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METHIONINE-INDUCED CHANGES IN GROWTH, GLYCINEBETAINE, ASCORBIC ACID, TOTAL SOLUBLE PROTEINS AND ANTHOCYANIN CONTENTS OF TWO *Zea mays* L. VARIETIES UNDER SALT STRESS

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ABSTRACT

A pot study was performed to evaluate the influence of methionine (0, 25 and 50 mg L^{-1}) as foliar spray on maize (*Zea mays* L.) varieties named as DTC (hybrid) and Malika under salt (90 mM NaCl) stress. Seeds of both maize varieties were sown in plastic pots under completely randomized design. Two-week-old maize plants were subjected to two levels of salt stress i.e., 0 and 90 mM (NaCl) in full strength Hoagland's nutrient solution. Foliar application of different methionine levels (0, 25 and 50 mg L^{-1}) was applied to four-week-old maize plants. A three way analysis of variance (ANOVA) of data of 11-week-old plants of maize plants showed that salt stress considerably reduced the growth, chlorophyll, relative water content (%), free amino acids and flavonoid contents, whereas improved membrane permeability (%), total leaf area per plant, glycine betaine (GB), total soluble proteins, free proline, anthocyanin and ascorbic acid contents of both maize varieties. Maize var. Malka was greater in fresh weight of root, total chlorophyll and total soluble protein contents, while DTC (hybrid) excelled in anthocyanin, total soluble sugars and flavonoid contents. Methionine as foliar application significantly improved dry shoot weight, fresh root weight, GB, total soluble protein, ascorbic acid and anthocyanin contents of two maize varieties under salinity stress. Of the varying methionine levels, 50 mg L^{-1} proved better in decreasing the adverse influence of NaCl stress on both varieties [DTC (hybrid) and Malika] of maize.

Key words: proteins, salinity, oxidative stress, vitamins, ascorbic acid.

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INTRODUCTION

Salinity stress is among the main abiotic strains that significantly decrease growth as well as yield of major food cultivations globally. Salinity stress disturbs metabolic processes by the formation of reactive oxygen species that damage membrane system of cytoplasmic organelles (Nazar *et al*., 2011). Plants tolerance towards salt stress greatly depends upon the capability of its cytoplasmic structure and its interaction with external environment. This capability can be improved and enhanced by the use of amino acids under salt stress (Wang *et al*., 2010). For example, exogenous applications of amino acids has been reported to reduce abiotic stresses in various crop species such as wheat (El-Said and Mahdy, 2016), maize (Alam *et al*., 2017), rice (Xiaochuang *et al*., 2017), barley (Genisel *et al*., 2015), potato (Awad *et al*., 2007), faba bean (Sadak *et al*., 2015), *Arabidopsis thaliana* (Dominguez-Solis *et al*., 2004) and canola (Lei *et al*., 2016) etc..

About 80% human food is derived from crops in which only cereals (wheat, rice and maize) contribute 50% of total food production throughout the world (Langridge and Fleury, 2011). However, major food crops are deficient in vital amino acids such as lysine plus tryptophan in cereals (Pfefferle *et al*., 2003), isoleucine, cysteine and methionine in potatos (Stiller *et al*., 2007), and threonin and methionine in soybeans (Muntz *et al*., 1998). Malnutrition in micronutrients (iron, zinc, vitamins) and macronutrients (amino acids methionine and lysine) can affect more than 40% of human population globally (Hesse *et al*., 2004). Human beings can synthesize only ten of the twenty amino acids, while the remaining ten are obtained from diet. However, some food crops like cereals are deficient in crucial amino acids e.g., tryptophan, threonine and lycine, while leguminous crops did not contain adequate supply of sulphure-containing amino acids like cysteine and methionine. In developing countries, people depend upon plant-derived food and 90% of energy intake can be derived from a single plant species that could be low in methionine contents leading to protein energy malnutrition (PEM) in humans. Plant derived food that is deficient in methionine can decrease growth of animals (Xu *et al*., 1998). In order to increase nutritional quality contents of essential amino acids like tryptophan, methionine, and lysine must be increased in cereal grains (Lee *et al*., 2001).

Methionine is among the four essential amino acids that are produced from aspartic acid pathway. Hesse and Hoefgen (2003) reported that amino acids that

contain sulphur their endogenous level can be increased by variation of biosynthetic path of methionine and cystein in cereals like rice. Cysteine as well as methionine has potential to change physiological processes in vegetation under saline stress (Khan *et al*., 2014).

Exogenous applications of amino acids regulate physio-chemical developments in crops under varied climatic conditions (Moreira and Moraes, 2017; Ros *et al*., 2014). For example, exogenous application of amino acids regulate antioxidant defense system in soybean when applied in combination at very low concentration e.g., alanine, glutamate, cysteine and glycine (Teixeira *et al*., 2017). Alam *et al.* (2017) reported that amino acids maintain nutrients balance in maize hybrids under salt stress. Some amino acids such as serine, glycine, valine and glutamate play role in improving plant health through therapeutic elements (Moran-Palacio *et al*., 2014). Proline and glycinebetaine (GB) improve crop production by reducing oxidative stress (Ashraf and Foolad, 2007). Exogenous application of cysteine increase growth in *Ocimum basilicum* L. under cobalt stress (Azarakhsch *et al*., 2015), salt stress tolerance in barley (Genisel *et al*., 2015), and cadmium (Cd) stress in *Arabidopsis thaliana* (Dominguez-Solis *et al*., 2004);); glutamic acid increased salt (NaCl) and cold stress tolerance in *Brassica napus* L. (Lei *et al*., 2015, 2016); a mixture of glycine, methionine and tryptophan increased growth of gladiolus plant (Khattab *et al*., 2016); tryptophan enhanced growth of chickpea under rainfed conditions (Abbas *et al*., 2013); and proline increased growth of chilli under salinity (Butt *et al*., 2016).

Methionine contents decreased (upto 40%) under abiotic stresses (Pavlíková *et al*., 2014; Zemanová *et al*., 2014). However, plants treated with methionine have increased resistance to disease. Many efforts have been made to produce food and forage crops with higher methionine contents for both humans and animals respectively. Methionine is an excellent source of essential mineral sulfure that acts as a free radical scavenger in the body (Kumar *et al*., 2014). So feed with higher level of methionine contents must be applied in the form of forage crops to monogastric animal feed (Bagga *et al*., 2004). The major goal of recent study is to evaluate the influence of foliar treatments of methionine on the growth as well as physiochemical attributes of maize plants under control and NaCl stress situations.

MATERIALS AND METHODS

To assess the influence of foliar use of methionine on two maize varieties Malka and DTC (hybrid) a pot experiment was conducted at the Botanical Garden Government College University, Faisalabad natural climatic environments. Experimental design was completely randomized with three replicates. Seeds of both maize varieties were obtained from Ayub Agricultural Research Institute (AARI) and sown in sand filled plastic pots. There were total 36 pots and six seeds were sown in each pot. Thinning was performed after one week of germination and four plants per pot were kept. Two-week-old maize plants were subjected to two levels of salt stress i.e., 0 and 90 mM (NaCl). Salt treatment was applied in full strength Hoagland's nutrient solution in aliquots of 45 mM for consecutive two days until final volume 90 mM was achieved. Foliar treatment of methionine (0, 25 and 50 mg L^{-1}) was applied to four-week-old maize plants. Several growth and physio-biochemical parameters were taken of the 11-week-old maize plants.

Growth attributes: Two plants from each pot were collected and measured shoot and root fresh weights and shoot and root lengths. Then oven-dried the leaf samples at 72°C for 48 hours and measured the shoot and root dry weights. Total leaf area per plant was calculated by using the formula of Carleton and Foote (1965)

Total leaf area (cm^2) = leaf length × leaf width × correction factor (0.75)

Determination of physiobiochemical parameters: Relative water contents (%): Jones and Turner (1978) method was used for the determination of relative water contents (%). Fresh leaf (0.5 g) kept in deionized water for 24 hours and turgid weight (Tw) was calculated. Then leaf samples were kept in oven at 80°C for 48 hours and measured dry weight (Dw). Following formula was used for relative water contents (%) determination

$$RWC\ (\%) = [(Fw\text{-}Tw)/Fw\text{-}Dw)] \times 100$$

Determination of chlorophyll contents: The method of Arnon (1949) was used for the determination of chlorophyll contents. Fresh leaves (0.5 g) were chopped in 10 ml acetone (80%) and kept at 0-4°C for overnight. Then absorbance of extract was recorded at optical density of 645 and 663 nm with a spectrophotometer.

Determination of membrane permeability (%): Fresh leaves 0.5 (g) were cut into small pieces, kept in the test tubes containing 10 ml distilled water, vortexed and measured the electrical conductivity (EC_o). Then kept the samples in refrigerator overnight, vortexed and measured the electrical conductivity (EC_1). After that autoclaved the samples for one hour, vortexed and measured the electrical conductivity (EC_2) of dead leaf tissues. Following formulae was used for the estimation of membrane permeability (%).

$$RMP\ (\%) = (EC_1 - EC_o / EC_2 - EC_o) \times 100$$

Hydrogen peroxide (H_2O_2): Velikova *et al*. (2000) protocol was used for the determination of H_2O_2 contents. Fresh leaf (0.5 g) finely homogenized in 5 ml of 0.1% trichloroacetic acid (TCA), centrifuged at 12000 × *g* for 15 min and absorbance of reaction mixture (0.5 ml

extract + 0.5 ml buffer + 1 ml KI) was measured at 390 nm using a spectrophotometer.

Total free amino acids: Determination of total free amino acids was made according to the procedure reported by Moore and Stain (1957). Citrate buffer (10 ml pH 5.0) was used for the extraction of fresh leaf material (0.5 g). The supernatant was centrifuged at 15,000 × *g* for 10 min and added 1 ml each of extract, pyridine and ninhydrin solution in test tubes. Test tubes with samples were kept in water bath at 90ºC for 30 min and read the optical density of solution at 570 nm using a spectrophotometer.

Glycinebetaine determination: Grieve and Grattan (1983) protocol was used for the determination of glycinebetaine. For this fresh leaf (0.5 g) was extracted in 10 ml of distilled water. To 1 ml of leaf extract added 1 ml of $2NH_2SO_4$ and to 0.5 ml of this mixture added 0.2 ml K-I_3 in an ice bath and cooled for 90 min at 4ºC. To this mixture added 2.8 ml cold distilled water and 6 ml of 1-2 dichloromethane and read the absorbance at 365 nm using a spectrophotometer.

Determination of proline: For the determination of lead free proline contents Bates *et al.* (1973) procedure was used. Fresh leaf 500 mg was homogenized in 10 ml of 3% sulphosalcylic acid and filtered. To 2 ml of filtrate added 2 ml each of glacial acetic acid and acid ninhydrin and kept the samples in water bath at 80ºC for 1 hour. After that terminated the reaction by putting the samples in an ice bath, added 4 ml of toluene in each sample and read the absorbance of colored layer at 520 nm with a spectrophotometer.

Total soluble sugars: Fresh leaf tissue (0.1 g) was homogenized in phosphate buffer (5ml of 0.2%). To 0.1 ml of supernatant added 3 ml anthron's reagent, vortexed and kept this mixture at 95ºC for 15 min. After cooling the absorbance of colored extract was taken at 625 nm using a spectrophotometer.

Determination of total soluble proteins: Bradford (1976) procedure was used for the appraisal of total soluble protein contents. Fresh leaf (500 mg) was finely homogenized in phosphate buffer, centrifuged at 2000 × *g* for 10 min. To 1 ml of supernatant added 5 ml Coomassie Brilliant blue reagent, vortexed for 20 s and measured the absorbance of solution at 595 nm on a spectrophotometer (Hitachi-U-1800, Japan).

Total phenolics: Protocol of Julkenen-Titto (1985) was used for the determination of total phenolic contents. Fresh leaf (0.5g) was homogenized in 2 ml of 80% acetone, homogenized at 10,000 × *g* for 15 min and stored at 20ºC. A mixture of 0.1 ml supernatant + 2 ml distilled water + 0.5 ml Folin-Ciocalteau's phenol (FC-reagent) + 2.5 ml sodium carbonate (20%) prepared and final volume made upto 5 ml with distilled water. Absorbance of mixture was observed at 750 nm using a spectrophotometer.

Ascorbic acid contents: For the appraisal of ascorbic acid contents method of Mukherjee and Choudhuri (1983) was used. Fresh leaf samples w finely homogenized in liquid nitrogen followed by in a cocktail containing 2% dinitrophenyl-hydrazine and 6% trichloroacetic acid prepared in half-strength H_2SO_4 and 10% thiourea dissolved in 70% ethanol. Then the solution was boiled, cooled at room temperature, centrifuged (1000 × *g*) for 10 min and resultant pellet dissolved in 80% $H2SO_4$. The absorbance of solution was taken at 530 nm using a spectrophotometer.

Flavonoids: Method of Zhishen *et al.* (1999) was used for the determination of flavonoids. Acetone (80%) was used for the extraction of 0.1 g fresh leaf samples. Reaction mixture consists of 0.5 ml of supernatant + 2 ml distilled water + 0.6 ml of 5% $NaNO_2$ + 0.5 ml 10% $AlCl_3$ and 2ml of 1M NaOH and reading of solution was made at 510 nm with a spectrophotometer.

Anthocyanin contents: For the determination of anthocyanin contents fresh leaf sample (0.1 g) was extracted in phosphate buffer (5 ml), after centrifugation the absorbance of supernatant was performed at 600 nm with a spectrophotometer

Statistical analysis: A three-way analysis of variance (ANOVA) was performed on the data using statistical package, CoStat program (version v6.303) and means were compared by LSD (least significant difference) (Snedecor and Cochran, 1980).

RESULTS

Salt stress (90 mM NaCl) reduced shoot as well as root fresh and dried masses, shoot length, and total leaf area per plant markedly in two maize (*Zea mays* L.) varieties i.e., DTC (hybrid) and Malika (Table 1; Fig. 1). Two maize cultivars did not display any major alteration but in root fresh weight that was high in var. Malika. Foliar application of methionine (Met) significantly improved dry mass of shoot in addition to root and fresh weight of root in stressed or control states (Table 1; Fig. 1).

Relative water content lessened under stress of 90 mM NaCl significantly (Table 1; Fig. 1), while all other factors remain unchanged. Photosynthetic pigments such as total chlorophyll contents and chlorophyll *a* and *b* molecules diminished under salt (NaCl) stress considerably. Maize varieties exhibited noticeable variations as var. Malika remained greater in chl. *a*, *b* and total chlorophyll matters in salinity stressed environments (Table 1; Fig. 2).

Hydrogen peroxide (H_2O_2) contents increased in var. DTC (hybrid), while decrease in var. Malika under NaCl stress. Foliar application using different levels of Met enhanced H_2O_2 contents noticeably in var. DTC (hybrid), while decrease in var. Malika in both stress

positions. Salt stress importantly enhanced membrane permeability (%) in both varieties of maize. Total amino acid reduced under salt stress in both maize varieties significantly, while glycine betaine (GB) and proline contents significantly increase in both maize varieties. However, var. DTC (hybrid) accumulated more GB contents under NaCl stress conditions (Table 1; Fig. 2).

Total soluble sugars decreased by foliar application of Met in var. DTC (hybrid) under salt stress, while in var. Malika under non stress conditions (Table 1; Fig. 3). Total soluble proteins increased in var. DTC (hybrid) under salt stress conditions, while var. Malika accumulated more proteins in non-stressed situations. Foliar usage of Met enhanced soluble proteins in these two maize varieties under two stress level, whereas reduced in var. DTC (hybrid) under control conditions (Table 1; Fig. 3). DTC (hybrid) and Malika varieties of maize crop indicated important change as var. DTC (hybrid) accumulated high soluble proteins contents under salt stress conditions, while var. Malika excelled under non stress conditions. Both maize varieties also showed significant difference towards Foliar application of Met decreased soluble proteins contents in var. DTC (hybrid), while increased in var. Malika under non stress conditions (Table 1; Fig. 3).

Foliar use of different Met levels improved total phenolic contents in var. Malika significantly, while lessened in var. DTC (hybrid) under saline or control (non-stress) conditions (Table 1; Fig. 3). Ascorbic acid contents enhanced under NaCl stress condition and through foliar use of Met in DTC (hybrid) and Malika varieties of maize significantly (Table 1; Fig. 3). Anthocyanin contents noticeably enhanced both under salinity and foliar treatment of Met in maize varieties (Table 1; Fig. 3). Both varieties exhibited significant alteration as DTC (hybrid) accumulated high anthocyanin contents than Malika under NaCl stress application (Table 1; Fig. 3). Flavonoid contents significantly reduced in salt stressed environment in var. Malika. Maize varieties presented significant variance as DTC (hybrid) accumulated high contents of flavonoid under NaCl stress or control conditions (Table 1; Fig. 3).

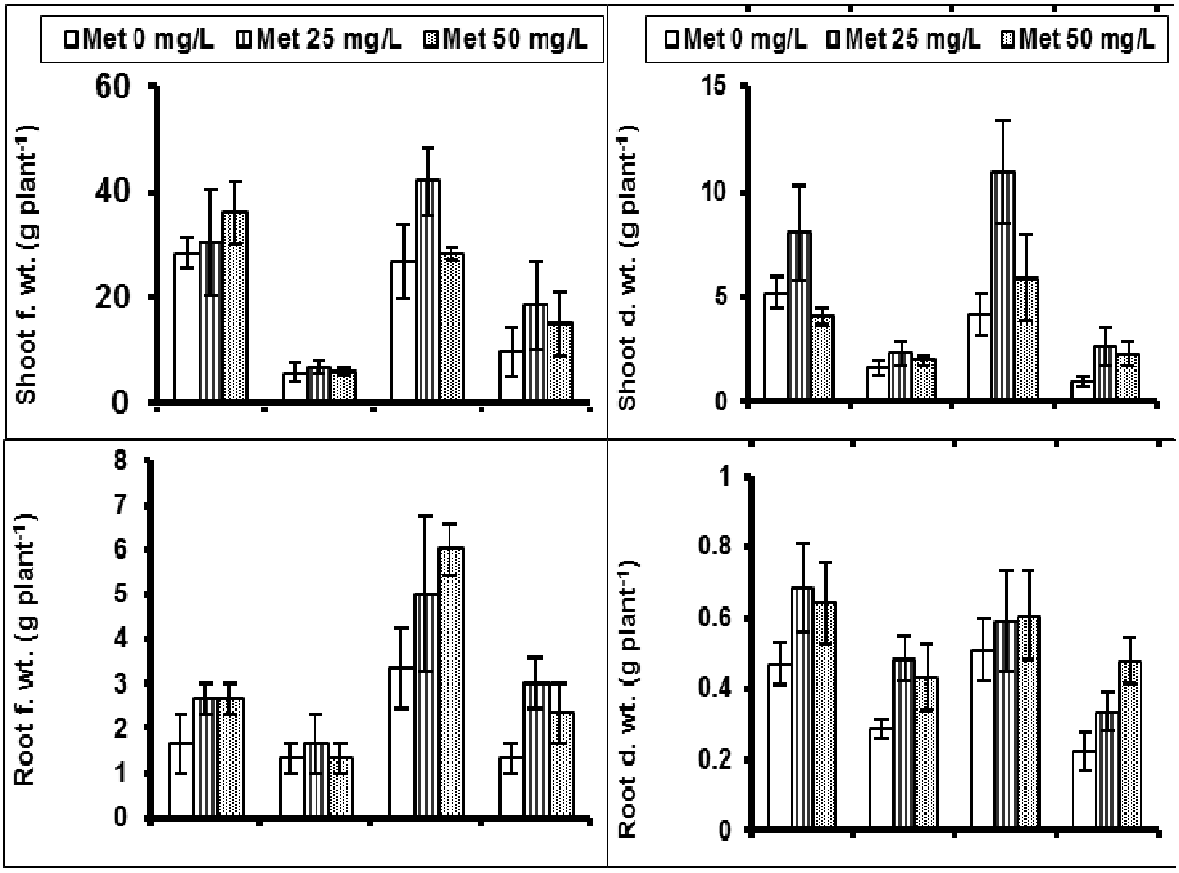
Table 1. Analysis of variance of the parameters of growth, relative water content (%), chlorophyll, hydrogen peroxide, membrane permeability (%), amino acids, glycinebetaine, proline, total soluble proteins, total soluble sugars, total phenolics, anthocyanin, ascorbic acid and flavonoid contents of maize plants foliarly sprayed with varying levels of methionine (Met) under non saline and saline conditions.

Source of variation	df	Shoot fresh wt.	Shoot dry wt.	Root fresh wt.	Root dry wt.	Shoot length
Varieties (Var)	1	182.25ns	3.497ns	23.36***	0.016ns	286.7ns
Salinity-Stress (SS)	1	4246.6***	174.3***	26.69***	0.397***	7140.2***
Methionine (M)	2	137.2ns	30.19**	5.444*	0.102*	219.4ns
Var × SS	1	124.6ns	3.297ns	6.25ns	0.001ns	140.8ns
Var × M	2	115.7ns	4.957ns	1.444ns	0.015ns	47.25ns
SS × M	2	11.027ns	15.44ns	1.444ns	0.004ns	14.12ns
Var × SS × M	2	53.86ns	1.685ns	0.333ns	0.007ns	30.36ns
Error	24	92.13	4.72	1.583	0.025	79.61
Source of variation	**df**	**Root length**	**Total leaf area plant^{-1}**	**RWC% contents**	**Chlorophyll *a***	**Chlorophyll *b***
Varieties (Var)	1	0.587ns	8041.6ns	117.9ns	0.0379ns	0.018ns
Salinity-Stress (SS)	1	2.454ns	35661***	5849.6***	0.0889**	0.172**
Methionine (M)	2	25.25ns	1345ns	239.5ns	0.009ns	0.029ns
Var × SS	1	39.27ns	4220**	29.95ns	0.2048***	0.090*
Var × M	2	2.138ns	9149ns	65.33ns	0.0028ns	0.0002ns
SS × M	2	20.79ns	2081ns	12.60ns	0.007ns	0.025ns
Var × SS × M	2	31.18ns	3068ns	0.169ns	0.0123ns	0.039ns
Error	24	11.64	5117	80.95	0.0104	0.017
Source of variation	**df**	**Total chlorophyll**	**H_2O_2 contents**	**MP (%)**	**Amino acids**	**Proline contents**
Varieties (Var)	1	0.110*	22.34ns	17.26ns	0.302ns	61.76ns
Salinity-Stress (SS)	1	0.509***	3.18ns	752.80***	37.22*	4758.7***
Methionine (M)	2	0.072ns	7.593ns	107.48ns	13.79ns	181.0ns
Var × SS	1	0.568***	114.1**	170.65ns	10.35ns	18.34ns
Var × M	2	0.002ns	30.97*	109.97ns	1.056ns	25.89ns
SS × M	2	0.016ns	14.33ns	79.51ns	2.631ns	228.52ns
Var × SS × M	2	0.095*	11.05ns	22.21ns	0.499ns	27.113ns

Error	24	0.021	8.667	43.43	5.960	210.03
Source of variation	**df**	**GB contents**	**Soluble sugars**	**Soluble proteins**	**Ascorbic acid**	**Total phenolics**
Varieties (Var)	1	34.32ns	1847.1*	97.26***	1.736ns	4.429ns
Salinity-Stress (SS)	1	317.5***	2.115ns	41.85**	9.588***	5.62ns
Methionine (M)	2	135.9**	522.0ns	42.38***	3.177*	3.401ns
Var × SS	1	113.9*	608.9ns	141.92***	0.019ns	2.018ns
Var × M	2	51.63ns	852.4*	24.39**	0.0919ns	11.87*
SS × M	2	5.335ns	195.10ns	8.646ns	0.213ns	2.31ns
Var × SS × M	2	13.71ns	689.5*	3.335ns	0.402ns	9.13ns
Error	24	21.48	162.0	3.036	0.584	3.034

Source of variation	**df**	**Anthocyanin**	**Flavonoids**
Varieties (Var)	1	1.761ns	2.353***
Salinity-Stress (SS)	1	6.347*	1.647***
Methionine (M)	2	5.814*	0.062ns
Var × SS	1	7.098*	1.183***
Var × M	2	0.327ns	0.054ns
SS × M	2	0.633ns	0.160ns
Var × SS × M	2	0.023ns	0.030ns
Error	24	1.073	0.064

df = degrees of freedom; *, **, and *** significant at 0.05, 0.01 and 0.001 levels respectively.
ns = non-significant; RWC (%) = relative water content (%); MP (%) = membrane permeability; H_2O_2 = hydrogen peroxide; GB = glycinebetaine



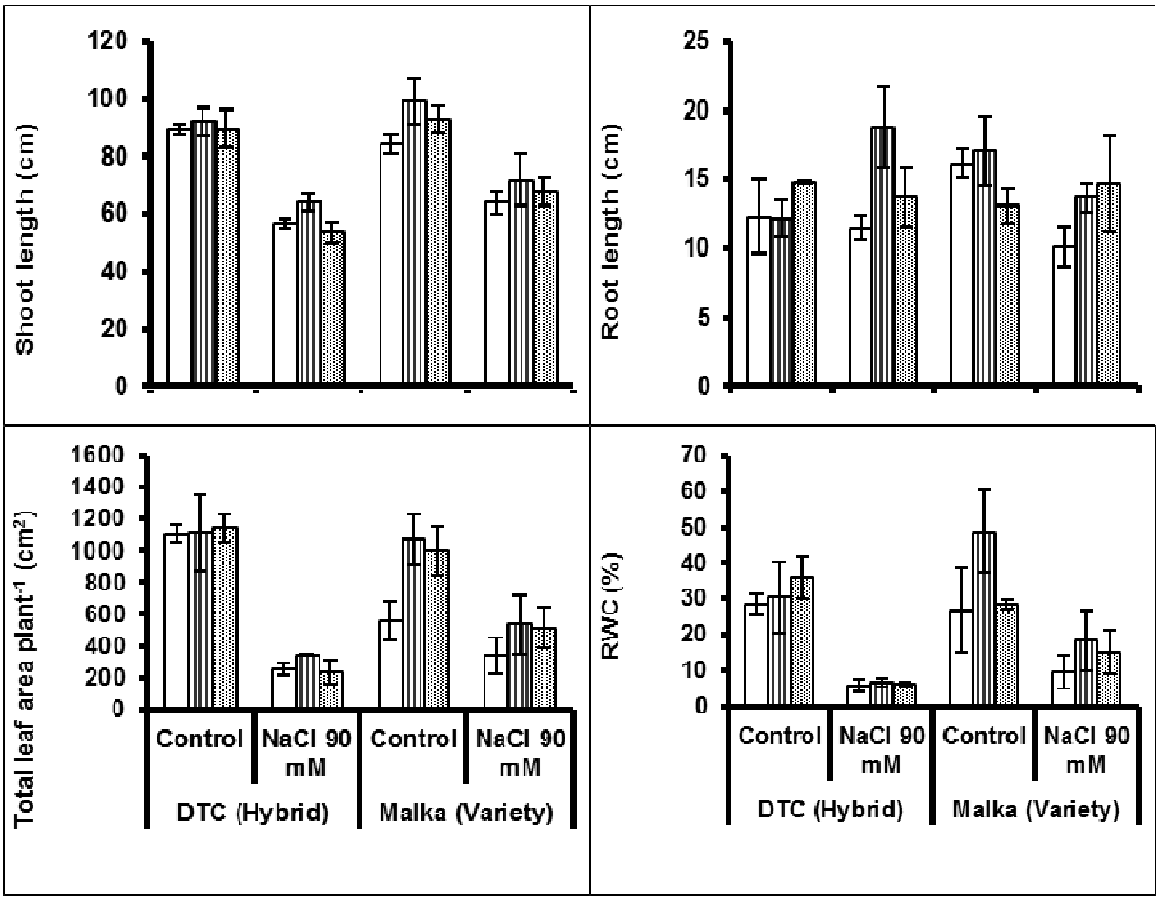
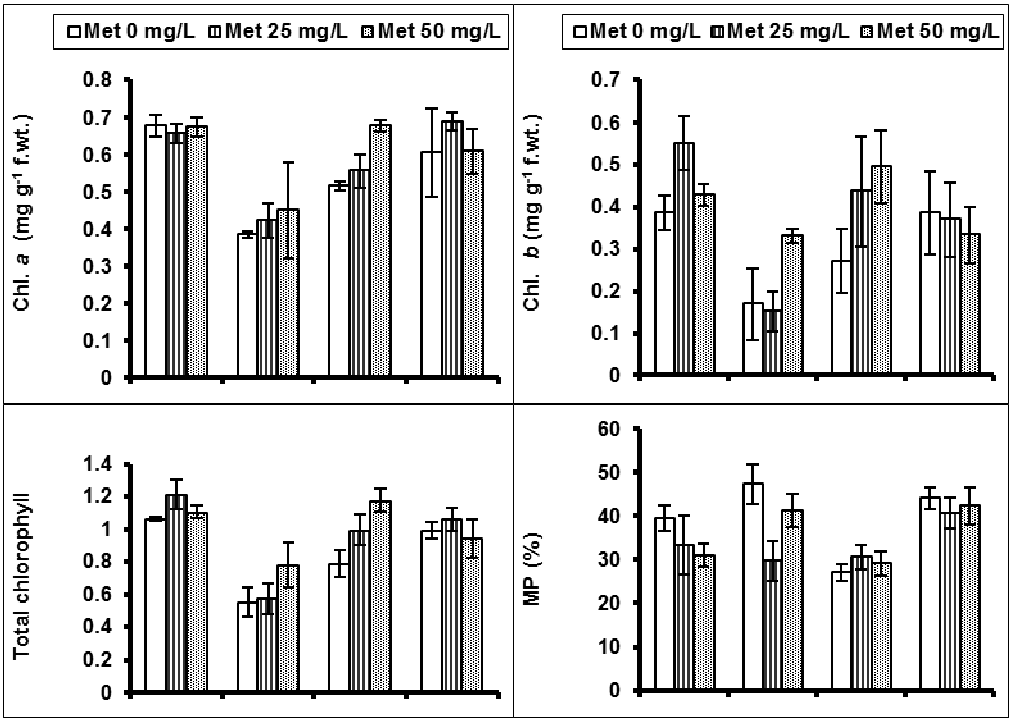


Fig. 1. Growth parameters and relative water contents (%) of maize (*Zea mays* L.) plants foliarly-sprayed with methionine under non saline and saline conditions.



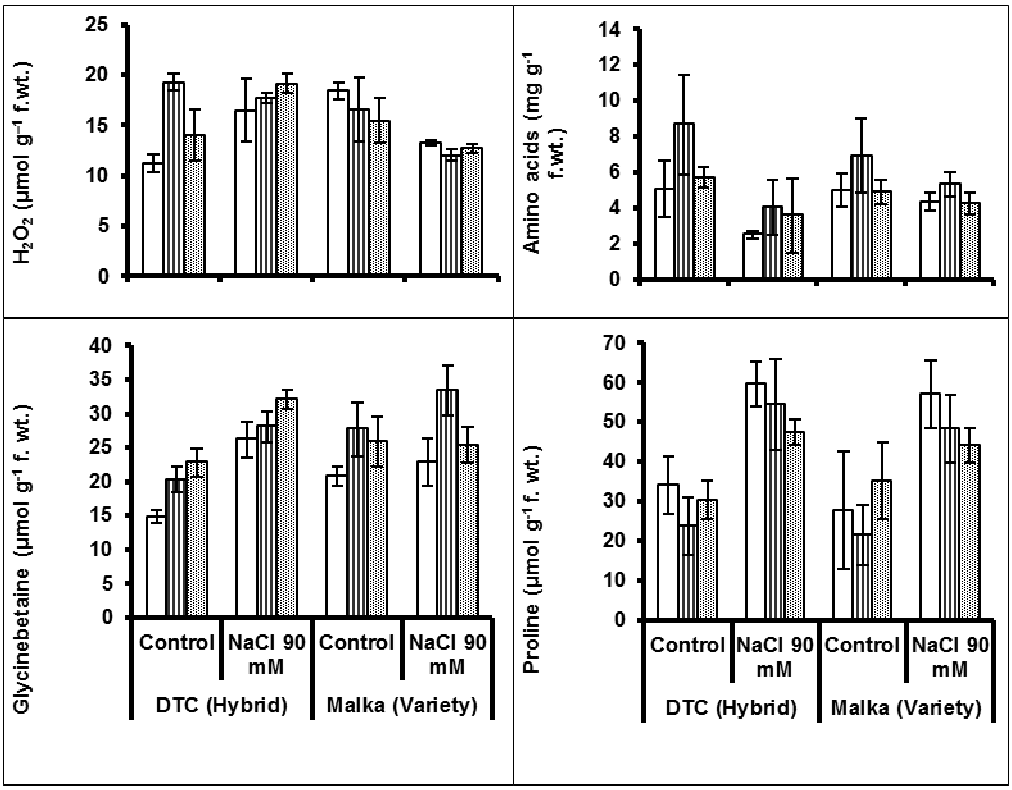
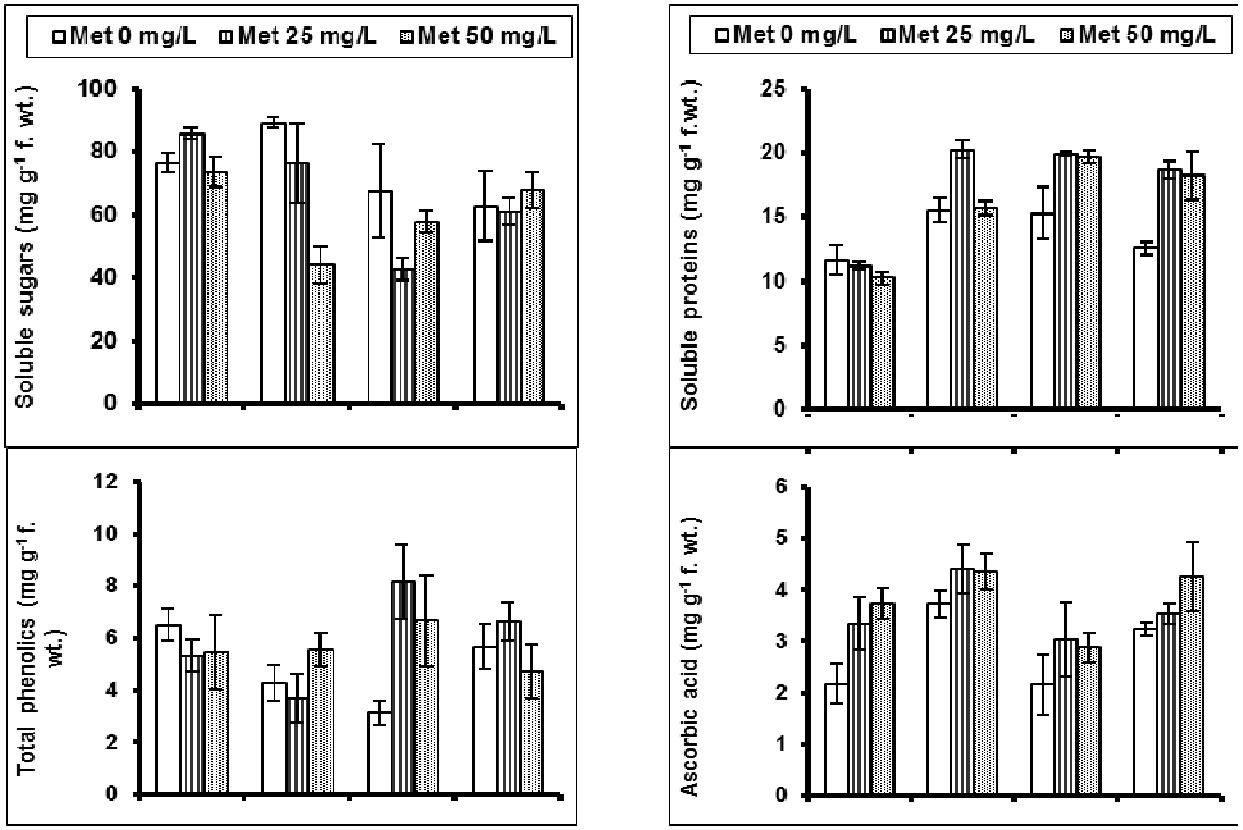


Fig. 2. Chlorophyll, membrane permeability (%), hydrogen peroxide, amino acids, glycinebetaine and proline contents of maize (*Zea mays* L.) plants foliarly-sprayed with methionine under non saline and saline conditions.



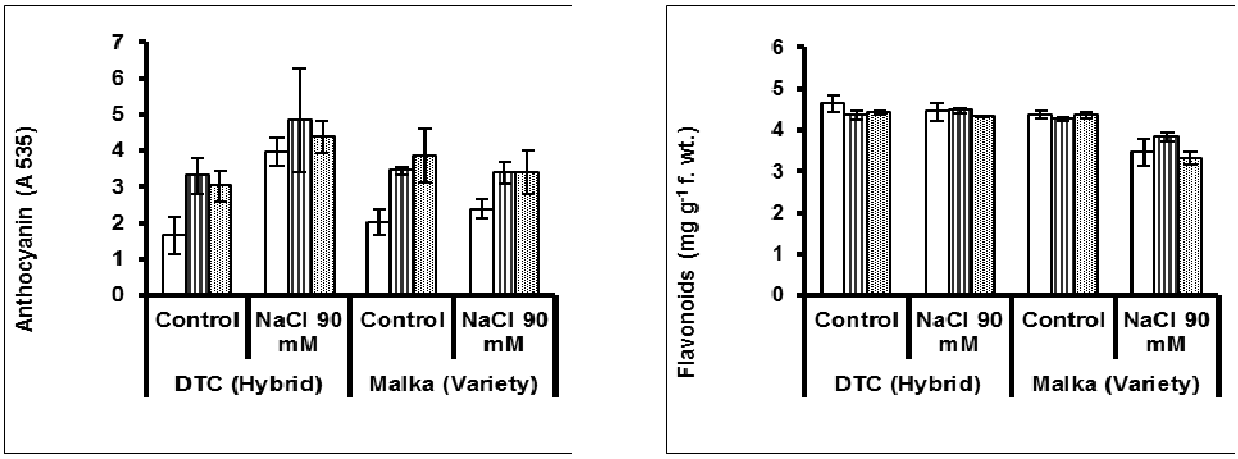


Fig. 3. Contents of total soluble sugars, total soluble proteins, total phenolics, ascorbic acid, flavonoids and anthocyanin of maize (*Zea mays* L.) plants foliarly-sprayed with methionine under non saline and saline conditions.

DISCUSSION

Abiotic stresses like salinity, drought and climate change can decrease about 50-70% crop yield production throughout the world (Mittler, 2006). Salinity stress can afflict 20% cultivated land and 33% irrigated land with 10% annual increase in agricultural area globally (Jamil *et al*., 2011). Methionine is a sulphure-containing, non-polar amino acid that has a straight side chain containing S-methyl (-CH_3) thio-ether at γ-carbon (Guedes *et al*., 2011). It enters body through dietary protein and participates in the synthesis of other S-containing amino acids (Ulrey *et al*., 2005). It is nutritionally vital amino acid whose low quantity in plants reduces its nutritional protein value for humans and animals (Amir and Hacham, 2008). Methionine oxidized to methionine sulfoxide due to oxidative stress of monooxygenases under abiotic stresses (Zhu *et al*., 2015; Jacques *et al*., 2015; Manta and Gladyshev, 2017).

Salt stress decrease growth of shoot by diminishing initiation and expansion of leaf and internodes growth and through increasing leaf abscission (Qu *et al*., 2012). Decline in relative water content (RWC) has been reported in all maize varieties in saline environments (Sairam *et al*., 2002). El Sayed (2011) reported decline in the photosynthetic pigments in maize (*Zea mays* L.) crop under NaCl stress conditions. Hydrogen peroxide (H_2O_2) level is maintained inside the cell by various types of antioxidant enzymes like peroxidase (POD) and catalase (CAT) (Foyer and Noctor, 2003). Whereas, the concentration of antioxidant enzymes and metabolites can increase or decrease during various environmental conditions (Yu and Rengel, 1999). Parvaiz and Satyawati (2008) reported that total free amino acids in plants leaves to be greater in salt resistant as compared to salt sensitive cultivars of sunflower. In this study, ascorbic acid contents increased under NaCl stress and by usage of methionine as foliar application in both cultivars of maize. Ascorbic acid has been reported to improve growth via increase in fresh and dry weights, photosynthetic pigments i.e., carotenoids, chlorophyll *a* and *b* contents, relative water content, proline and activities of antioxidant enzymes, while decreased membrane permeability, malondialdehyde and sodium and chloride ions in red cabbage plants (Hegazi and El-Shraiy, 2017).

In present study, contents of glycinebetaine increase in salt stress and foliar treatments of methionine in both varieties of maize. Exogenous treatments of glycinebetaine has been described to enhance salinity tolerance in safflower seedling through improving antioxidant defense system and maintaining nutrients balance (Alasvandyari *et al*., 2017). Glycinebetaine (GB) containing lines of maize (*Zea mays* L.) showed less growth inhibition under salt stress conditions. Glycinebetaine (GB) content increased during salinity stress in many plants though it may increase in shoots and may not vary significantly in roots (Wang and Nil, 2000). Kaya *et al*. (2010) described that proline accumulation increases in maize (*Zea mays* L.) plants suffering from salinity stress. Ayaz *et al*. (2000) reported that flavonoids and phenolic compounds are amongst the utmost in effect and widely dispersed secondary metabolites in plants. They show significant eco-physiological roles by involving in tolerance mechanisms of plants against different types of stresses.

In this study, protein contents improved under NaCl stress and by foliar usage of methionine in two maize varieties. It has been described that plants species accumulate greater contents of protein under salinity stress (Abd El-Samad *et al*., 2017). Anthocyanins are coloring pigments that belong to flavonoid family of plants and play role against many oxidants under abiotic stresses (Parvaiz *et al*., 2017). According to

Kaliamoortiiy and Rao (1994) due to high salt levels anthocyanins accumulate as a stress response. According to Ali and Abbas (2003) Barley (*Hordeum vulgare* L.) seedlings exposed to sodium chloride (NaCl) suffer from an oxidative stress as seen from its effect on flavonoids, phenolic compounds and oxidant enzymes analyzed in roots and shoots.

Bahmani *et al.* (2015) reported that the exogenous treatments of sulfur metabolites such as vitamins (thiamine and biotin), amino acids such as methionine and cysteine, thioredoxin system (glutathione, lipoic acid, and glucosinolats) can increase the plants salt stress tolerance. A gene which involved in over-expression of methionine biosynthesis has been revealed to increase tolerance against salinity (Gläser *et al*., 1993). Genomic study of *Arabidopsis thaliana* (*t365*) mutant decreased in S-adenosyl-L-methionine, phosphorethanolamine N-methyltransferase (*PEAMT*) genetic factor intricate in biosynthesis of glycine betaine (GB) presented hyper-sensitivity to salinity (NaCl) stress (Mou *et al*., 2002).

Methionine plays various roles in cellular metabolism e.g., as mRNA initiation, translation, protein constituent and as S-adenosylmethionine (SAM) bio-regulatory element. It is a precursor of S-adenosylmethionine that is a cofactor and play versatile roles in methyl group transfer and synthesis of polyamines, ethylenes (Fontecave *et al.,* 2004). According to an estimate about 20% methionine used in protein synthesis and 80% is converted into S-adenosylmethionine (SAM) formation (Hesse and Hofgen, 2003). *S*-adenosylmethionine synthetase involved in S-adenosylmethionine synthesis from methionine and ATP is one of the salt stress responsive gene (Ma *et al*., 2017). SAM is involved in the synthesis of metabolites (polyamines) and of the gaseous plant hormone (ethylene) that play part in regulation of plant retorts to different biotic or abiotic stresses (Gong *et al*., 2014; Hu *et al*., 2012; Kollner *et al*., 2010; Nagel *et al*., 2008).

In current study, foliar treatment of methionine significantly improved shoot dry mass, fresh root mass, GB, total soluble protein, ascorbic acid and anthocyanin contents of maize varieties (DTC (hybrid) and Malika) in salt stress or controlled conditions. Of the varying methionine levels, 50 mg L^{-1} proved better in decreasing harmful effect of NaCl stress in both varieties of maize.

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