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Improving Salinity Tolerance in *Brassica* (*Brassica napus* var. Bsa and *Brassica campestris* var. Toria) by Exogenous Application of Proline and Glycine Betaine

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Abstract. The pot culture experiment was conducted to determine the influence of proline and glycine betaine on *Brassica* under saline conditions. Different salinity levels (0, 65, 130 mM) were created according to the saturation percentage of the soil. Proline (0, 4, 8 mM) and glycine betaine (0, 5, 10 mM) were exogenously applied to find out their effects on growth and physiological changes produced in *Brassica* under salinity stress. Salinity stress reduced the growth of the plants and induced the physiological and biochemical changes. Different growth parameters of plants such as plant height, shoot, root fresh and dry weight was decreased with the increase of salinity stress. Salinity has also reduced the chlorophyll content, protein content and nitrate reductase activity of the *Brassica*. But the application of proline and glycine betaine was more effective to reduce the effect of salinity. Collected data from the present experiment indicated that adverse effects of salinity were counteracted by proline and glycine betaine. Overall, it was observed that exogenous application of both proline and glycine betaine has reduced the effect of salinity.

Keywords: *Brassica napus*, *Brassica compestris*, proline, glycine betaine

Introduction

Soil salinity along with a variety of environmental stresses is now a very serious problem all over the world due to its adverse effects on plant growth and physiology (Taie *et al.*, 2013). Salt stress is a great challenge for agriculture. Yield of the crop is reduced because crops fail to cope with salinity stress (Aymen and Cherif, 2013). There is 22 million hectares arable land in Pakistan. About 24% of crops of Pakistan are grown on rainfed land, whose area is about 4.6 million hectares (Muhammad and Muhammad, 2007). All over the world 35% agricultural production has been decreased due to salinity. Salinity affects 7% of the world's entire land area (Chaum *et al*., 2012). Salinization of arable land is increasing day by day and it is expected that after 25 years 30% of the total land area will face the problem of salt stress (Latef and Chaoxing, 2014; Kapoor *et al*., 2013). Crop growth and productivity are decreased by soil salinity (Cominelli *et al*., 2013).

Plants protect themselves from injurious and destructive effects of salt stress by producing different compatible osmoprotectant metabolites such as proline and glycine betaine (Chelli-Chaabouni *et al*., 2010). These osmolytes gather in the plant and protect tissue and cellular membranes of the plant (Anjum *et al*., 2012). It has been reported that foliar spray of proline and glycine betaine is valuable for plants in mitigating salt induced injuries (Ahmad *et al*., 2012; Hoque *et al*., 2007).

Proline and glycine betaine are also source of carbon and nitrogen. They stabilize the structures of membranes. Proline metabolism has a main role in storage and transfer of energy (Gilberti *et al*., 2014). The effects of salinity stress can be decreased by foliar application of the osmolytes i.e. proline and glycine betaine. In the plants stress tolerance quality is enhanced by the application of foliar spray and it is also a beneficial plan (Ali and Ashraf, 2011).

Brassica species are present in family Cruciferae. Members of the family Cruciferae are known as mustard plants. The petals of the plants belonging to this family are in a cross manner i.e. four petals are cross shaped. Canola (*Brassica napus* and *Brassica campestris* L.) is an important oil seed crop, its world average is 1,820 kg/ha (Chambo *et al*., 2014). 13% of the world's demand of oil is obtained from canola. Oil content of canola seeds is 40% (Snowdon *et al*., 2007). The experiment

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was conducted to determine the effect of proline and glycine betain on brassica under saline conditions.

Materials and Methods

The pot culture experiment was conducted in Department of Botany, University of Sargodha, Sargodha Pakistan. Proline and glycine betaine were applied exogenously to improve salinity tolerance in plants. The experiment was laid out in completely randomized design (CRD) with three replications.

Physiological parameters. *Chlorophyll content*. Method proposed by Davies (1976) was used to compute the chlorophyll contents. The extraction from the 0.5 cm chopped leave pieces was done with 5 mL acetone (80%) and kept at 10 °C. The absorbance of the supernatant was measured at 645 and 663 nm on spectrophotometer. Chl a, Chl b and total chlorophyll were calculated by using the following formula:

Chl. a = [12.7 (OD 663) -2.69 (OD 645)] × V/1000 × W
Chl. b = [22.9 (OD 645) -4.68 (OD 663)] × V/1000 × W
Total Chl. = [20.2 (OD 645) + 8.02 (OD 663)] × V/100 × W
V = Volume of the extract
W = Weight of the sample

Total free amino acids. Method proposed by Hamilton and Van Slyke (1943) was used to compute the total free amino acids. Chopped segments of leaves were extracted with 0.2M phosphate buffer of 7.0 pH. 1 mL from extract, 10% pyridine and 2% ninhydrin solution were put in the test tube. Distilled water was used to make the volume upto 50 mL. The optical density of this coloured solution was seen at 570 nm on spectrophotometer (Hitatchi, 220, Japan). A standard curve was made with Leucine and then calculation for free amino acids was done by this formula:

$$\text{Total amino acids (mg/g fresh wt)} = \frac{\text{Graph reading of sample} \times \text{volume of sample} \times \text{dilution factor}}{\text{Weight of fresh tissue}} \times 100$$

Nitrate reductase activity (NRA). Method proposed by Sym (1984) was used to compute the nitrate reductase activity.

Procedure. Phosphate buffer with the molarity of 0.02 M was added in leaf sample. From this mixture 1 mL was taken out and 0.02 M KNO_3 solution was entered in it. The amount of KNO_3 used for this purpose was 1 mL and 1-naphthyl ethylene diamine dihydrochloride (0.02%) was added in the solution after vigorous shaking of 1-naphthyl ethylene diamine dihydro-chloride. With NO_2 diazocomplex, a pink colour was produced. Spectrophotometer was used to determine the absorbance at 542 nm.

Total soluble sugars. Method proposed by Yemm and Willis (1954) was used to compute the total soluble sugars.

Procedure. In the test tubes of 25 mL, plant extract (0.3 mL) was added. 6 mL of anthrone reagent was added in the test tubes. Then the test tubes were warmed in the boiling water bath for 10 min. These test tubes were cooled down by placing them in chilled water for 10 min and then incubated for 20 min by maintaining the temperature at 25 °C. Spectrophotometer was used to measure the optical density at 625 nm (Hitatchi, 220, Japan). Standard curve was developed for the calculation of soluble sugars.

Na and K analysis. *Digestion*. In the digestion tubes concentrated H_2SO_4 was added with 0.5 g of ground material (Wolf and Stahl, 1982). 35% hydrogen peroxide was added in the digestion tubes. After this they were heated at 350 °C in the digestion block. This process of heating was continued for 30 min. 0.5 mL of hydrogen peroxide was added in it. For making the digested material colourless 0.5 mL hydrogen peroxide was added and the tubes were placed again in the digestion block. This step was done again and again until the solution became colourless. The volume was kept 50 mL in volumetric flasks by adding distilled water.

Estimation of cations (Na^+ and K^+). Method proposed by Jenway (PFP 7) was used to determine sodium (Na^+) and potassium (K^+) with the help of flame photometer PFP7 (Yilmaz and Yavuz, 1999).

Calcium determination. Method proposed by salinity laboratory (Kunze and Dixon, 1986) was used to determine calcium.

Yield and yield components. Plant height was recorded using a meter rod. Data for yield and yield components were recorded at maturity.

Statistical analysis. Analysis of variance of the data from each attribute was computed using three factor factorial design (Steel *et al*., 1997).

Results and Discussion

As shown by Table 1 that salinity stress has significantly reduced the growth and production of brassica but the

exogenous application of proline and glycine betaine minimizes the effect of salinity. Salinity has decreased plant height (Fig. 1), shoot fresh weight (Fig. 2), shoot dry weight (Fig. 3), root fresh weight (Fig. 4), root dry weight (Fig. 5), Chl a (Fig. 6), Chl b (Fig. 7), total Chl (Fig. 8), total soluble sugars (Fig. 9), protein (Fig. 10), total free amino acids (Fig. 11), NRA (Fig. 12), potassium (Fig. 13), calcium (Fig. 14) and sodium (Fig. 15) increased under salt stress. As shown that 8 mM and 10 mM concentration of proline and glycine betaine were more effective to reduce the effect of salinity as compared to 4 mM and 5 mM concentrations of proline and glycine betaine. Sodium ions present in the growth mediums showed antagonistic effect on calcium and potassium ions.

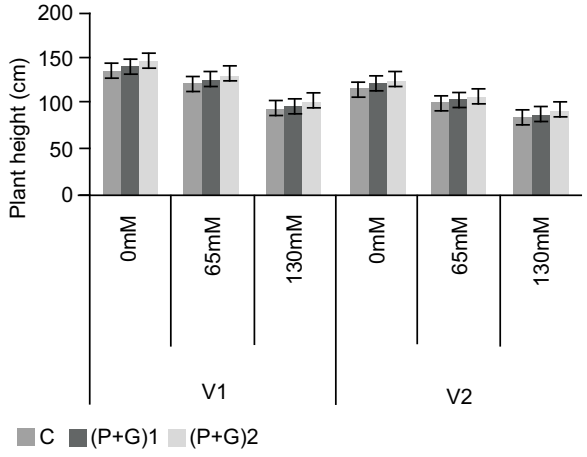

Fig. 1. Concentration of proline/glycine betaine.

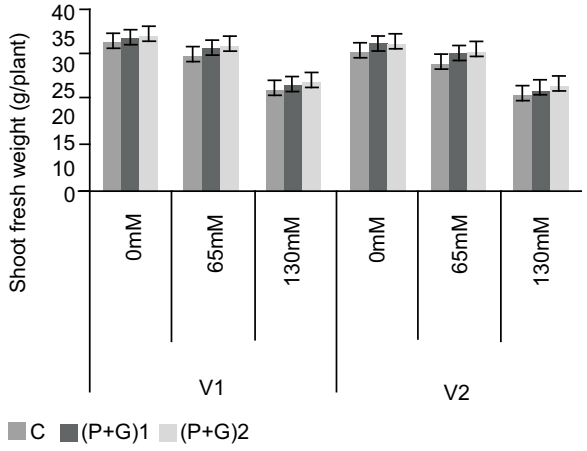

Fig. 2. Concentration of proline/glycine betaine.

Table 1. Salinity effects on *Brassica* growth and production

SOV	DF	Parameters														
		Plant height	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Chl a	Chl b	Total Chl	Sugars	Proteins	A.A	NRA	Na	Ca	K
Variety (V)	1	2361.92**	14.10**	20544.7**	297.47**	482.23**	20.49**	20.72**	142841**	1202.56**	8517.43**	538452**	7618.79**	4593750**	80.60**	4511001**
Treatment (T)	2	3667.83**	263.18**	131470**	1786.21**	5189.66**	2516.62**	6440.18**	668953**	10245.7**	99259.5**	6194365**	226283**	1.1E+08**	4918.25**	4.0E+07**
Chemicals (C)	2	188.26**	12.16**	1477.30**	36.41**	464.87**	32.59**	195.34**	157850**	137.93**	5292.14**	1349845**	13771.5**	1.4E+07**	379.26**	2998814**
V × T	2	115.95**	0.14NS	372.45**	6.71**	38.32**	0.00NS	0.00NS	109487**	274.53**	892.85**	279862**	2045.37**	656250**	0.00NS	377554**
V × C	2	1.93NS	0.26NS	6.01**	0.14NS	10.59**	0.00NS	0.00NS	110148**	5.51**	67.22**	3070.57**	123.20**	0.00NS	0.00NS	12138.5**
T × C	4	2.63*	0.32NS	9.36**	0.46NS	19.05**	1.91*	13.17**	137291**	1966.87**	112.45**	73593.8**	928.13**	143569**	13.89**	92754.2**
V × T × C	4	0.54NS	0.09NS	3.33*	0.34NS	5.42**	0.00NS	0.00NS	121428**	16.84**	93.59**	39303.2**	816.04**	0.00NS	0.00NS	2969.79**
Error	36															
Total	53															

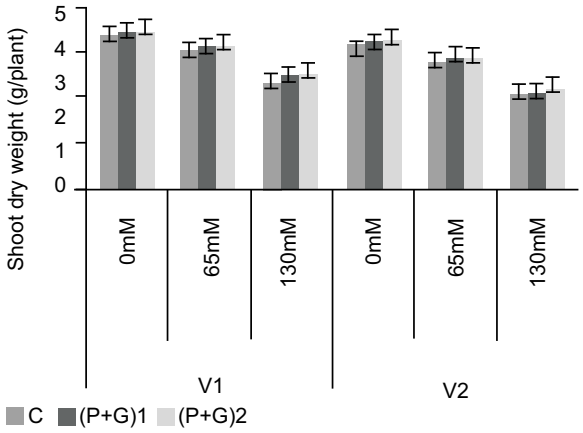

Fig. 3. Concentration of proline/glycine betaine.

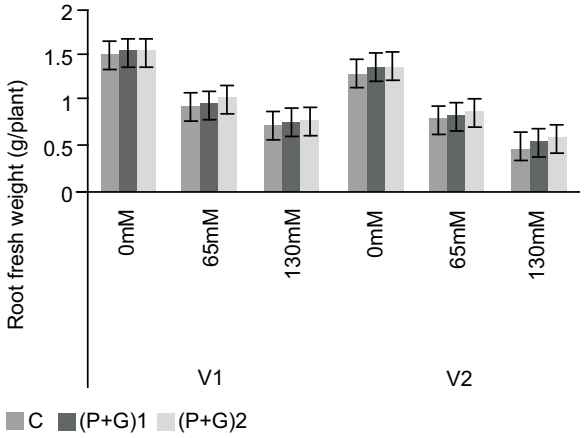

Fig. 4. Concentration of proline/glycine betaine.

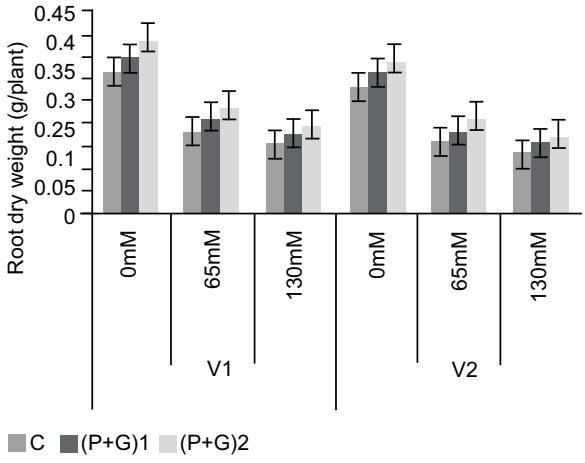

Fig. 5. Concentration of proline/glycine betaine.

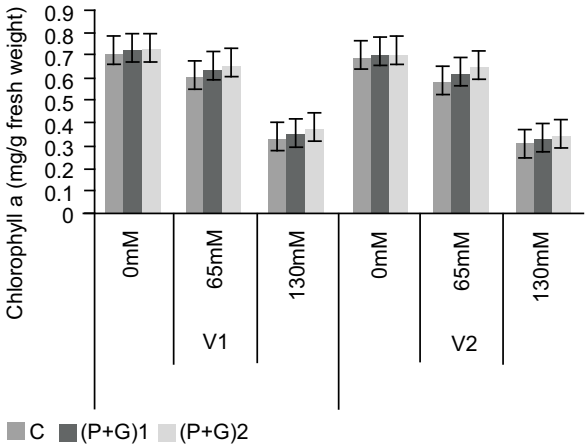

Fig. 6. Concentration of proline/glycine betaine.

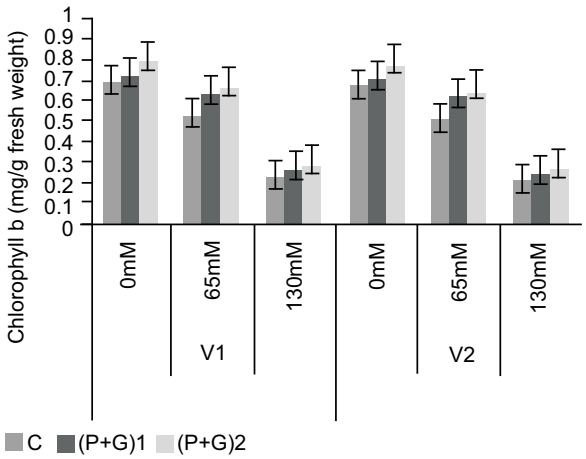

Fig. 7. Concentration of proline/glycine betaine.

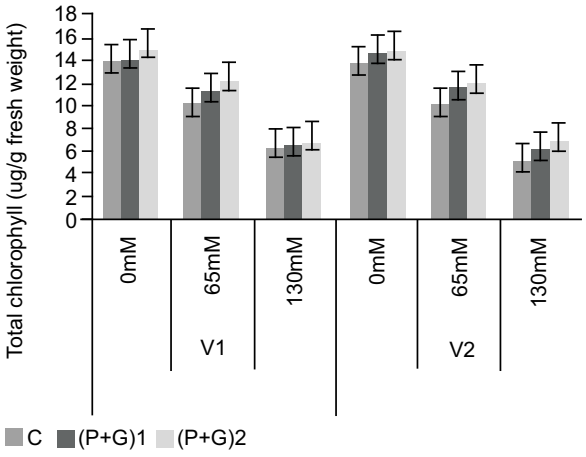

Fig. 8. Concentration of proline/glycine betaine.

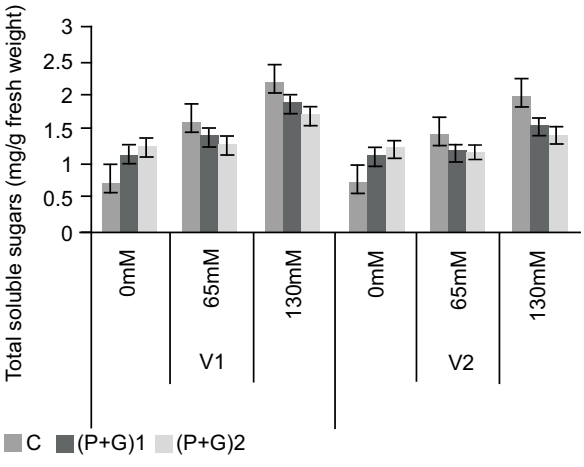

Fig. 9. Concentration of proline/glycine betaine.

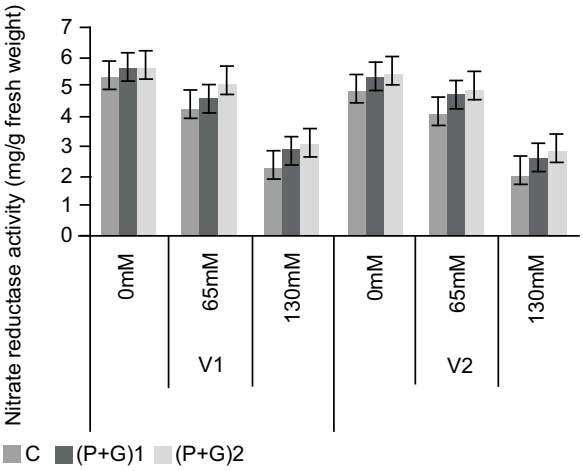

Fig. 12. Concentration of proline/glycine betaine.

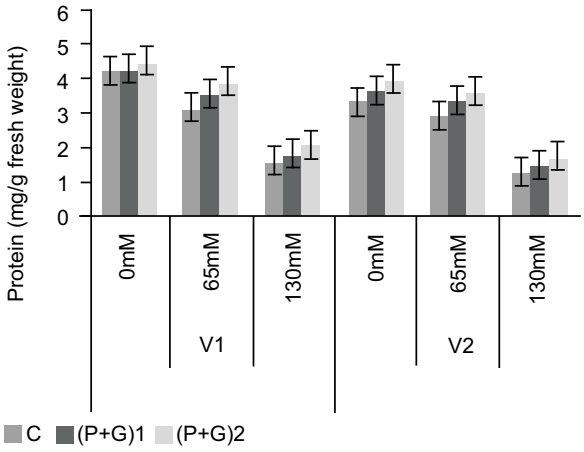

Fig. 10. Concentration of proline/glycine betaine.

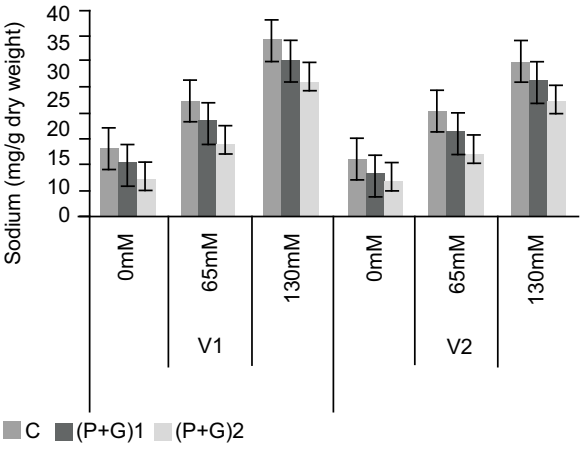

Fig. 13. Concentration of proline/glycine betaine.

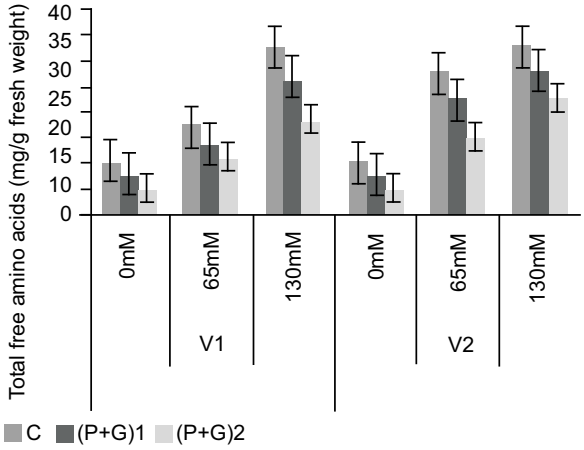

Fig. 11. Concentration of proline/glycine betaine.

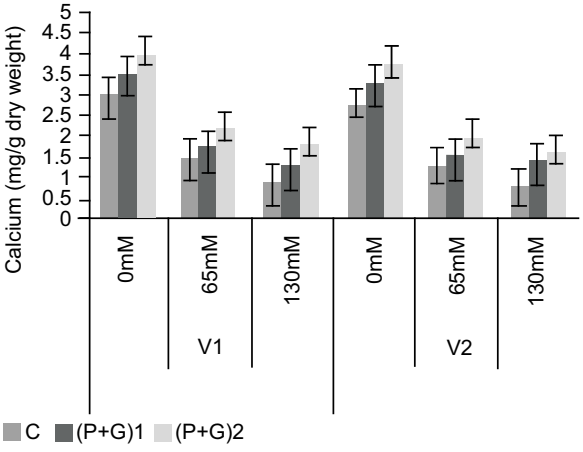

Fig. 14. Concentration of proline/glycine betaine.

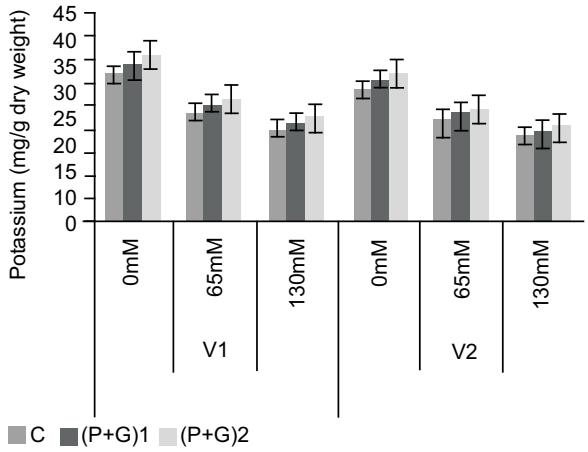

Fig. 15. Concentration of proline/glycine betaine.

Figure 1-15 shows the effect of exogenous application of proline and glycine betaine on plant height, shoot and root fresh and dry weight, shoot, Chl a, Chl b, total Chl, protein, total free amino acids, NRA, sodium, calcium, potassium of brassica under saline and non-saline conditions.

Salinity tolerance in brassica increased by exogenous application of proline and Glycine betaine. Glycine betaine content was observed to be increased under salt stress (Amandeep *et al*., 2014).

Glycine betaine was found effective to decrease salt stress damage when it was exogenously applied on canola (Athar *et al*., 2015). With glycine betaine, proline also play role in improving plant growth. The application of 1 and 5 mM proline improved the growth of brassica varieties (Posmyk and Janas, 2007). Salinity levels increases uptake of Na^+ in all plant parts including root, stem and leaf. Simultaneously it decreases the calcium and potassium uptake due to antagonistic effects. The addition of Na^+ in plant parts increased as the level of salinity was increased. The amount of proline, soluble carbohydrates and reduced sugar increased as salinity increased (Mostajeran and Gholaminejad, 2014).

Proline is a widespread compatible solute. There are many roles for proline in saving plants from harmful and damaging effects of salinity. It can stabilize the membranes and guard them from harmful ions which can destroy their structure (Khan *et al*., 2009). Proline and glycine betaine can be used to check the salt tolerance ability of different plant species (Ahmad *et al*., 2009).

Foliar spray of proline and glycine betaine enhances the development of both salt affected and non-stressed plants of canola varieties. Same results have been observed in maize (Nawaz and Ashraf, 2007) and wheat (Raza *et al*., 2007).

It is clear from the outcomes of current study that salinity stress reduced plant growth, plant height, dry biomass and fresh weight. Growth of all the plants increased by applications of proline and glycine betaine, either the plants were facing salinity or were grown under non saline conditions. Chl a, Chl b and total chlorophyll were also reduced under salt stress but their value increased when proline and glycine betaine were applied on the plants. The 4 and 5 mM levels of proline and glycine betaine are less effective to reduce salinity than 8 and 10 mM levels of proline and glycine betaine. Proline and glycine betaine decreased the harmful effects of salinity stress and enhanced the growth of plants. The encouraging effects are clear from the above results.

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