

Original Article

Antioxidant status and their enhancements strategies for water stress tolerance in chickpea

Status antioxidante e suas estratégias de aprimoramento para tolerância ao estresse hídrico no grão-de-bico

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Abstract

Water stress executes severe influences on the plant growth and development through modifying physio-chemical properties. Therefore, a field experiment was designed to evaluate the antioxidant status and their enhancements strategies for water stress tolerance in chickpea on loam and clay loam soils under agro-ecological conditions of Arid Zone Research Institute, Bahawalpur (29.3871 °N, 71.653 °E) and Cholistan farm near Derawer (28.19°N, 71.80°E) of Southern Punjab, Pakistan during winter 2014-15. Experimental treatments comprised of two chickpea cultivars i.e. Bhakhar 2011 (drought tolerant) and DUSHT (drought sensitive), two water stress levels i.e. water stress at flowering stage and water stress at flowering + pod formation + grain filling stage including well watered (control) and two exogenous application of osmoprotectants i.e. glycine betaine (GB) 20 ppm and proline 10 uM including distilled water (control). Results indicated that water stress at various growth stages adversely affects the growth, yield and quality attributes of both chickpea cultivars. Exogenous application of GB and proline improved the growth, yield and quality parameters of both chickpea cultivars even under water stress conditions. However, superior results were obtained with exogenously applied GB on Bhakhar 2011 under well-watered conditions. Similarly, foliar spray of GB on chickpea cultivar Bhakhar 2011 under stress at flowering + pod formation + grain filling stage produced maximum superoxide dismutase, peroxidase and catalase contents. These results suggested that application of GB mitigates the adverse effects of water stress and enhanced tolerance in chickpea mainly due to higher antioxidant enzymes activity, demonstrating the protective measures of plant cells in stress condition. Hence, antioxidants status might be a suitable method for illustrating water stress tolerance in chickpea.

Keywords: superoxide dismutase, peroxidase, catalase, glycine betaine, proline.

Resumo

O estresse hídrico exerce fortes influências no crescimento e no desenvolvimento das plantas, modificando as propriedades físico-químicas. Portanto, a presente atividade de pesquisa foi projetada para avaliar o *status* antioxidante e suas estratégias de aprimoramento para tolerância ao estresse hídrico no grão-de-bico em condições agroecológicas, no Instituto de Pesquisa da Zona Árida, Bahawalpur (29.3871 ° N, 71.653 ° E) e fazenda do Cholistan, perto de Derawer (28.19 ° N, 71.80 ° E), no sul de Punjab, Paquistão, durante Rabi 2014-15. Tratamentos experimentais compostos de dois genótipos de grão-de-bico, como Bhakhar 2011 (tolerante à seca) e DUSHT (sensível à seca), dois níveis de estresse hídrico, ou seja, estresse hídrico no estágio de floração, estresse hídrico na fase de floração e estresse hídrico na fase de floração + formação de vagem + estágio de enchimento de grãos, incluindo água bem controlada (controle) e duas aplicações exógenas de osmoprotetores, isto é, glicina betaína 20 ppm e prolina 10 uM, incluindo água destilada (controle). Os resultados indicaram que o estresse hídrico em vários estágios de crescimento afeta negativamente os atributos de crescimento, rendimento e qualidade de ambas as cultivares de grão-de-bico. A aplicação exógena de glicina betaína e prolina melhorou os parâmetros de crescimento, rendimento e qualidade de ambos os genótipos de grão-de-bico, mesmo sob condições de estresse hídrico. No entanto, resultados superiores foram obtidos com glicina betaína aplicada exogenamente em Bhakhar 2011, em condições bem regadas. Além disso, o *spray* foliar de glicina betaína na cultivar de grão-de-bico Bhakhar 2011, sob estresse na fase de floração + formação de vagem + enchimento de grãos, produziu o máximo de superóxido dismutase, peroxidase e catalase. Esses resultados sugeriram que a aplicação de glicina betaína atenua os efeitos adversos do estresse hídrico e aumenta a tolerância no grão-de-bico, principalmente pela maior atividade de enzimas antioxidantes, demonstrando medidas protetoras das células vegetais em condições de estresse. Portanto, o *status* de antioxidantes pode ser um método adequado para ilustrar a tolerância ao estresse hídrico no grão-de-bico.

Palavras-chave: superóxido dismutase, peroxidase, catalase, glicina betaína, prolina.

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1. Introduction

Chickpea (*Cicer arietinum* L.) belongs to leguminosae family, plays an important role in human nutrition for large population sectors in the developing world and is considered as a healthy food in many developed countries (Merga and Haji, 2019). In addition to its high protein content (20-28%) and carbohydrates (60-65%), chickpea is a good source of essential amino acids and minerals (Merga and Haji, 2019). Moreover, chickpea is a crucial source of fodder and also fixes atmospheric nitrogen through symbiotic nitrogen fixation, hence plays a key role in enhancing and sustaining the overall productivity of the cropping systems (Khaitov et al., 2016).

Chickpea is traditionally cultivated in arid and semi-arid tropical regions under rain-fed condition on residual moisture preserved after monsoon rains (during July and August). It is susceptible to terminal water stress due to depletion of stored soil moisture towards maturation, and experiences up to 50% yield loss (Sinha et al., 2019). Plants experience water stress either when the water supply to roots is interrupted or when transpiration rate becomes very high. When plants are subjected to water stress conditions, show lot of morpho-physiological and biochemical changes (Reddy and Raghavendra, 2006). Under such conditions, some reactive oxygen species (ROS) are produced (Munne-Bosch and Penuelas, 2003). While the amount of ROS production beats the antioxidant defense competency of the cell, ensuing in cellular damages (Almeselmani et al., 2006). Imbalance between the production of ROS and the antioxidant defense inhibits the growth and photosynthetic abilities of plants and injure the cellular components of proteins, membrane lipids and nucleic acids under water stress conditions (Peifang et al., 2015).

Plants adapted to water stress accumulate particular molecules, which have a cryo-protective role (Farooq et al., 2009). Glycine betaine (GB) is one such cryo-protective solute that stabilizes membranes and photosynthetic apparatus, detoxification of ROS, cellular osmotic adjustment and protects the activities of proteins and enzymes involved in the antioxidant defense system under water stress conditions (Chen and Murata, 2011). GB is an organic compound present in plants, that is an amphoteric quaternary amine, plays an imperative part as a compatible solute in plants under water stress conditions (Ashraf and Foolad, 2007). It accumulates in response to water stress in the leaves of some plants that may confer tolerance to the osmotic stress (Farooq et al., 2008). It is primarily abundant in chloroplast where it plays a dynamic role in adjustment and protection of thylakoid lamella, thereby improving the photosynthetic efficiency under water stress conditions (Wang et al., 2010).

In addition to GB, exogenous application of proline is attaining important consideration to cope with water stress (Sadak and Ahmed, 2016). Proline is a proteinogenic amino acid, extremely important for numerous vital metabolic processes within the plant tissues. At optimum level, it has various roles such as osmoprotectant, balance of redox status, storage of carbon and nitrogen, turgor generation, maintenance the structure of proteins and cytosolic pH, acting as a part of stress signal, an inhibitor of

lipid membrane peroxidation and antioxidant as effective quencher of ROS formed under stress conditions in plants (Hayat et al., 2012). Proline can act as a signaling molecule to influence cell proliferation, modify mitochondrial functions and trigger specific gene expression, which can be vital for plant recovery from water stress conditions (Hayat et al., 2012). Limited number of studies was conducted on the influence of GB and proline on the chickpea productivity under stress conditions. It is hypothesized that exogenous application of GB and proline may positively affect the physiological attributes of chickpea and improved the growth and productivity under water stress conditions. Keeping these facts in view, the present investigation was therefore undertaken to study the role of osmoprotectants to mitigate the adverse effect of water stress on chickpea genotypes through the modulation of antioxidant enzymes status.

2. Materials and Methods

2.1. Experimental detail

The present research activity was designed to evaluate the antioxidant status and their enhancements strategies for water stress tolerance in chickpea under agro-ecological conditions of Arid Zone Research Institute, Bahawalpur (29.3871 °N, 71.653 °E) and Cholistan farm near Derawer (28.19 °N, 71.80 °E) of Southern Punjab, Pakistan during winter 2014-15. Experimental treatments comprised of two chickpea genotypes i.e. Bhakhar 2011 (drought tolerant) and DUSHT (drought sensitive), two water stress levels i.e. Water stress at flowering stage and water stress at flowering + pod formation + grain filling stage including well watered (control) and two exogenous application of osmoprotectants i.e. glycine betaine 20 ppm (Bardhan et al., 2007) and proline 10 uM (Kaur et al., 2011) including distilled water (control). Foliar spray was applied twice at 50% flowering and at 50% pod formation stage. Field experiment was arranged in a Randomized Complete Block Design with factorial experiment having three repeats.

Prior to sowing, composite soil samples were collected from experimental areas at the depth of 0-30 cm and examined for physico-chemical features. The soil texture of experimental areas were loam and clay loam, having pH 8.2 and 7.9, organic matter 0.77 and 0.93%, EC 2.5 and 2.1 dS m⁻¹, total nitrogen 0.034 and 0.049%, available phosphorus 6.3 and 4.5 ppm and exchangeable potassium 192 and 94 ppm in Bahawalpur and Cholistan, respectively.

2.2. Crop husbandry

Pre-soaking irrigation was applied before seedbed preparation. When the soil reached to a workable moisture level, seedbed was prepared by cultivating the field twice with tractor-mounted cultivator each followed by planking during winter 2014-15. Seeds of both chickpea cultivars was sown by hand drill using 60 kg ha⁻¹ seed rate by keeping row to row distance of 25 cm and plant to plant distance 10 cm during last week of October 2014. Seeds were placed at 3 to 5 cm depth in each row. The seedlings were thinned out by maintaining one plant per

hill at 15 days after sowing. Recommended dose of Urea @ 20 Kg N ha⁻¹ and Di-ammonium phosphate (DAP) @ 22 Kg P ha⁻¹ was applied at the time of seedbed preparation. Plant protection measures and agronomic practices were kept uniform and normal for all the experimental units throughout the growing season.

2.3. Data collection

Thirty days after sowing, ten randomly selected plants were tagged to record the secondary branches per plant, number of pods per plant and 100 grain weight.

Growth parameters were examined three times during growing seasons at both locations. Data regarding CGR was recorded by adopting the following Formula 1 of Watson (1952).

$$\text{CGR} (\text{g m}^{-2}\text{day}^{-1}) = (W_2 - W_1) / (T_2 - T_1) \quad (1)$$

whereas, W_1 and W_2 is the oven-dried weight at first and second sampling, T_1 and T_2 is the time of first and second sampling.

Relative growth rate (RGR) was calculated by using Equation 2 proposed by Radford (1967).

$$\text{RGR} (\text{g g}^{-1}\text{day}^{-1}) = (\text{Ln}W_2 - \text{Ln}W_1) / (T_2 - T_1) \quad (2)$$

whereas, Ln= Natural Log, W_1 and W_2 is the oven-dried weight at first and second sampling, T_1 and T_2 is the time of first and second sampling.

Total soluble protein (mg g⁻¹) was determined according to procedure followed by Bradford (1976). The K⁺ content in leaves were determined by flame photometer (Richards, 1954). Superoxide dismutase (IU min⁻¹mg⁻¹ protein) was calculated by adopting the methodology followed by (Giannopolitis and Ries, 1977). Moreover, peroxidase (IU min⁻¹mg⁻¹ protein) and catalase (IU min⁻¹mg⁻¹ protein) was calculated by adopting the procedure followed by Chance and Maehly (1955). 1m² from each experimental unit was harvested and threshed manually to record the grain yield per unit area on 11th April 2015.

2.4. Statistical analysis

All the collected data was analyzed by using the MSTAT software. The Duncan's Multiple Range test (DMRt) was used to compare the differences among treatment means at 5% probability level (Steel et al., 1997).

3. Results

3.1. Crop growth rate (CGR) (g m⁻² day⁻¹)

CGR is the rate of dry matter accumulation per unit ground area. Exogenous application of osmoprotectants on chickpea genotypes under water stress conditions significantly affected the CGR recorded at different growth stages at both locations (Figure 1a and 1b). The crop growth rate (CGR) followed an increasing trend in the initial stages of crop growth and decreased thereafter. Exogenous application of glycine betaine on chickpea

cultivar Bhakhar 2011 cultivated under well-watered condition produced significantly higher CGR at all growth stages. Chickpea genotype DUSHT cultivated under water stress at flowering + pod formation + grain filling stage produced minimum CGR throughout the growing season.

3.2. Relative Growth Rate (RGR) (g g⁻¹ day⁻¹)

RGR is the increase in dry mass per unit of plant mass over a specified period of time. Exogenous application of osmoprotectants on chickpea genotypes under water stress conditions significantly affected the RGR recorded at different growth stages at both locations (Figure 2a and 2b). Exogenous application of glycine betaine on chickpea cultivar Bhakhar 2011 cultivated under well-watered condition produced significantly higher RGR at all growth stages. Chickpea cultivar DUSHT cultivated under water stress at flowering + pod formation + grain filling stage produced minimum RGR throughout the growing season at both locations.

3.3. Number of secondary branches per plant

Secondary branches develop at buds located on the primary branches and determines the total number of leaves, and hence the total photosynthetic area. Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the number of secondary branches per plant at both locations (Table 1). Exogenous application of glycine betaine on Bhakhar 2011 cultivated under well watered conditions produced maximum number of secondary branches per plant against the minimum was observed from DUSHT cultivated under water stress condition at flowering + pod formation + grain filling stage.

3.4. Number of pods per plant

Number of pods per plant as the major yield parameters reflects the plant performance during previous growth stages, which depend mainly on the vigorous of vegetative growth and flowering status. Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the number of pods per plant at both locations (Table 1). Exogenous application of glycine betaine on Bhakhar 2011 cultivated under well watered conditions produced maximum number of pods per plant against the minimum was observed from DUSHT cultivated under water stress condition at flowering + pod formation + grain filling stage.

3.5. 100 grains weight (g)

Grains weight is crucial yield boosting attribute and has been given due consideration during varietal assessing process. Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the 100 grains weight at both locations (Table 1). Exogenous application of glycine betaine on Bhakhar 2011 cultivated under well watered

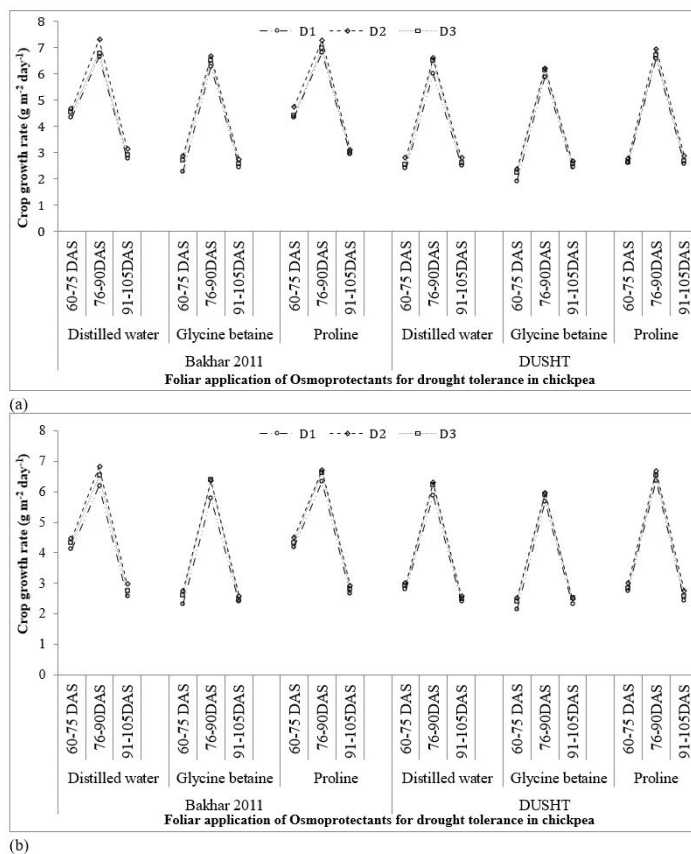


Figure 1. (a) Influence of exogenous application of osmoprotectants on crop growth rate (g m⁻² day⁻¹) of chickpea genotypes in Bahawalpur; (b) Influence of exogenous application of osmoprotectants on crop growth rate (g m⁻² day⁻¹) of chickpea genotypes in Cholistan. Whereas D1= well watered; D2= Drought at flowering+ pod formation + grain filling stage; D3= Drought at flowering stage; DAS, Days after sowing.

conditions produced maximum 100 grains weight against the minimum was observed from DUSHT cultivated under water stress condition at flowering + pod formation + grain filling stage.

3.6. Grain yield (kg ha⁻¹)

Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the final grain yield at both locations (Table 2). Exogenous application of glycine betaine on Bhakhar 2011 cultivated under well watered conditions produced maximum grain yield against the minimum was observed from DUSHT cultivated under water stress at flowering + pod formation + grain filling stage.

3.7. Leaf total soluble protein (mg g⁻¹)

Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the leaf total soluble protein at both locations (Table 2). Exogenous application of glycine betaine on Bhakhar 2011 cultivated under well watered conditions produced maximum leaf total soluble protein. While minimum leaf total soluble protein was observed

from DUSHT cultivated under water stress condition at flowering + pod formation + grain filling stage.

3.8. Leaf K⁺ contents

Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the leaf K⁺ contents at both locations (Table 2). Exogenous application of glycine betaine on Bhakhar 2011 cultivated under water stress at grain filling stage produced maximum leaf K⁺ contents. While minimum leaf K⁺ contents was observed from DUSHT cultivated under water stress condition at flowering + pod formation + grain filling stage.

3.9. Superoxide dismutase (SOD) (IU min⁻¹mg⁻¹ protein)

Superoxide dismutase (SOD) establishes the first line of protection through detoxification of superoxide radicals, hence sustain membranes of plant tissue (Sairam and Saxena, 2000). Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the SOD at both locations (Table 3). Exogenous application of glycine betaine on Bhakhar 2011 cultivated under water stress at flowering + pod

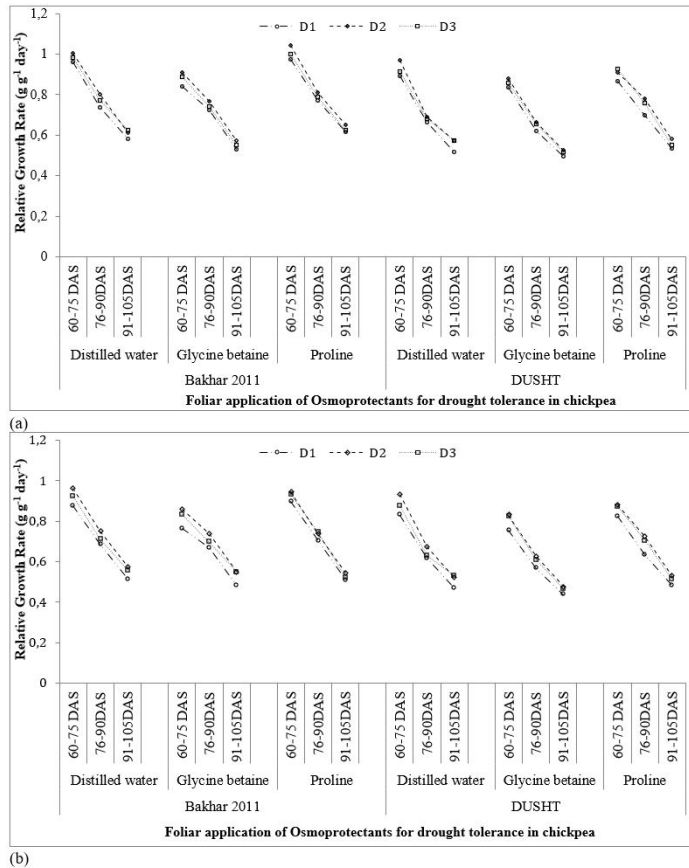


Figure 2. (a) Influence of exogenous application of osmoprotectants on relative growth rate ($\text{g g}^{-1} \text{day}^{-1}$) of chickpea genotypes in Bahawalpur; (b) Influence of exogenous application of osmoprotectants on relative growth rate ($\text{g g}^{-1} \text{day}^{-1}$) of chickpea genotypes in Cholistan. Whereas D1= well watered; D2= Drought at flowering+ pod formation + grain filling stage; D3= Drought at flowering stage; DAS, Days after sowing.

formation + grain filling stage produced maximum SOD. While minimum SOD was observed from DUSHT cultivated under well-watered condition.

3.10. Peroxidase (POD) ($\text{IU min}^{-1}\text{mg}^{-1} \text{protein}$)

Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the POD at both locations (Table 3). Exogenous application of glycine betaine on Bhakar 2011 cultivated under water stress at flowering + pod formation + grain filling stage produced maximum POD. While minimum POD was observed from DUSHT cultivated under well-watered condition.

3.11. Catalase (CAT) ($\text{IU min}^{-1}\text{mg}^{-1} \text{protein}$)

Catalase (CAT) eradicates H_2O_2 by breaking it down directly to water and oxygen. CAT and POD are the essential enzymes involved in regulation of intracellular level of H_2O_2 . Comparison of the treatment means showed that exogenous application of osmoprotectants on chickpea genotypes under water stress at different growth stages significantly affected the CAT at both locations (Table 3).

Exogenous application of glycine betaine on Bhakar 2011 cultivated under water stress at flowering + pod formation + grain filling stage produced maximum CAT. While minimum CAT was observed from DUSHT cultivated under well-watered condition.

4. Discussion

Water stress inhibited the growth and development of chickpea plants and showed several morphological and physiological modifications (Raheleh et al., 2012). In present study, water stress at flowering + pod formation + grain filling stage significantly reduced the crop and relative growth rate of chickpea cultivars at both locations. Crop growth is accomplished through cell division, cell enlargement and cell differentiation and involves genetic, physiological, ecological and morphological events and their complex interactions. Under water stress conditions, cell elongation of plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Awari et al., 2017). However, application of osmoprotectants improves the crop growth both under stressed and well-watered

Table 1. Influence of exogenous application of osmoprotectants on secondary branches, number of pods and 100-grain weight (g) of chickpea.

Chickpea genotypes	Water stress level	Foliar spray	Secondary branches per plant		Number of pods per plant		100-grain weight (g)	
			Bahawalpur	Cholistan	Bahawalpur	Cholistan	Bahawalpur	Cholistan
Bhakhar 2011	Well watered	Distilled water	9.50 ac	9.76 ac	26.27 bd	28.46 ad	202.60 ac	204.94 ab
		Glycine betaine	9.85 a	10.16 a	31.16 a	31.52 a	208.19 a	209.86 a
		Proline	9.27 ad	9.95 ab	29.10 ac	30.44 ab	206.33 ab	205.67 ab
	Water stress at flowering + pod formation + grain filling stage	Distilled water	8.18 df	8.51 eh	21.68 eg	24.40 cf	186.19 cd	189.86 cd
		Glycine betaine	8.93 ad	8.96 df	27.61 ad	28.27 ad	201.12 ac	202.70 ab
		Proline	8.31 ce	8.71 dg	26.37 bd	26.72 ae	197.37 ad	194.03 ad
	Water stress at flowering stage	Distilled water	8.61 be	8.84 df	26.31 bd	26.69 ae	198.63 ac	197.30 ac
		Glycine betaine	9.60 ab	9.36 bd	29.45 ab	29.88 ac	206.90 ab	205.23 ab
		Proline	8.97 ad	9.04 df	28.09 ad	28.76 ad	201.35 ac	202.01 ab
DUSHT	Well watered	Distilled water	8.45 be	9.02 de	25.71 be	26.37 ae	194.18 ad	196.51 ac
		Glycine betaine	8.90 ad	9.23 cd	27.12 ad	28.22 ad	199.73 ac	203.07 ab
		Proline	8.55 be	9.13 ce	25.50 bf	27.13 ae	195.52 ad	198.76 ac
	Water stress at flowering + pod formation + grain filling stage	Distilled water	7.01 f	6.94 j	19.29 g	20.12 f	163.32 e	169.98 e
		Glycine betaine	8.27 de	7.99 hi	24.28 df	23.62 df	186.17 cd	182.84 ce
		Proline	8.19 df	8.05 gi	21.14 fg	21.81 ef	179.85 de	178.85 de
	Water stress at flowering stage	Distilled water	7.67 ef	7.83 i	24.83 cf	25.49 bf	185.16 cd	178.56 de
		Glycine betaine	9.13 ad	8.96 df	26.45 bd	27.14 ae	195.71 ad	193.18 ad
		Proline	8.28 ce	8.45 fi	25.89 be	26.96 ae	188.51 bd	190.51 bd
LSD 0.05p=			1.2204	0.6690	4.3911	5.5971	18.481	16.937

LSD= Least significant difference.

conditions. Exogenous application of GB on chickpea cultivar Bhakhar 2011 cultivated under well-watered conditions produced significantly higher crop and relative growth rate at all growth stages. Earlier studies showed the positive influence of GB on plant growth (Ashraf and Foolad, 2007). Foliar spray of GB can promptly penetrate through leaf surface and be easily transferred to other parts, where it would contribute to enhancement in water stress tolerance (Ashraf and Foolad, 2007). Foliarly applied GB induced tolerance might be credited to reducing of the osmotic potential due to net solute accumulation in response to water stress, which could assist to preserve

the metabolic processes, contribute to growth of plants through maintaining turgor in cells and ultimately increase drought tolerance, thus maintaining higher photosynthetic activities (Hussain et al., 2009).

The results of present investigation revealed that the number of branches per plant was reduced significantly due to the water stress as compared to the well-watered condition. However, exogenous application of GB produced significantly higher number of branches plant even under water stress conditions. As it was earlier literature showed that GB application at early stage of crop was found to be more effective in ameliorating the negative impact of water

Table 2. Influence of exogenous application of osmoprotectants on grain yield (kg ha⁻¹), leaf soluble protein (mg g⁻¹) and leaf K⁺ contents of chickpea.

Chickpea genotypes	Water stress level	Foliar spray	Grain yield (kg ha ⁻¹)		Leaf total soluble protein (mg g ⁻¹)		leaf K ⁺ contents	
			Bahawalpur	Cholistan	Bahawalpur	Cholistan	Bahawalpur	Cholistan
Bhakhar 2011	Well watered	Distilled water	1532.7 bd	1792.1 ac	1.07 ac	1.02 a	49.52 ad	44.85 ab
		Glycine betaine	1928.6 a	1950.7 a	1.15 a	1.11 a	52.62ac	48.28 a
		Proline	1732.0 ab	1844.7 ac	1.09 ac	1.06 a	50.30 ad	46.97 a
	Water stress at flowering + pod formation + grain filling stage	Distilled water	1165.2 eg	1261.7 fh	0.46 d	0.43 b	47.41 ad	42.41 ab
		Glycine betaine	1537.1 bd	1527.6 cf	0.53 d	0.50 b	51.38 ac	45.38 ab
		Proline	1457.5 be	1427.0 eg	0.51 d	0.46 b	50.13 ad	44.13 ab
	Water stress at flowering stage	Distilled water	1458.5 be	1468.3 df	1.08 ac	1.05 a	51.65 ac	41.65 ab
		Glycine betaine	1884.8 a	1862.6 ab	1.12 ab	1.08 a	54.69a	47.36 a
		Proline	1689.5 ac	1760.2 ad	1.08 ac	1.06 a	53.68 ab	45.01 ab
DUSHT	Well watered	Distilled water	1411.3 bf	1544.4 cf	0.99 c	0.97 a	47.98ad	39.31 ab
		Glycine betaine	1529.9 bd	1832.6 ac	1.03 ac	1.01 a	52.24ac	44.91 ab
		Proline	1525.1 bd	1656.3 ae	1.01 bc	1.00 a	51.03 ac	41.03 ab
	Water stress at flowering + pod formation + grain filling stage	Distilled water	1029.9 g	963.1 h	0.43 d	0.41 b	41.76 d	37.16 b
		Glycine betaine	1352.3 dg	1254.3 fh	0.49 d	0.46 b	45.40 bd	42.07 ab
		Proline	1123.5 fg	1129.3 gh	0.45 d	0.43 b	44.86 cd	41.53 ab
	Water stress at flowering stage	Distilled water	1385.2 cf	1373.2 eg	0.99 c	0.98 a	48.70 ad	42.03 ab
		Glycine betaine	1628.8 ad	1580.3 be	1.02 bc	1.04 a	52.43 ac	45.77 ab
		Proline	1556.2 bd	1533.1 cf	1.01 bc	1.03 a	51.43 ac	43.93 ab
LSD 0.05p=			326.67	318.00	0.1225	0.1467	8.6519	9.2161

LSD= Least significant difference.

stress by maintenance of leaf water status due to enhanced osmotic adjustment and improved photosynthesis, mainly due to a higher stomatal conductance (Iqbal et al., 2008).

Productivity of a crop mainly depends on its effective utilization of available moisture. Application of irrigation water at reproductive stage of chickpea improved the photosynthetic rate and also increased the grain filling period, consequently improving the number of pods per plant, grain weight and ultimately the grain yield (Allu et al., 2014). Conversely, water stress restricted the chickpea

productivity by reducing the plant growth, canopy structure as indicated by leaf senescence, photosynthesis, chlorophyll contents that ultimately produced lower photosynthates and inefficient remobilization of the photo-assimilates from source to pods that resulted in short and shriveled grains (Sankar et al., 2007). Exogenous application of GB improved the number of pods per plant, grain weight and grain yield both under water stress and well-watered conditions. Foliar spray of GB improved the productivity by improving the water potential, osmotic potential,

Table 3. Influence of exogenous application of osmoprotectants on antioxidant enzymes status of chickpea.

Chickpea genotypes	Water stress level	Foliar spray	Superoxide dismutase (IU min ⁻¹ mg ⁻¹ protein)		Peroxidase (mmol min ⁻¹ mg ⁻¹ protein)		Catalase (μ mol min ⁻¹ mg ⁻¹ protein)	
			Bahawalpur	Cholistan	Bahawalpur	Cholistan	Bahawalpur	Cholistan
Bhakhar 2011	Well watered	Distilled water	72.01 g	63.98 ef	7.29 de	6.63 de	40.32 eg	34.49 fh
		Glycine betaine	95.45 e	74.10 e	9.57 c	7.90 de	48.24 de	45.47 e
		Proline	91.19 ef	69.93 ef	8.54 cd	7.47 de	45.25 de	37.98 eh
	Water stress at flowering + pod formation + grain filling stage	Distilled water	195.95 c	184.10 c	11.80 b	13.46 b	84.31 bc	77.21 cd
		Glycine betaine	227.23 a	217.09 a	13.47 a	17.23 a	105.31 a	101.04 a
		Proline	214.95 ab	203.62 ab	12.48 ab	16.11 a	102.41 a	95.97 a
	Water stress at flowering stage	Distilled water	71.58 g	67.89 ef	7.14 de	6.91 de	43.80 df	36.20 eh
		Glycine betaine	91.49 e	73.03 ef	8.61 cd	8.11 de	52.94 d	44.49 ef
		Proline	81.01 eg	71.39 ef	8.47 cd	7.88 de	47.55 de	42.77 ef
DUSHT	Well watered	Distilled water	65.82 g	60.15 f	5.66 e	6.13 e	32.29 g	28.27 h
		Glycine betaine	78.58 eg	68.57 ef	7.26 de	6.93 de	42.37 dg	40.76 eg
		Proline	70.79 g	66.29 ef	6.32 e	6.49 de	40.81 eg	34.62 fh
	Water stress at flowering + pod formation + grain filling stage	Distilled water	166.64 d	159.88 d	9.47 c	10.15 c	74.51 c	71.90 d
		Glycine betaine	207.49 bc	196.59 bc	11.63 b	13.16 b	90.56 b	92.05 ab
		Proline	194.99 c	184.25 c	11.29 b	12.62 b	78.36 c	82.69 bc
	Water stress at flowering stage	Distilled water	66.86 g	62.23 ef	6.54 e	6.41 de	33.03 fg	30.73 gh
		Glycine betaine	74.21 fg	67.28 ef	8.42 cd	8.10 de	40.97 eg	43.44 ef
		Proline	72.26 g	64.73 ef	8.25 cd	8.15 d	37.98 eg	39.34 eg
LSD 0.05p=			17.074	13.647	1.6400	1.9988	11.118	10.374

LSD= Least significant difference.

turgor pressure, water use efficiency that induced water stress tolerance in crop, thus keeping high photosynthetic activities, which then lead to improved proficiency of the plant to distribute more photoassimilates to developing grains and produced higher yield (Hussain et al., 2009).

Water stress significantly reduced the leaf soluble protein and K⁺ contents of chickpea genotypes. However, exogenously applied proline and GB could considerably compensate this loss. Exogenous application of GB produced significantly higher total leaf soluble protein and leaf K⁺ contents. As its application was found to be more effective in ameliorating the negative impact of water stress (Azam et al., 2005). Foliar spray of GB improved the total leaf soluble protein and leaf K⁺ contents of plants

subjected to moisture stress by maintenance of leaf water status due to enhanced osmotic adjustment, improved photosynthesis and enhanced root growth that result in higher uptake of nutrients (Iqbal et al., 2008).

Antioxidant protection system plays an imperative role in plant's response to stress situations. The accumulation of ROS was related to be sensed as an 'alarm' signal that initiates pre-emptive defense responses (Shao et al., 2005). In present investigation the antioxidant activities increase in both chickpea cultivars under water stress situation at both locations. Higher antioxidants activities during water stress can protect plants from oxidative injury (Laxa et al., 2019). Under water stress condition, antioxidant enzymes (SOD, POX and CAT) protect the cells from ROS and they

were found maximum in Bhakhar 2011. Lower activity of antioxidant was found in DUSHT (Zaefyzadeh et al., 2009). Foliar application of GB significantly improved the antioxidant enzyme activities in chickpea plants cultivated under moisture stress. It might be due to the fact that GB application induced enzymatic activities in seedlings particularly SOD and CAT activities and it proved to enhance the cellular integrity and allowed the plants to maintain its water status resulting in improving of photosynthesis and general metabolism activities (Farooq et al., 2008).

5. Conclusion

Results of present study showed that water stress flowering + pod formation + grain filling stage of chickpea adversely affects its yield and physiological parameters. However, exogenous application of GB mitigates the adverse effects of water stress and enhanced tolerance in chickpea mainly due to higher antioxidant enzymes activity, demonstrating the protective measures of plant cells in stress condition. Hence, antioxidants status might be a suitable method for illustrating water stress tolerance in chickpea.

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