16a	7.10a	6.72a
T <sub>3</sub> =150 mM	5.97c	6.13c	6.19b
<b>K application rate (K)</b>			
K <sub>0</sub> =0%	5.76d	6.04c	6.08d
K <sub>1</sub> =0.5%	5.93c	6.12b	6.24c
K <sub>2</sub> =1.0%	6.36a	6.65a	6.43b
K <sub>3</sub> =1.5%	6.05b	6.70a	6.53a
<b>T x K</b>			
T <sub>0</sub> K <sub>0</sub> =0 mM GB+0% K	5.72k	5.93h	5.98f
T <sub>0</sub> K <sub>1</sub> =0 mM GB+0.5% K	5.78jk	6.03fgh	6.17cde
T <sub>0</sub> K <sub>2</sub> =0 mM GB+1% K	5.98fg	6.13c-f	6.21cde
T <sub>0</sub> K <sub>3</sub> =0 mM GB+1.5% K	5.93gh	6.14c-f	6.25cd
T <sub>1</sub> K <sub>0</sub> =50 mM GB+0% K	6.02ef	6.06efg	6.09def
T <sub>1</sub> K <sub>1</sub> =50 mM GB+0.5% K	6.13c	6.12d-g	6.19cde
T <sub>1</sub> K <sub>2</sub> =50 mM GB+1% K	6.22b	6.26c	6.23cde
T <sub>1</sub> K <sub>3</sub> =50 mM GB+1.5% K	6.11cd	6.42b	6.34c
T <sub>2</sub> K <sub>0</sub> =100 mM GB+0% K	5.45L	6.17cde	6.23cde
T <sub>2</sub> K <sub>1</sub> =100 mM GB+0.5% K	5.88hi	6.21cd	6.34c
T <sub>2</sub> K <sub>2</sub> =100 mM GB+1% K	7.19a	7.99a	7.04b
T <sub>2</sub> K <sub>3</sub> =100 mM GB+1.5% K	6.11cd	8.02a	7.28a
T <sub>3</sub> K <sub>0</sub> =150 mM GB+0% K	5.84ij	5.99gh	6.05ef
T <sub>3</sub> K <sub>1</sub> =150 mM GB+0.5% K	5.92ghi	6.11d-g	6.21cde
T <sub>3</sub> K <sub>2</sub> =150 mM GB+1% K	6.07cde	6.21cd	6.24cde
T <sub>3</sub> K <sub>3</sub> =150 mM GB+1.5% K	6.03def	6.22cd	6.26cd
V x T, V x K, V x T x K	Non significant		

Means not sharing the same letters within a column differ significantly at 5% probability.

Following the similar trend of P uptake, uptake of K was significantly improved with GB at 100 mM as compared to its other dose levels in all the three trials (Table 3). Potassium applied at 1 and 1.5% in field trial during 2009-10 showed significantly high K uptake compared to other K levels whereas K at 1% in pot trial and K at 1.5% in field trial during 2010-11 showed significant improvement in K uptake (Table 3). Combined application of 100 mM GB and 1% K significantly improved K uptake in pot and field trial during 2009-10 whereas treatment (100 mM GB + 1.5% K) showed significant increase in K uptake in both field trials (Table 3).

**Table 4. Interactive effect of GB and K spray under drought at grain filling stage on calcium uptake (mg/g) of wheat**

Varieties (V)	Pots trial	2009-10	2010-11
V <sub>1</sub> =Lasani-2008	2.73 a	3.13 a	3.09 a
V <sub>2</sub> =Auqab-2000	2.62 b	3.02 b	3.03 b
<b>GB application rate (T)</b>			
T <sub>0</sub> =0 mM	2.61 b	3.01 b	2.96 b
T <sub>1</sub> =50 mM	2.72 a	3.12 a	3.09 a
T <sub>2</sub> =100 mM	2.72 a	3.13 a	3.10 a
T <sub>3</sub> =150 mM	2.66 ab	3.06 ab	3.08 a
<b>K application rate (K)</b>			
K <sub>0</sub> =0%	2.41 c	2.81 c	2.79 b
K <sub>1</sub> =0.5%	2.70 b	3.10 b	3.13 a
K <sub>2</sub> =1.0%	2.79 ab	3.22 a	3.14 a
K <sub>3</sub> =1.5%	2.82 a	3.19 ab	3.16 a
<b>T x K</b>			
T <sub>0</sub> K <sub>0</sub> =0 mM GB+0% K	2.36 g	2.76	2.68
T <sub>0</sub> K <sub>1</sub> =0 mM GB+0.5% K	2.59 de	2.99	2.95
T <sub>0</sub> K <sub>2</sub> =0 mM GB+1% K	2.73 bcd	3.13	3.06
T <sub>0</sub> K <sub>3</sub> =0 mM GB+1.5% K	2.76 bc	3.17	3.15
T <sub>1</sub> K <sub>0</sub> =50 mM GB+0% K	2.34 g	2.74	2.82
T <sub>1</sub> K <sub>1</sub> =50 mM GB+0.5% K	2.74 bcd	3.14	3.10
T <sub>1</sub> K <sub>2</sub> =50 mM GB+1% K	2.87 ab	3.27	3.17
T <sub>1</sub> K <sub>3</sub> =50 mM GB+1.5% K	2.94 a	3.34	3.26
T <sub>2</sub> K <sub>0</sub> =100 mM GB+0% K	2.55 ef	2.95	2.86
T <sub>2</sub> K <sub>1</sub> =100 mM GB+0.5% K	2.80 abc	3.20	3.24
T <sub>2</sub> K <sub>2</sub> =100 mM GB+1% K	2.78 abc	3.18	3.19
T <sub>2</sub> K <sub>3</sub> =100 mM GB+1.5% K	2.76 bc	3.16	3.13
T <sub>3</sub> K <sub>0</sub> =150 mM GB+0% K	2.39 fg	2.79	2.83
T <sub>3</sub> K <sub>1</sub> =150 mM GB+0.5% K	2.69 cde	3.09	3.20
T <sub>3</sub> K <sub>2</sub> =150 mM GB+1% K	2.76 bc	3.16	3.18
T <sub>3</sub> K <sub>3</sub> =150 mM GB+1.5% K	2.80 abc	3.20	3.13
V x T, V x K, V x T x K	Non significant		

Means not sharing the same letters within a column differ significantly at 5% probability.

**Table 5. Interactive effect of GB and K spray under drought at grain filling stage on sodium uptake (mg/g) of wheat**

Varieties (V)	Pots trial	2009-10	2010-11
V <sub>1</sub> =Lasani-2008	7.40b	8.21	8.54
V <sub>2</sub> =Auqab-2000	7.52a	8.37	8.68
<b>GB application rate (T)</b>			
T <sub>0</sub> =0 mM	8.15a	9.32a	9.75a
T <sub>1</sub> =50 mM	7.19c	7.99b	8.37b
T <sub>2</sub> =100 mM	7.14d	7.71c	7.84c
T <sub>3</sub> =150 mM	7.36b	8.12b	8.49b
<b>K application rate (K)</b>			
K <sub>0</sub> =0%	8.36a	9.37a	9.76a
K <sub>1</sub> =0.5%	7.20b	8.09b	8.41b
K <sub>2</sub> =1.0%	7.16c	7.92bc	8.23bc
K <sub>3</sub> =1.5%	7.13c	7.77c	8.05c
<b>T x K</b>			
T <sub>0</sub> K <sub>0</sub> =0 mM GB+0% K	11.07a	12.77a	13.45a
T <sub>0</sub> K <sub>1</sub> =0 mM GB+0.5% K	7.26d	8.26b	8.77bc
T <sub>0</sub> K <sub>2</sub> =0 mM GB+1% K	7.18efg	8.20b	8.51b-e
T <sub>0</sub> K <sub>3</sub> =0 mM GB+1.5% K	7.11hi	8.05bc	8.25d-g
T <sub>1</sub> K <sub>0</sub> =50 mM GB+0% K	7.24de	8.26b	8.72bcd
T <sub>1</sub> K <sub>1</sub> =50 mM GB+0.5% K	7.23def	8.00bc	8.42b-f
T <sub>1</sub> K <sub>2</sub> =50 mM GB+1% K	7.16fgh	7.89bc	8.28def
T <sub>1</sub> K <sub>3</sub> =50 mM GB+1.5% K	7.15gh	7.83bc	8.08efg
T <sub>2</sub> K <sub>0</sub> =100 mM GB+0% K	7.34c	8.08bc	8.08efg
T <sub>2</sub> K <sub>1</sub> =100 mM GB+0.5% K	7.10hi	7.96bc	7.95fgh
T <sub>2</sub> K <sub>2</sub> =100 mM GB+1% K	7.08i	7.54cd	7.79gh
T <sub>2</sub> K <sub>3</sub> =100 mM GB+1.5% K	7.06i	7.25d	7.54h
T <sub>3</sub> K <sub>0</sub> =150 mM GB+0% K	7.77b	8.37b	8.81b
T <sub>3</sub> K <sub>1</sub> =150 mM GB+0.5% K	7.22def	8.13b	8.50b-e
T <sub>3</sub> K <sub>2</sub> =150 mM GB+1% K	7.22def	8.05bc	8.32c-f
T <sub>3</sub> K <sub>3</sub> =150 mM GB+1.5% K	7.20d-g	7.95bc	8.32c-f
V x T, V x K, V x T x K	Non significant		

Means not sharing the same letters within a column differ significantly at 5% probability.

Calcium uptake significantly increased with the application of GB in plants under drought at grain filling stage but highest uptake among the treatments was with 100 mM GB application (Table 4). In the same way, K application significantly increased Ca uptake in plants where highest increases were recorded by K at 1.5% in pot and field trial (year 2010-11). In field trial during 2009-10, K application at 1% gave highest increase in the Ca uptake among the treatments (Table 4). The combined inoculation of GB and K at 50 mM GB and 1.5%, showed significantly high Ca uptake in plant under drought at grain filling stage under pot conditions. However, Ca uptake in both field trials (2009-10 and 2010-11) remained statistically similar among all the interactive treatments of GB and K (Table 4).

Highest uptake of Na in plants was recorded under drought at grain filling stage in pot as well as field conditions where no GB or K was applied (Table 5). However, the application

of GB and K at 100 mM and 1.5% significantly reduced the uptake of Na under drought at grain filling stage of the crop (Table 5).

## DISCUSSION

Drying soils lose water rapidly, destroying the connectivity between soil particles and matric potential of soil reaches to maximum negative. Decreased soil matrix potential exposes the crop plants to a dilemma of osmotic and water deficit stress which ultimately inhibits crop growth and productivity. Similarly, hydraulic conductivity of root membranes is deteriorated due to the impaired functioning of water channels with the impact of Na<sup>+</sup> (Carvajal *et al.*, 2000). Drought stress leads to nutrient deficiency or toxicity problems in crop plants which reduce the crop productivity. However, the survivability and tolerance of plants to drought is crucial for sustainable productivity where mineral nutrition plays a vital role (Mengel and Kirkby, 2001; Aslam *et al.*, 2013). In wheat, drought at grain filling stage is most detrimental to growth, productivity and mineral nutrition (Raza *et al.*, 2012) therefore, in the present experiments significantly reduced activity of plants was recorded in terms of nutrient uptake. But exogenous application of glycine betaine or potassium significantly improved the uptake of N, P, K and Ca in plants for regulating osmotic adjustment and plant growth. Conversely, reduced Na uptake decreased its lethal impacts on wheat plants. It was demonstrated in a study (Abd El-Hadi *et al.*, 1997) that the reduction in crop yield due to restricted irrigation can be eliminated by the application of K which was further supported by Valadabadi and Farahani (2009) study.

Highest significant increases in the N uptake were recorded up to 67, 72 and 54% in pot, first and second year field trials, respectively, due to the foliar application of K at 1.5% and without glycine betaine. These increases in N contents and plant uptake can be due to the accumulation of free amino acids in plants which could not be synthesized into proteins under water deficit stress (Alam, 1994) because under drought conditions the enzymes responsible for the conversion of nitrate into ammonia *i.e.* nitrate reductase are badly affected (Sinha and Nicholas, 1981) or increased protease activity. Most of the nitrate accumulations come from the leaves and other plant tissue (Sarwar *et al.*, 1991) which might be due to inhibition of N translocation from root to shoot under stress (Khalil and Mandurah, 1990). Similar increase in N uptake (about 27%) was observed by Raza *et al.* (2013) when they applied K on wheat plants under drought at grain filling stage whereas 51% decrease in the accumulation was observed due to the application of glycine betaine. These increases in N contents in crop plants are involved in osmotic adjustment in wheat under drought (Raza *et al.*, 2012).

Reduced moisture in soil decreased the uptake of P in plants (Baligar *et al.*, 2001) which ultimately appeared the P deficiency symptoms under low or moderate drought stress (Alam, 1994). When in the present studies, K and glycine betaine at 1.5% and 100 mM, respectively, were applied to wheat crop under drought at grain filling stage, a significant improvement in the P uptake was observed. However, the highest increases were recorded up to 63, 59 and 65% in pot, first and second year field trials, respectively, with K and glycine betaine (at 1.5% and 100 mM) application. In the same way, Raza *et al.* (2012) observed significant improvement in P uptake of wheat under drought where glycine betaine was applied as foliar spray. In another study, Raza *et al.* (2013) have demonstrated that the foliar application of K to wheat plants under drought at grain filling stage increased the P uptake up to 36% over untreated control.

Potassium a very essential nutrient for plant growth, development and involved in plant defense against biotic and abiotic stresses. Potassium is also accumulated in plants to regulate osmotic adjustment under drought or osmotic stress (Maser *et al.*, 2002; Aslam *et al.*, 2013). In the present studies, combined application of glycine betaine and K (100 mM and 1%, respectively) significantly increased the K uptake in wheat plants under drought at grain filling stage where highest increases were 26, 35 and 18% in pot, first and second year field trials, respectively. Potassium is accumulated in meristematic regions of plants due to their higher mobility. It moves from older to younger leaves and get accumulated in ears for osmotic adjustment (Arnon, 1972). Similar increases in the K uptake were demonstrated by Raza *et al.* (2013) in wheat where they applied K under drought stress. Ashraf (1998) and Khondakar *et al.* (1983) have also noted increases in the K contents in plants facing drought stress. Contrary to our results, Raza *et al.* (2012) showed decrease in the accumulation of K in plants under drought where glycine betaine was applied as foliar.

Calcium plays vital role in plant response to drought stress. Therefore, in our studies, about 24, 21 and 22% increases in Ca uptake of wheat plants in pot, first and second year field trials, respectively, under drought at grain filling stage where glycine betaine and K (50 mM and 1.5%) were applied in combination. In wheat, Ca has been reported to decrease the devastating impact of drought by increasing the concentration of compatible solutes (glycine betaine and proline) which help in the amelioration of stress in the growth of seedlings, improve plant water status and reduce membrane injury (Nayyar, 2003). Calcium is of great significance in many plant physiological functioning and responses to abiotic stresses. It acts as a signal transmitter to help in the regulation of plant growth and development under stress. Most importantly it regulates the opening and closing of stomata and adaptation of plant to drought through ABA homeostasis (Song *et al.*, 2008; Aldesuquy *et*

al., 2013). In a similar study, significant increase in the Ca uptake was recorded in wheat plants under drought stress where the plants were sprayed with glycine betaine solution (Raza et al., 2012). But the application of K showed 18% increase in the Ca uptake in wheat plants under drought at grain filling stage (Raza et al., 2013).

Drought stress increases the uptake of Na in plants where it may act as protector or destructor of plant biosystems because it can replace K in many plant functions. Sometimes it is required for osmotic adjustment and the regulation of plant photosystems (Brownell and Bielig, 1996; Carvajal et al., 2000). However, in our studies, Na uptake in wheat decreased significantly up to 36, 40 and 44% in pot, first and second field trials, respectively, due to the combined application of 100 mM glycine betaine and 1.5% K under drought at grain filling stage. Similar results were obtained by Raza et al. (2012) when they applied glycine betaine on wheat plant under drought at grain filling stage of wheat and about 26% decrease in Na accumulation was recorded. However, Ashraf (1998) could not define any relationship between Na concentration and drought stress in plants. Moreover, highest increases in Na contents (up to 31%) were recorded by Raza et al. (2013) under drought stress at grain filling stage whereas the application of K reduced its concentration.

**Conclusion:** Drought at grain filling stage of wheat crop is detrimental to crop mineral nutrition because of the reduced soil moisture which inhibits the optimal crop growth and development. The water deficit situation increase or decrease the uptake of N, P, K, Ca and Na in plants. But the application of K at 1.5% in combination with glycine betaine at 100 mM seems to be most beneficial in diluting the impact of drought at grain filling stage on the mineral nutrition of the crop and drought tolerance ability. Further it is suggested that the responses of wheat crop to these osmolytes should be studied at molecular level under drought to identify the mechanisms which are tangled for variable plant responses.

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