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EFFECT OF SALT STRESS (NACL) ON SOME MORPHO-PHYSIOLOGICAL PROPERTIES OF MAIZE (*ZEA MAYS* L.)

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Abstract

The lower crop productivity in most of the cases is attributed to various abiotic stresses. Salt stress is one of the abiotic stresses in worldwide that inhibit the crop's growth and productivity which is going to increasing day by day. To keep this problem in mind the present study was carried out to see the impact of salt stress on some morpho-physiological and biochemical characteristics in 4 maize inbred lines (2012-13R # 838 HUZM-88, 2012-13R # 839 HUZM-147, 2012-13R 841 # HUZM-242 and 2012-13R # HUZM-386). The maize seedlings were transplanted to plastic pots contained sterilized sandy soil that continuously aerated full-strength Hoagland nutrient solution. Salt stress was applied to the plants at four levels (0, 4, 8 and 12 dS m^{-1}) from source sodium chloride (NaCl). The plants were harvested for experimental analysis after 30 days of treatment. Results indicated that salt stress significantly decreased shoot, root length, fresh and dry weight, leaf area, anthocyanin and chlorophyll content and relative water content (RWC) of maize plants.

Key words: *Zea mays* L., salt stress, anthocyanin, chlorophyllcontent, leaf area, morpho- physiological characteristics

Introduction

World agriculture is facing a lot of challenges like producing 70% more food for an additional 2.3 billion people by 2050 while at the same time fighting with poverty and hunger, consuming scarce natural resources more efficiently and adapting to climate change (FAO 2009). However, the productivity of crops is not increasing in parallel with the food demand. The lower productivity in most of the cases is attributed to various abiotic stresses. Curtailing crop losses due to various environmental stressors is a major area of concern to cope with the increasing food requirements (Shanker and Venkateswarlu 2011).

Maize (*Zea mays* L.) is the important cereal crop which is the basic need of food and oil for human intake. It is also used as feed for livestock (Hussain *et al*. 2010) throughout the world but this crop is normally submissive to salt stress. It is estimated that about 20% of the irrigated land in the present world is affected by salt stress that is exclusively classified as arid and desert lands comprising 25% of the total land of our planet (Rasool *et al*., 2013). Effects of salt stress on crop productivity are more severe in arid and semiarid regions where limited rainfall, high evapo-transpiration, high temperature, poor water quality, and poor soil management practices exacerbate salt stress effect (Neto *et al*., 2006).

Salt stress is an abiotic stress that can affect the plant growth and physiological and biochemical activities such as photosynthesis activity and chlorophyll content (Hajer *et al*. 2006; Saleh, 2012). Leaf chlorophyll under salt stress damage and its cause of decreasing of photosynthesis (Turan *et al*. 2009). have mentioned the reduction of fresh and dry weights of shoot and root, stem length, leaf area, and chlorophyll contentmaize plants in salt stress conditions (Rohanipoor *et al*. 2013).

Salt stress can be distinguished at several levels such as shoot, root, and tissues (Tester and Davenport 2003). It creates both ionic as well as osmotic stress on plants (Parvaiz and Satyawati 2008). stated that increased sodium chloride salt stress causes the decrease in vegetation growth and the rate of photosynthesis (Munns, 1993). A large amount of salt can cause various modifications in plant metabolism such as inhibition of

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enzyme activity, changes in phosphorylation state and production of reactive oxygen species (Allakhverdiev *et al*. 2000, Blumwald *et al*. 2000). Osmotic stress is caused due to the excess of Na^+ and Cl^- in the environment that decreases the osmotic potential of the soil solution and hence water uptake by plant root (Rasool *et al., 2013)*.

Anthocyanin synthesis is one of the subsequent production and localization of anthocyanin in root, stem and especially leaf tissues may allow the plant to develop resistance to a number of environmental stresses (Scott, 1999; Shirley, 2002; Steyn and Wand, 2002). Maize is one of the cereals whose anthocyanin composition is better defined (Escribano-Bailon *et al*. 2004). Anthocyanins are purple flavonoid pigments that are synthesized in many vegetative plant organs, including leaf, stem, anthers, glumes of the cob tassel, coleoptiles and the aleurone layer of maize (Cone *et al*. 1986). Anthocyanin in purple corn have been reported to have various biological activities, such as antibacterial, antifungal activity, antioxidant, antimutagenic, the prevention of obesity, diabetes, ameliorating hyperglycemia in mice (Tsuda *et al*. 2003) and have higher antioxidant properties than blueberries (Cevallos-Casals and Cisneros-Zevallos 2003). Thus, purple corn can play a role in functional food and may be also useful as a contributor to food safety.

In this study, we hypothesized that salt stress (NaCl) decrease the growth of maize plants. Therefore, this study aimed to investigate the effects of salt stress in maize, and it evaluated the morpho-physiological and biochemical characteristics such as shoot, root length, fresh and dry weight, leaf area, anthocyanin, and chlorophyll content and relative water content (RWC) of maize plants.

Materials and methods

Pot experiment under glass house conditions

This study was carried out is one of the abiotic stress in greenhouse condition in Banaras Hindu University Varanasi, India with natural light, daily photoperiod 12 h, day average temperature 24°C, night average temperature 18°C and mean relative humidity 70 ± 5%. The seed of 4 maize inbreed lines i.e., 2012-13R # 838 HUZM-88, 2012-13R # 839 HUZM-147, 2012-13R 841 # HUZM-242 and 2012-13R # HUZM-386 were obtained from the department of Genetics and Plant Breeding, Institute of Agricultural Sciences, B.H.U., Varanasi, (U.P.), India. The healthy and bold seed of these inbreeds were surface sterilized with 95% ethanol and 0.1% mercuric chloride ($HgCl_2$) followed by 6-7 washing with sterile deionized water. Seeds were incubated in dark at 28^0C for 2 days for germination on sterilized Petri dish with wet paper.

Afterward, germinated selected seedlings (with 1-2 cm long radicals) of equal size and vigor were transferred to each plastic pot (in depth of 2 cm) having 22cm height and 20 cm opening mouth diameter containing sterilized sandy soil in a ratio of 1:2 Wt/Wt. Salt stress was applied before soil sterilization at four levels (0, 4, 8 and 12 dS m-1) from the source of sodium chloride (NaCl). For soil sterilization, salt treated and untreated (control) field soil was autoclaved twice for 20 min at 120°C with a 24 h interval. The pot contained 1000 ml of continuously aerated full-strength Hoagland nutrient solution (Hoagland and Arnon, 1950), which was renewed every other day. All plants were harvested after one-month (4 weeks) treatments and separated into shoot and root. Shoot and root samples were dried at 70°C for 48 h, in a forced-air oven were dried and dry weight was determined. The fresh and dry weight of shoot and root recorded using electronic precision balance (0.001g).

Leaf area was measured with the help of leaf area meter systronics made in India, model no- 211 by averaging the value taken from four plant samples. Leaf chlorophyll content was measured by using SPAD containing hand held chlorophyll content meter (model ccm-200). At each evaluation, the content was repeated 5 times from leaf tip to base and the average was used for analysis.

Measurement of anthocyanin

Anthocyanin content calculated by a known weight of fresh leaf tissues was soaked in 3 ml of acidified methanol (1% v/v HCl) for 24 h in darkness at 4°C with occasional shaking. About 2 ml of distilled water and 4.8 ml of chloroform were mixed and added to the extract then filtered. The mixture was centrifuged for 15 min at 5000 rpm. The light absorbance of the upper phase was determined by an UV-visible spectrophotometer as the difference between the absorbance at 530 and 657 nm wavelengths. The concentration of anthocyanin as mg g^{-1} dry weight of the differently treated plants using the following equation:

$$Anthocyanin = \frac{(OD539 - 0.25\,OD657 \times TV}{(DW \times 1000)}$$

Where: OD= optical density (nm); TV= total volume of the extract (ml); DW= dry weight of the leaf tissue (g).

Determination of RWC

Plants were uprooted for calculating leaf relative water content (RWC). The RWC, stated by (Slatyer, 1967), express as a percentage of water content at a

given time and tissue related to the water content at full turgor. After taking the fresh weight (FW in gm), plants were immersed in tap water for 4 hours for making the cells turgid and leaves were removed, the surface water was blotted out and the turgid weight recorded. Dry weight (DW in g) of the plant was recorded after placing the plants the in the oven at 70^0C for 6 h. Leaf relative water content was calculated using the following formula (Turner, 1981):

$$Relative\,water\,content(\%) = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Where: FW., fresh weight of the shoot; DW., dry weight of the shoot; TW., turgid weight of the shoot.

Statistical analysis

A greenhouse experiment was arranged in a completely randomized design (CRD) with four replications. All parameters were investigated by one-way analysis of variance (ANOVA) using SPSS software. The control and treatment means were compared at 95% and 99% probability level (P = 0.05 and 0.01), and the same set of data was further analyzed to calculate the critical difference (C.D.) at P = 0.05 and 0.01, respectively.

Results

Effect of salt stress on maize inbreed lines at the initial growth stages

The effects of salt stress (NaCl) of increasing level (0, 4, 8 and 12 dS m^{-1}) on maize inbred lines were observed by shoot and root length, fresh and dry weight, leaf area, anthocyanin and chlorophyll content and relative water content (RWC) after 30 days exposure to salt stress. The seedlings of these inbreed lines died after 15 days at 12 dS m^{-1} supplied salt stress.

Effect of salt stress on shoot length

A significantly decreased shoot length was recorded over control after 30 days applied salt stress with all inbreeds (Table 1). Inbreed line 4 showed maximum significant decreased (29.77%) shoot length and inbreed line 2 observed minimum significant decreased (14.02%) shoot length at 4 dS m^{-1} applied (Table 1). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (64.38%) and minimum (38.05%) shoot length (Table 1) observed in 4 and 2 inbred lines, respectively.

Effect of salt stress on root length

A significantly decreased root length was recorded over control after 30 days applied salt stress with all inbreeds (Table 1). Inbreed line 4 showed maximum significant decreased (44.77%) root length and inbreed line 1 observed minimum significant decreased (37.65%) root length at 4 dS m^{-1} applied (Table 1). The simultaneous application of salt stress 8 dS m^{-1}

Table 1. Mean comparison and analysis of variance effects salt stress on leaf area, shoot length and root length of four maize inbreed lines (greenhouse conditions).

	Shoot length (cm)				Root length (cm)				leaf area (cm^2)			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	66.45**	82.00**	65.85**	74.75**	51.13**	64.38**	57.38**	43.00**	394.65**	448.85**	379.90**	365.00**
4 dS m^{-1}	52.87 (-20.43)**	70.50 (-14.02)**	56.60 (-14.05)**	52.50 (-29.77)**	31.88 (-37.65)**	39.00 (-39.42)**	32.00 (-44.23)**	23.75 (-44.77)**	254.13 (-35.61)**	341.05 (-24.02)**	338.10 (-11)**	92.20 (-74.74)**
8 dS m^{-1}	27.47 (-58.65)**	50.80 (-38.05)**	26.75 (-59.38)**	26.63 (-64.38)**	23.38 (-54.28)**	28.50 (-55.73)**	23.23 (-59.52)**	16.10 (-62.56)**	110.85 (-71.91)**	209.00 (-53.44)**	87.40 (-76.99)**	41.75 (-88.56)**
12 dS m^{-1}	0	0	0	0	0	0	0	0	0	0	0	0
SEM±	1.43	0.87	1.06	1.22	1.79	1.46	2.22	0.67	1.13	1.53	4.13	1.85
CD 5%	3.12	1.91	2.32	2.66	3.9	3.17	4.83	1.45	2.45	3.33	9	4.04
CD 1%	4.37	2.67	3.25	3.73	5.47	4.45	6.77	2.04	3.44	4.67	12.62	5.66
CV %	11.02	4.87	8.07	8.97	19.05	12.5	22.26	9.1	1.68	1.73	5.8	4.2

Note: 1= 2012-13R # 838 HUZM-88; 2= 2012-13R # 839 HUZM-147; 3= 2012-13R 841 # HUZM-242 and 4= 2012-13R # HUZM-386 inbreed lines, respectively; CD = Critical difference; SEM = Standard error of mean. Values in parentheses indicate % increase over control. **significant at 1%

Table 2. Mean comparison and analysis of variance effects salt stress on anthocyanin and chlorophyll content of four maize inbreed lines (greenhouse conditions).

	Chlorophyll content (SPAD)				Anthocyanin content (mg/g)			
	1	2	3	4	1	2	3	4
Control	35.23**	30.25**	21.36**	35.40**	0.0121**	0.0331**	0.0571**	0.0079**
4 dS m^{-1}	25.15 (-28.6)**	25.73 (-14.96)**	16.73 (-21.75)**	25.63 (-27.61)**	0.0110 (-9.24)**	0.0263 (-20.44)**	0.0516 (-9.61)**	0.0071 (-9.84)**
8 dS m^{-1}	17.75 (-49.61)**	16.10 (-46.78)**	13.25 (-38.01)**	13.55 (-61.72)**	0.0073 (-40.22)**	0.0248 (-25.20)**	0.0160 (-71.99)**	0.0051 (-35.92)**
12 dSm^{-1}	0	0	0	0	0	0	0	0
SEM±	0.95	0.51	0.64	0.69	0.0003	0.0004	0.0007	0.0003
CD 5%	2.08	1.12	1.39	1.49	0.0006	0.0008	0.0016	0.0006
CD 1%	2.91	1.57	1.94	2.09	0.0009	0.0012	0.0022	0.0008
CV %	13.82	8.06	14.02	10.39	10.5916	5.1499	6.674	15.5618

Note: 1= 2012-13R # 838 HUZM-88; 2= 2012-13R # 839 HUZM-147; 3= 2012-13R 841 # HUZM-242 and 4= 2012-13R # HUZM-386 inbreed lines, respectively; CD = Critical difference; SEM = Standard error of mean. Values in parentheses indicate % increase over control. **significant at 1%

[1] showed that significantly decreased maximum (62.56%) and minimum (54.28%) root length (Table 1) observed in 4 and 1 inbred lines, respectively.

Effect of salt stress on leaf area

A significantly decreased leaf area was recorded over control after 30 days applied salt stress with all inbreeds (Table 1). Inbreed line 4 showed maximum significant decreased (49.56%) leaf area and inbreed line 3 observed minimum significant decreased (21.91%) leaf area at 4 dS m^{-1} applied (Table 1). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (80.97%) and minimum (48.82%) leaf area (Table 1) observed in 1 and 3 inbred lines, respectively.

Effect of salt stress on chlorophyll content

A significantly decreased chlorophyll content was recorded over control after 30 days applied salt stress with all inbreeds (Table 2). Inbreed line 1 showed maximum significant decreased (28.60%) chlorophyll content and inbreed line 2 observed minimum significant decreased (14.96%) chlorophyll content at 4 dS m^{-1} applied (Table 2). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (61.72%) and minimum (38.01%) content for chlorophyll content (Table 2) observed in 4 and 3 inbred lines, respectively.

Effect of salt stress on anthocyanin content

A significantly decreased anthocyanin content was recorded over control after 30 day's applied salt stress with all inbreeds (Table 2). Inbred line 2 showed maximum significant decreased (20.44%) anthocyanin content and inbreed line 1 observed minimum significant decreased (9.24%) anthocyanin content at 4 dS m^{-1} applied (Table 2). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (71.99%) and minimum (25.20%) content for anthocyanin content (Table 2) observed in 3 and 2 inbred lines, respectively.

Effect of salt stress on fresh shoot weight

A significant decreased fresh shoot weight was recorded over control after 30 day's applied salt stress with all inbreeds (Table 3). Inbreed line 4 showed maximum significant decreased (49.56%) fresh shoot weight and inbreed line 3 observed minimum significant decreased (21.91%) fresh shoot weight at 4 dS m^{-1} applied (Table 3). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (80.97%) and minimum (48.82%) fresh shoot weight (Table 3) observed in 1 and 3 inbred lines, respectively.

Effect of salt stress on fresh root weight

A significant decreased fresh root weight was recorded over control after 30 day's applied salt stress with all inbreeds (Table 3). Inbreed line 3 showed maximum significant decreased (73.31%) fresh root weight and inbreed line 4 observed minimum significant decreased (68.74%) fresh root weight at 4 dS m^{-1} applied (Table 3). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (87.53%) and minimum (84.16%) fresh root weight (Table 3) observed in 1 and 4 inbred lines, respectively.

Effect of salt stress on turgid weight

A significant decreased turgid weight was recorded over control after 30 day's applied salt stress with all inbreeds (Table 3). Inbreed line 4 showed maximum significant decreased (44.67%) turgid weight and inbreed

Table 3: Mean comparison and analysis of variance effects salt stress on fresh shoot weight,fresh root weight and turgid weight of four maize inbreed lines (greenhouse conditions).

	Fresh shoot weight (g)				Fresh root weight (g)				Turgid weight (g)			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	18.00**	17.85**	12.7**	10.91**	2.13**	1.69**	1.71**	1.77**	19.29**	19.05**	13.84**	12.20**
4 dS m^{-1}	11.49 (-36.18)**	13.47 (-24.54)**	9.92 (-21.91)**	5.50 (-49.56)**	0.57 (-73.06)**	0.47 (-72.08)**	0.46 (-73.31)**	0.55 (-68.74)**	12.36 (-35.93)**	14.58 (-23.46)**	11.17 (-19.29)**	6.75 (-44.67)**
8 dS m^{-1}	3.43 (-80.97)**	8.21 (-54.03)**	6.50 (-48.82)**	3.30 (-69.74)**	0.27 (-87.53)**	0.24 (-85.97)**	0.23 (-86.66)**	0.28 (-84.16)**	4.13 (-78.59)**	9.21 (-51.65)**	9.41 (-32.01)**	4.50 (-63.11)**
12 dS m^{-1}	0	0	0	0	0	0	0	0	0	0	0	0
SEM±	0.27	0.49	0.45	0.41	0.05	0.06	0.02	0.09	0.28	0.43	0.34	0.32
CD 5%	0.6	1.07	0.98	0.9	0.11	0.12	0.04	0.2	0.61	0.94	0.74	0.69
CD 1%	0.84	1.5	1.38	1.26	0.16	0.17	0.05	0.28	0.85	1.32	1.04	0.97
CV %	9.41	14.1	17.51	23.63	19.55	26.41	8.33	40.05	8.78	11.45	11.16	15.38

Note: 1= 2012-13R # 838 HUZM-88; 2= 2012-13R # 839 HUZM-147; 3= 2012-13R 841 # HUZM-242 and 4= 2012-13R # HUZM-386 inbreed lines, respectively; CD = Critical difference; SEM = Standard error of mean. Values in parentheses indicate % increase over control. **significant at 1%

Table 4. Mean comparison and analysis of variance effects salt stress on dry shoot weight, dry root weight and RWC (%)of four maize inbreed lines (greenhouse conditions).

	Dry shoot weight (g)				Dry root weight (g)				RWC (%)			
	1	2	3	4	1	2	3	4	1	2	3	4
Control	3.67**	3.17**	2.72**	2.88**	0.89**	0.80**	0.79**	0.76**	91.79**	93.72**	91.59**	87.20**
4 dS m^{-1}	1.84 (-49.90)**	1.60 (-49.68)**	1.18 (-56.62)**	1.25 (-56.55)**	0.47 (-46.91)**	0.40 (-50.78)**	0.42 (-46.35)**	0.38 (-49.50)**	91.77 (-0.02)**	92.71 (-1.08)**	88.93 (-2.90)**	76.89 (-11.82)**
8 dS m^{-1}	0.94 (-74.34)**	0.93 (-70.82)**	0.84 (-69.12)**	0.63 (-78.32)**	0.16 (-82.30)**	0.13 (-84.11)**	0.09 (-88.57)**	0.07 (-91.09)**	76.71 (-16.43)**	87.77 (-6.35)**	66.94 (-26.91)**	68.49 (-21.46)**
12 dS m^{-1}	0	0	0	0	0	0	0	0	0	0	0	0
SEM±	0.08	0.07	0.09	0.06	0.01	0.01	0.02	0.01	2.73	4.34	6.47	5.06
CD 5%	0.17	0.15	0.19	0.14	0.03	0.02	0.03	0.02	5.94	9.45	14.09	11.03
CD 1%	0.24	0.2	0.27	0.2	0.04	0.03	0.05	0.03	8.33	13.25	19.75	15.47
CV %	13.87	13.31	20.84	15.3	9.16	8.99	13.75	8.02	11.86	17.89	29.57	24.63

Note: 1= 2012-13R # 838 HUZM-88; 2= 2012-13R # 839 HUZM-147; 3= 2012-13R 841 # HUZM-242 and 4= 2012-13R # HUZM-386 inbreed lines, respectively; CD = Critical difference; SEM = Standard error of mean. Values in parentheses indicate % increase over control. **significant at 1%

Fig. 1: Anthocyanin content in maize inbreeds 2012-13R # 838 HUZM-88, 2012-13R # 839 HUZM-147, 2012-13R 841 # HUZM-242 and 2012-13R # HUZM-386 plant, salt stress during 30 days with NaCl (0, 4 and 8 dS m^{-1}).

Fig. 2. Effects on maize inbreed 2012-13R # 838 HUZM-88 plant of salt stress during 30 days with NaCl (0, 4 and8 dS m^{-1}).

Fig. 3. Effects on maize inbreed 2012-13R # 839 HUZM-147 plant of salt stress during 30 days with NaCl (0, 4 and8 dS m^{-1}).

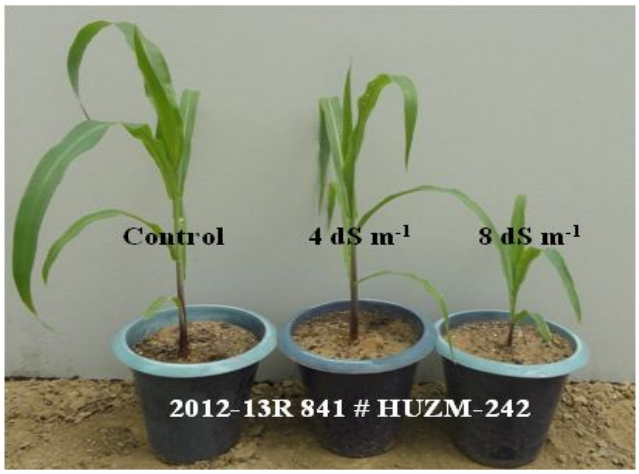

Fig. 4. Effects on maize inbreed 2012-13R 841 # HUZM-242 plant of salt stress during 30 days with NaCl (0, 4 and8 dS m^{-1}).

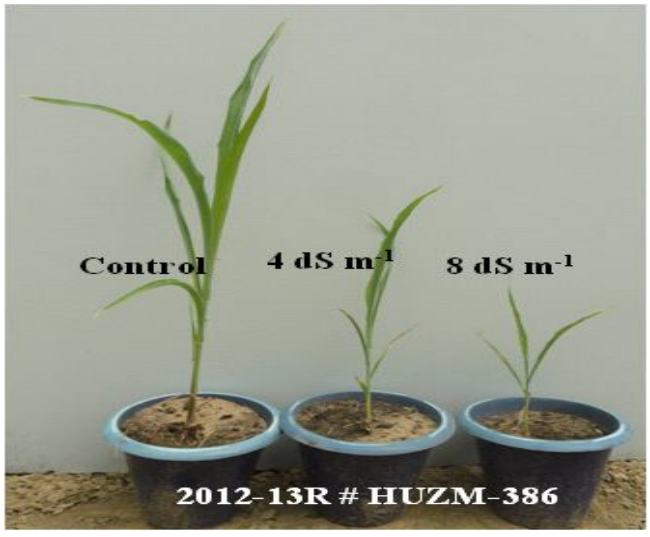

Fig. 5. Effects on maize inbreed 2012-13R # HUZM-386 plant of salt stress during 30 days with NaCl (0, 4 and8 dS m^{-1}).

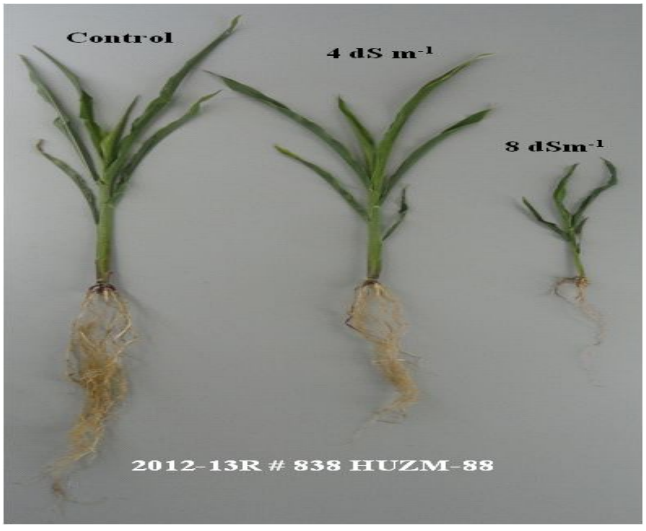

Fig. 6. Effect on shoot and root length maize inbreed 2012-13R # 838 HUZM-88 plant of salt stress during 30 days with NaCl (0, 4 and 8 dS m^{-1}).

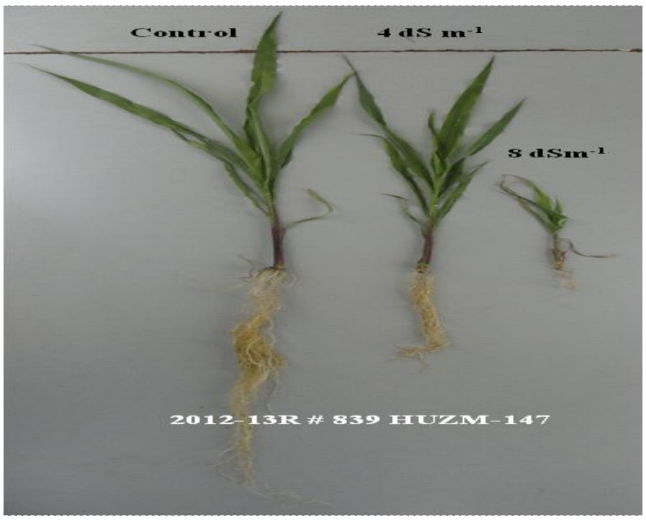

Fig. 7. Effect on shoot and root length maize inbreed 2012-13R # 839 HUZM-147 plant of salt stress during 30 days with NaCl (0, 4 and 8 dS m^{-1}).

Fig. 8. Effect on shoot and root length maize inbreed 2012-13R 841 # HUZM-242 plant of salt stress during 30 days with NaCl (0, 4 and 8 dS m^{-1}).

line 3 observed minimum significant decreased (19.29%) turgid weight at 4 dS m^{-1} applied (Table 3). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (78.59%) and minimum (32.01%) turgid weight (Table 3) observed in 1 and 3 inbred lines, respectively.

Effect of salt stress on dry shoot weight

A significant decreased dry shoot weight was recorded over control after 30 day's applied salt stress with all inbreeds (Table 4). Inbreed line 3 showed maximum significant decreased (56.62%) dry shoot weight and inbreed line 3 observed minimum significant decreased (49.68%) dry shoot weight at 4 dS m^{-1} applied (Table 4). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum

Fig. 9. Effect on shoot and root length maize inbreed 2012-13R # HUZM-386 plant of salt stress during 30 days with NaCl (0, 4 and 8 dS m^{-1}).

(78.32%) and minimum (69.12%) dry shoot weight (Table 4) observed in 4 and 3 inbred lines, respectively.

Effect of salt stress on dry root weight

A significant decreased dry root weight was recorded over control after 30 day's applied salt stress with all inbreeds (Table 4). Inbreed line 2 showed maximum significant decreased (50.78%) dry root weight and inbreed line 3 observed minimum significant decreased (46.35%) dry root weight at 4 dS m^{-1} applied (Table 4). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (91.09%) and minimum (82.30%) dry root weight (Table 4) observed in 4 and 1 inbred lines, respectively.

Effect of salt stress on relative water content (RWC)

A significant decreased relative water content (RWC) was recorded over control after 30 day's applied salt stress with all inbreeds (Table 4). Inbreed line 4 showed maximum significant decreased (11.82%) RCW and inbreed line 3 observed minimum significant decreased (0.02%) RCW at 4 dS m^{-1} applied (Table 4). The simultaneous application of salt stress 8 dS m^{-1} showed that significantly decreased maximum (26.91%) and minimum (6.35%) RCW% (Table 4) observed in 3 and 2 inbred lines, respectively.

Discussion

In this study, 4 inbreed lines were used for the effect of salt stress (NaCl) at different levels by conducting pot experiment on maize (*Zea mays* L.). It was observed that salt stress at all levels significantly decreased shoot, root length, fresh and dry weight of maize (Table 1, 3 and 4; Figure 2, 3, 4, 5, 6, 7, 8 and 9). In general, plant growth

was decreased with increase in salt stress. However, plants were effective (dead) in the presence of higher salt stress level (12 dS m^{-1}). Application of salt stress (NaCl) at different levels significantly decreased all growth parameters with increase in salt stress on maize (Cicek and Cakirlar 2002; Xinghong and Congming 2005; Giaveno *et al*. 2007; Parvaiz and Satyawati 2008; Hussain *et al*. 2010; Khatoon *et al*. 2010; Zahoor *et al*. 2011; Khodarahmpour *et al*. 2012; Niu *et al*. 2012; Usman *et al*. 2012).

Leaf area significantly decreased under salt stress (NaCl) at different levels (Table 1). Salt stress (NaCl) concentrations increased observed significantly decrease leaf area on maize (*Zea mays* L.) (Cramer *et al*. 1994; Giaveno *et al*. 2007; Rohanipoor *et al*. 2013). Leaf area significantly contributed toward physiological indices, which boosted up crop growth and accumulation of more photo assimilates from source to sink and consequently, it led to higher grain yield (Ahmed *et al*. 2012).

Anthocyanin and chlorophyll content significantly decreased under salt stress (NaCl) at different levels (Table 2 and Fig. 1). Anthocyanin accumulation as a stress response under higher salt stress was studied at different stages of seedling growth of maize (Kaliamoorthy and Rao, 1994; Dutt *et al*. 1991; Ramanjulu *et al*. 1993; Rohanipoor *et al*. 2013). Applied salt stress at four levels (0, 3, 6 and 9 dS m^{-1}) from source sodium chloride (NaCl) and observed significantly decreased chlorophyll content. Leaf chlorophyll content under salt stress reduced and it causes decreasing of photosynthesis (Delfine *et al*. 1999; Turan *et al*. 2009). Imposition of salt stress significantly reduced chlorophyll a, b and total chlorophyll content was found with all maize varieties (Zahoor *et al*. 2011). In field conditions, salt stress significantly reduced shoot dry mass, cob yield, total kernel yield, weight of 1000 kernels, chlorophylls "a" and "b" and relative water content in the maize plants (Kaya and Okant, 2013).

We found that salt stress (NaCl) also affected significantly decreased relative water content (RWC) on maize (Table 4). Application of salt stress (NaCl) at different levels significantly decreased relative water content with increase in salt stress on maize (Cicek and Cakirlar 2002; Rohanipoor *et al*. 2013). Many important physiological and morphological processes, such as leaf enlargement, stomatal opening, and associated leaf photosynthesis are directly affected by the reduction of leaf turgor potential which accompanies the loss of water from leaf tissue (Jones and Turner, 1978). Water stress and turgor loss through inadequate osmotic adjustment slow cellular expensive growth and lead to a reduction in leaf cell size (Curtis and Lauchli 1987).

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