EFFECTS OF SALT STRESS ON THE PHYSIOLOGICAL TRAILS OF MAIZE (ZEA MAYS)

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Abstract

Salinity is one of the abiotic stresses that can affect the plant growth and productivity. For this purpose, the present study was conducted at Department of Botany, University of Malakand, Khyber Pakhtunkhwa Pakistan, to evaluate the responses of different zea mays genotypes for physiological traits under salt stress. A total of four zea mays genotypes: LP, DLW, DLH and YC were evaluated for physiological study. A pot experiment was conducted, after the germination different concentrations (Control, 50, 100 and 200mM) of salt were applied at appropriate stages. For physiological traits the Proline content (62.35%) was recorded in DLW (100mM) minimum reduction DLH (100mM) 14.23%. Maximum reduction in Chlorophyll a content was recorded in DLW (50mM), minimum reduction (28.71%) was recorded in DLW (100mM). Chlorophyll b content (44.17%) was recorded in LP (200mM), minimum reduction (1.81%) was recorded in DLH (200mM). Maximum reduction (81.73%) regarding protein content was recorded in DLW (200mM) whereas minimum reduction (24.14%) in DLW (100mM), maximum reduction (92.68%) regarding hydrogen peroxide was recorded in DLW (50mM) minimum reduction (27.93%) was recorded in DLW (200mM) respectively. Owing to these characters, the representative selection of resistant genotype is suggested to be used as a salt tolerant genotype.

Key words: Zea Mays, Abiotic stress, NaCl, Proline, Chlorphyll.

Introduction

The maize is one three crops that are most important as a food resource for the people and is widely cultivated crop in Pakistan. Maize provides many essentials mineral, multiple vitamins B, and is a good fibers source but is lacking in vitamin C, vitamin B12, calcium and iron etc. abiotic stresses such as sun, salinity, temperature and drought conditions have aggressive effects on the production and growth of crops (Ahuja *et al*., 2010; Shahbaz *et al*., 2013)**.** Abiotic pressures or non-living causes include drought or water shortage, salinity, temperature rise, chemical contaminants, oxidative agents, which pose dramatic plant threats and cause environmental degradation. Abiotic stress is the first factor that induces crop degradation and decreases crop productivity by more than 50% (Caddell *et al*., 2019). Increase salt concentrations in water or soil are referred to as salinity, salinity stress is a major environmental factor that inhibits plant growth and development, and its negative consequences are posing a serious threat to our most important cropland and urban greenbelts (Jouyban, 2012). Salinity is abiotic factor that has a severe impact on crop loss worldwide, affecting about 800 million hectares of agricultural cropland (Tang *et al*., 2012).In world region both arid and semi- arid, little rainfall, high evaporation, high transpiration rate and temperature are the primary causes of salinity, which reduces germination and seedling vigor, lowers germination rates and delays seed germination (Chohan *et al*., 2012).Salt stress has been shown to affect seed germination in a variety of plants, including rice (Xu *et al*., 2011), wheat (Akbari Moghaddam *et al*., 2011), maize (Fang Y, *et al*., 2017), and mustard (Polash *et al*., 2019; Ulfat *et al*., 2017). Salt stress has a variety of effects on seed germination. It also produces toxicity, which reduces seed intake by affecting the activity of nucleic acid metabolism enzymes (Gomes-Filho *et al*., 2008) and protein metabolism (Dantas *et al*., 2007; Al-Tawaha *et al*., 2019). One of the multiple salinity consequences involves a reduction in plant growth rate. The

soil's saline water inhabits development in two aspects; the ability of roots to retain water is interrupted by high salt levels in soil water, while high salt levels in plants are also toxic and cause interruption in several physiological and biochemical pathways, i.e. the absorption and incorporation of nutrients (Ali and Yun, 2017). Several readings approve the inhibitory effect of salinity on biological pathways, including photosynthesis, which is the basic and complex physiological process in all chlorophyll-bearing plants. In agriculture crop productivity, the most serious abiotic stress factor is salinity. As to improve the performance of plant in saline environments several techniques have been proposed which include seed physiology at cellular level as well as whole plant by producing ionic and osmotic stresses. Salinity affects the relations of water with plant which alter osmotic stress or physiological drought (Polash *et al*., 2017). During salinity stress the most severely affected process is photosynthesis (Sudhir and Murthy, 2004). Due to the affected process the plant growth and the productivity become decreased. A common consequence of most abiotic stresses, including salinity, is the increased production of reactive oxygen species (ROS) such as superoxide radicals (O_2), hydroxyl radicals (•HO) and hydrogen peroxide (H2O2), which are extremely toxic to plants and cause damage to DNA, proteins, lipids, and chlorophyll (D Anglo and Rosa, 2020).

Materials and Methods

This research was carried out in the Botanical Garden University of Malakand' i.e Glasshouse. maize improved verities were collected from the plant genetic resources institute (PGRI) Islamabad. During research four genotypes were selected and used in the present experiment. These genotypes were (LP, DLW, DLH, YC) to evaluate salt resistant and susceptible genotypes, in the botanical garden and herbarium at university of Malakand, district Dir Lower Chakdara, Khyber Pakhtunkhwa, Pakistan. To assess the effect of salt solution on zea mays, a pot experiment was conducted in the Botanical Garden. A total 80 pots of zea mays plant (20 of each variety) were acquired. Three seeds of each line were sown in each pot (3 cm deep) filled with equal weight of soil. Four treatments [Control (well-watered i.e., Normal watering as per requirement of the crop), 50mM, 100mM, 200mM]. The experiment was laid out in a completely randomized design with three replications of each experimental unit. The weight of each plastic pot with filled soil and watering at the time of sowing, therefore moisture contents present in soil in pots. The seed of each line was sown in pots. After 15 days of germination thinning of plants were done and 2 plants per pot were maintained. Plants were irrigated normally according to their requirements till to 28 days before treatment start. After 28 days of normal growth of plants, salt stress was applied. 4-pots of each variety were separated for each treatment. different concentration of salt solution was added to each pot (50mM), (100mM), (200mM), for the solution 1mole of Nacl was dissolved in 1litre of distal water, added 25ml of salt solution to each pot and control was watering with same concentration of distal water. The experiment was performed under completely natural environmental conditions. The chlorophyll content was determined using the Arnon (1949) method. Graduated beaker, test tube, aluminum foil, grinder, lambda bio machine, and centrifuge were among the tools used. For extraction and analysis of chlorophyll content following chemicals were used. • NH4OH • Distal H_2O2 • 80% acetone. For preparation of ammonium hydroxide solution form 0.1 N NH_4OH solution, 0.35g NH_4OH dissolves in 100 ml distilled water. For preparation of ammonium hydroxide Acetone solution Form NH_4OH acetone, 0.1 N NH_4OH solution mixed with acetone in a ratio of 1:9 (v: v). For preparation of the 80% aqueous acetone solution Form reagent-grade acetone mixed with distilled water in a ratio of 2:8. Photosynthetic tissue of plants were stuck in a solution of 1 part 0.1 Normal (N) ammonium hydroxide solution to parts acetone [volume to volume (v: v)] and were kept in a centrifuge. The supernatant was separated from mixture and raise their volume up to 10ml by 80% acetone solution. The solution then poured in a test tube and kept at 40c for 24hrs. Spectrophotometer was used and the absorbance reading was record at wave lengths of 663 nanometers (nm) and 645 nm. At these wavelengths, the absorbance was recorded for each solution and chlorophyll contents (a and b) were calculated. According to Bates *et al*., (1973), 0.5 g of leaf samples from each treatment will be homogenized in 3% (w/v) sulphosalycilic acid and then homogenized distilled using filtrate to assess free proline levels. After adding 2 ml of 1% ninhydrin and 2 ml of 75 percent glacial acetic acid, the mixture was heated in a water bath for 1 hour at 100°C. After that, an ice bath was used to cease the reaction. The solution was extracted with toluene, and the absorbance of the part removed from the liquid state with toluene was measured at 520 nm. The amount of proline was calculated using a calibration curve and represented as micromoles of proline per gram of fresh weight. Proline= (ABS (520) ×35×10)/sample fresh weight 36=k= absorption coefficient 10= Rate of dilution. Protein contents were determined by using Bovine serum albumin (BSA) as standard the determination of protein was made. Fresh plant tissue (approx. 0.5 g) was grinded with 10 ml of chilled phosphate buffer through mortar and pestle in an ice environment. Then extracted sample of 0.5ml was mixed with 3ml of 6 times diluted bio-red color dye and 0.5ml of distilled water (dH2O). Then vortexed the solution and read the absorbance at 595 nm with the help of UV Spectrophotometer (Biochem-2100). Using BSA, Protein concentration was calculated from standard

curve. Hydrogen peroxide (H_2O_2) content was estimated according to the methods of Bernt and Bergmeyer. Approximately 0.5 g of root and leaf samples from control and treatment groups were homogenized with liquid nitrogen and the powders were suspended in 1.5 ml of 100 mM potassium phosphate buffer (pH 6.8). The suspensions were then centrifuged at 18,000rpm for 20 min at 4C°. The enzymatic reaction was initiated with 0.25 ml supernatant and 1.25 ml peroxidase reagent, consisting of 83 mM potassium phosphate buffer (pH 7.0), 0.005% (w/v) O-dianisidine, and 40 mg peroxidase/ ml at 30C°. The reaction was stopped after 10 min by adding 0.25 ml of perchloric acid and the reaction mixture was centrifuged at 50006 g for 5 min. The absorbance of the supernatant was read at 436 nm.

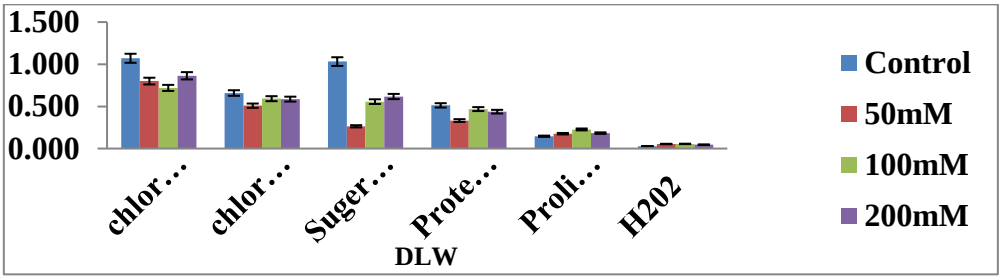
Results

The selected genotypes were tested for the below physiological traits to study their impact for these purposes only two genotypes were selected which showed resistance towards the salt or near to resistance DLH and the second variety was selected which more susceptible toward salt stress DLW.Descriptive statistical analysis of current results regarding H2O2 content leaves show significant differences from each other. The maximum value under control condition was (0.097 mg/g), minimum value (0.018mg/g), CV (59.87%) and standard error was (0.037) as shown in (table 3.10). Similarly, under salt stress condition maximum mean value (0.174mg/g), CV (42.41.32%) with standard error (0.036) was noted whilst minimum value (0.048mg/g) was noted in treatment one (50mM) respectively. Similarly in treatment two (100mM) maximum mean value (0.171 mg/g), CV (54.607) and standard error (0.059) was noted whilst minimum value (0.049mg/g) was recorded correspondingly. Likewise in treatment three (200mM) maximum mean value (0.671mg/g), CV (10.22%) and standard error (0.227) was noted whilst minimum value (0.057mg/g) was) recorded correspondingly. In the present studied parameter maximum reduction regarding H2O2 content was recorded in DLW treatment first (50mM) 92.68% follow by DLH treatment two (100MM) 73.77%, DLH treatment first (50mM) 56.30%, whereas minimum reduction was recorded in DLW treatment three (200mM) 27.93% respectively. Regarding proline contents of leaves descriptive statistic showed a remarkable variation between control group and drought stress condition group. In control condition the maximum value (1209.3umol/g), coefficient of variance (69.825%), mean (695.53) and standard error (485.52) was recorded whilst minimum mean value (248.5umol/g) was recorded. In the same case under different concentration of salt stress condition the maximum mean value in treatment first (50mM) (1743.00umol/g), coefficient of variance (76.339%), mean value (1015.9) and standard error (775.51) was recorded whilst minimum value (273.00umol/g), treatment two (100mM) the maximum mean value (1729.00umol/g), coefficient of variance (68.598%), mean value (1056.4) and standard error (724.68) was recorded whilst minimum value (311.5umol/g) and treatment three (200mM) the maximum mean value (1646.8umol/g), coefficient of variance (62.059%), mean value (1031.6) and standard error (640.22) was recorded whilst minimum value (379.75umol/g) was recorded respectively shown in the table. In the present studied parameter maximum reduction regarding proline content was recorded in DLW treatment two (100mM) 62.35% follow by DLW treatment first (50mM) 55.13%, whereas minimum reduction was recorded in DLH treatment first (50mM) 2.34% follow by DLH treatment two (100mM) 14.23% respectively. Regarding protein content of leaves descriptive statistics showed major differences between salt stress and control condition. In control condition the maximum value was recorded (0.518umol/g), CV% (9.111) and standard error (0.0432) whilst minimum mean value (0.422umol/g) was recorded. In the same case under different concentration of stress condition the maximum mean value in treatment first (50mM) (0.534umol/g), coefficient of variance (25.07) and standard error (0.108) was recorded whilst minimum value (0.321umol/g), treatment two (100mM) the maximum mean value (0.591umol/g), coefficient of variance (15.96) and standard error (0.078) was recorded whilst minimum value (0.357umol/g) and treatment three (200mM) the maximum mean value (0.672umol/g), coefficient of variance (17.98) and standard error (0.100) was recorded whilst minimum value (0.378umol/g) was recorded respectively shown in the table. In the present studied parameter maximum reduction regarding protein content was recorded in DLW treatment three (200mM) 81.73% follow by DLH treatment first (50mM) 51.89%, DLH treatment two (100mM) 33.14%, whereas minimum reduction was recorded in DLW treatment two (100mM) 24.14% follow by DLW treatment three (200mM) 30.86% respectively. During present work significant variation was observe in descriptive statistics of chlorophyll a content of leaves from each other. Under control condition the maximum value (1.114mg/g), CV (23.065%), mean value (0.8885) and standard error (0.2049) was recorded, whilst minimum value (0.691 mg/g) was noted as shown in table. Similarly, under salt stress condition maximum mean value (1.453mg/g), CV (29.762%), mean value (1.068) with standard error (0.3179) was noted whilst minimum value (0.622mg/g) was in treatment one (50mM) recorded respectively. Similarly, treatment two (100mM) maximum mean value (1.451mg/g), CV (36.95%) and standard error (0.3938) mean value

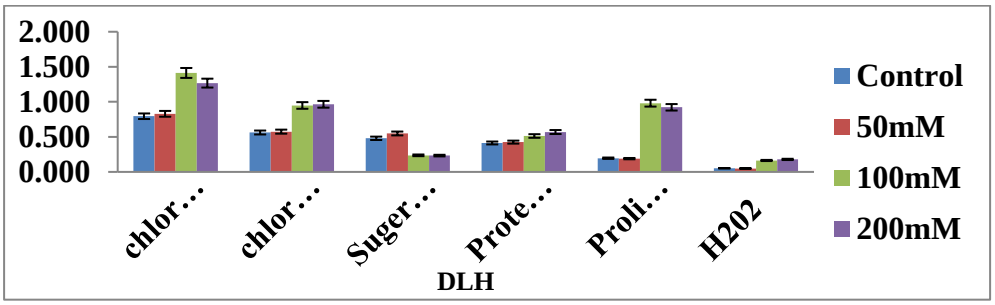
(1.0657) was noted whilst minimum value (0.59mg/g) was recorded correspondingly. Likewise in treatment three (200mM) maximum mean value (1.346mg/g), CV (31.62%) mean value (0.9918) and standard error (0.3136) was noted whilst minimum value (0.58mg/g) was) recorded correspondingly. In the present studied parameter maximum reduction regarding Chlorophyll a contents was recorded in DLW treatment first (50mM) 39.28% follow by DLW treatment three (200mM) 30.83%, whereas minimum reduction was recorded in DLW treatment two (100mM) 28.71% respectively. During present results of chlorophyll b content of leaves showed significant variation from each other. Under control condition the mean value (0.621), maximum mean value (0.811 mg/g), standard error (0.093) and CV (14978%) was recorded, whilst minimum value (0.567 mg/g) was noted as shown in (table). Similarly, under salt stress condition maximum value (0.874mg/g), CV (38.79) with standard error (0.198) mean value (0.689) was noted whilst minimum value (0.501 mg/g) was in treatment one (50mM) recorded respectively. Similarly treatment two (100mM) maximum mean value (0.987mg/g), CV% (25.809%) mean value (0.7698) and standard error (0.199) was noted whilst minimum value (0.572mg/g) was recorded correspondingly. Likewise in treatment three (200mM) maximum mean value (0.981mg/g), CV (25.812) and standard error (0.201) was noted whilst minimum value (0.566mg/g) was recorded condition respectively (Table). In the present studied parameter maximum reduction regarding Chlorophyll b content was recorded in LP treatment three (200mM) 44.17% follow by DLW treatment two (100mM) 38.88%, DLW treatment first (50mM) 26.75%, whereas minimum reduction was recorded in DLH treatment three (200mM) 1.81% respectively.

Table: Descriptive statistic for physiological studied attributes under induced salt stress of zea mays genotypes.

		Mean	SD	SE mean	CV%	Minimum	Maximum
H2O2							
	C	0.061	0.036	0.015	59.86	0.018	0.097
	50mM	0.086	0.035	0.014	41.32	0.048	0.1743
	100mM	0.109	0.059	0.243	54.6	0.049	0.171
	200mM	0.222	0.227	0.051	10.21	0.057	0.671
Proline							
	C	695.5	485.52	198.21	69.825	248.5	1209.3
	50mM	1016	775.51	316.6	76.339	273	1743
	100mM	1056	724.68	295.85	68.598	311.5	1729
	200mM	1032	640.22	261.37	62.059	379.75	1646.8
Protein							
	C	0.474	0.043	0.017	9.11	0.422	0.518
	50mM	0.43	0.107	0.044	25.007	0.321	0.534
	100mM	0.492	0.078	0.031	15.959	0.357	0.591
	200mM	0.558	0.1	0.041	17.995	0.378	0.672
Chlorophyll a							
	C	0.888	0.204	0.083	23.065	0.691	1.114
	50mM	1.068	0.317	0.129	29.762	0.622	1.453
	100mM	1.065	0.393	0.155	36.95	0.59	1.451
	200mM	0.991	0.3136	0.128	31.62	0.58	1.346
Chlorophyll b							
	C	0.621	0.093	0.038	14.978	0.567	0.811
	50mM	0.689	0.198	0.081	28.791	0.501	0.874
	100mM	0.769	0.199	0.081	25.809	0.572	0.987
	200mM	0.781	0.201	0.082	25.812	0.566	0.981



Graphical representation of physiological traits of DLW zea mays variety.



Graphical representation of physiological traits of DLH zea mays variety.

Accordingly, coefficient of correlation (Pearson) analysis between the studied physiological attributes was carried out and was given in Table. For physiological studied traits there was highly significant correlation between proline and H_2O_2 at control, treatment two (100mM), treatment one (50mM) condition r-value (0.95**), (0.98***), (0.98***) but treatment three (200mM) was negative correlate (-0.11) respectively. Similarly the correlation between H_2O_2 and protein there was less significant negative and positive correlation at control, treatment one (50mM), significant at treatment two and three (100mM & 200mM) r- value (-0.93), (0.98***), (0.36) and (-0.92), while the correlation between protein and proline was negative, highly significant and significant positive at control and all treatment condition r-value (-0.93) and (0.99***), (0.24) and (0.01) correspondingly. In the same way the correlation between chlorophyll a and H2O2 was negative at control condition, significant and high significant at all treatment r- value (-0.90), (0.93*), (0.98**), and (0.93*), although the correlation between proline and chlorophyll a negative at control and treatment one condition and highly significant and significant at both treatment two and three r- value (-0.96), (0.91*), (0.94**and (0.96**) respectively. Likewise there was significant, less significant and highly significant positive correlation between chlorophyll a and protein r- value (0.95**), (0.93**), (0.52) and (0.02), positive significant and highly significant. Similarly the correlation of sugar with H2O2 was negative, less significant and significant r-value (-0.93), (-0.92), (-0.69) and (0.01) and with proline content r- value (-0.98), (-0.95), (-0.61) and (-0.61), highly significant positive correlation with chlorophyll a and protein content, (0.98***), (-0.92), (-0.74and (-0.40) and (0.96**), (-0.95), (-0.59) and (0.10) r- value given at the table. Similarly there was less significant, significant and highly significant positive correlation of chlorophyll b content of leaves with H_2O_2, proline and protein content, chlorophyll a and sugar of leaves at control as well as all treatment environment correspondingly, significant r- value (-0.55), (0.98***), (0.96**) and (-0.29) and (-0.43), (0.99***), (0.97**) (0.97) and (0.42), (0.99***), (0.27), (0.19), chlorophyll a (0.23), (0.92*), (0.92), (0.95).

Table: Person coefficient correlation analysis of physiological studied attributes

		H2O2	Proline	Protein	Chlorophyll a	Sugar
Proline	C	0.95**				
	50Mm	0.98***				
	100Mm	0.98***				
	200Mm	-0.11				
Protein	C	-0.93	-0.93			
	50Mm	0.98***	0.99***			
	100Mm	0.36	0.24			
	200Mm	-0.92	0.01			
Chlorophyll a	C	-0.9	-0.96	0.95**		
	50mM	0.93*	0.91*	0.93**		
	100mM	0.98**	0.94**	0.52		
	200mM	-0.94	0.96**	0.022		
Chlorophyll b	C	-0.55	-0.43	0.42	0.23	0.29
	50mM	0.98***	0.99***	0.99***	0.92*	-0.95
	100mM	0.96**	0.97**	0.27	0.92	-0.58
	200mM	-0.29	0.97	0.19	0.95	-0.56

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