BIOCHEMICAL ATTRIBUTES OF SALT TOLERANT AND SALT SENSITIVE MAIZE CULTIVARS TO SALINITY AND POTASSIUM NUTRITION

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Maize (*Zea mays* L.) genotypes i.e. S-2002 (salt tolerant) and Akbar (salt sensitive) were grown under natural saline field condition to study the effect of salinity and potassium nutrition on protein contents, total soluble sugars, oil contents, crude fiber of the maize grains, proline and 1000 grain weight. The salinity level of the field was 9.95-10.60 dS m^{-1} and the original K concentration of the soil was 70-85.50 mg kg^{-1} and this level was kept as control. The experiment was triplicated and N P were applied @ 200:150 kg ha^{-1} and the potassium fertilizer was applied @ 200 kg ha^{-1}. At maturity grains were collected and the above mentioned biochemical parameters were studied. Salinity had deleterious effect on all the parameters in maize genotype Akbar while in most of cases the S-2002 had maximum levels while the application of K not only improved the yield but also improved the recovery of total soluble sugars (%) (TSS), proteins (%), oil contents (%) as well as 1000 grain weight, crude fiber (%) except proline contents.

Key word: Potassium nutrition, salinity, soluble sugars, proteins, proline, oil contents, crude fiber, maize;

INTRODUCTION

The world population is expanding rapidly and is expected to be around 8 billion by the year 2025 (Pinstrup-Andersen *et al.*, 1999). This represents an addition of nearly 80 million people to the present population every year. It is forecast that the increase in world population will occur almost exclusively in developing countries, where serious nutritional problems exist at present, and population pressure on agricultural soils is already very high. Environmental problems (e.g., water deficiency, extreme temperatures, salinity, flooding, soil acidity, and pathogenic infections) are increasing as a result of increasing world population and intensive use of natural recourses. These environmental stresses contribute significantly to reducing crop yields well below the potential maximum yields.

Maize (*Zea mays* L.) is one of the important crop in Pakistan, which serves three main purposes as food & corn oil for human consumption, feed for livestock and poultry, and raw material for agro-based industries. The average yield of maize in Pakistan is low as compared to other maize growing regions of the world. However, lower yield of maize in our country is not due to cultivars response but it seems to inadequate supply of water, imbalanced mineral nutrition and abiotic stresses. The soils of Pakistan are mostly calcareous in nature, alkaline in chemical reaction and low in organic matter (Sillanpaa, 1982 and Khattak, 1991).

Salt tolerant plants adopt many strategies that range from morpho-anatomical to physiological and biochemical in nature (Cheesemann, 1988; Zhu, 2001). Tolerant plants adjust osmotically by synthesis of highly water soluble compatible osmotica (e.g. glycinebetaine, free proline and low molecular weight sugars) and maintain turgor. Among these, free proline ameliorates salt induced oxidative damage to membranes (Ashraf and Harris, 2004 and Mansour *et al.*, 2005). Both reducing and non-reducing sugars contribute to turgor maintenance under salt or water stress (Cheeseman, 1988; Garg *et al.*, 2002).

Of the mineral nutrients, K plays a particular role for the translocation of nitrates to the root and shoot, increased rapid N-metabolism and maintenance of water potential in contributing to the survival of crop plants under environmental stress conditions. Potassium is essential for many physiological processes (Mengel and Kerkby, 2001).

Keeping in view the importance of maize crop, ameliorating and nutritional importance of potassium and a key role of K nutrition in many physiological processes in normal and particularly under saline environment, it was planned to cultivate the saline wasted land and to find the role of potassium nutrition on biochemical attributes (oil contents, total soluble sugars, protein, proline and crude fiber) under saline field environment.

MATERIALS AND METHODS

Growth conditions and experimental procedure

A field experiment was conducted in saline soil located near Faisalabad (latitude; 31^{o} $25^{/}$ North and longitude 73^{o} 90/East). The climate is arid to semi-arid with annual rainfall of about 250-500 mm and most of which occurs during monsoons season. The mean annual evaporation rate was 1625 mm and humidity ranged between 35 to 70%. Before laying out experiment, the soil samples were drawn to 0-30 cm soil depth from

field for basic physical and chemical analysis (Table-1). The field was ploughed and leveled with tractor drawn cultivator and leveler respectively.

Table 1. Pre-sowing soil analysis

Parameters	Unit	Soil depth	
		0-15 cm	15-30cm
EC_e	dS m^{-1}	9.95	10.60
pH	---	8.31	8.20
SAR	$(mmol\ L^{-1})^{1/2}$	18.65	17.10
K	mg kg^{-1}	70.0	85.50
Texture	----	Clay loam	

The field was properly laid out according to treatment plan following completely randomized block design with two treatments; i.e. Control (no added K and K @ 200 kg ha^{-1}) and three replications. The seed of salt tolerant (S-2002) and salt sensitive (Akbar) maize genotypes were surface-sterilised in 0.5% (v/v) sodium hypochlorite for 1 min before sowing. These seeds were sown with drill at RxR and PxP distance in their allotted plots having dimension 4x4m. The recommended dozes of NP fertilizers (200:150) were applied by using DAP, Urea as source respectively, while, SOP was used as source in K treatments. The canal water was used for irrigation and was applied according to crop water requirement.

Measurements

The plants were harvested at maturity manually and cobs were separated from shoot to air dry and grains were weighed to record 1000 grains weight. For determining proline (Bates *et al.*, 1973) in maize grain about 0.5 g of tissue was placed in a container with 5 ml water plus 0.2 ml toluene at room temperature and shaken for 60 min. The extract was decanted, and the extraction repeated two more times. For proline measurement, the reaction with ninhydrin was carried out and the absorbance was read at 520 nm using toluene as a blank. After extraction of solutes from apices, the final volume of the supernatant was 5 ml. The proline concentration was determined from a standard curve and calculated on a fresh weight basis. Protein contents were determined using the method of Lowery *et al.* (1951). 0.2 g seed material was taken and chopped in 5 ml phosphate buffer 0.2 M (pH 7.0). Two tubes containing 0.5 ml and 1.0 ml of seed extract were prepared for protein estimation. 0.5, 0.1, 0.2, 0.4, 0.6 and 1.0 ml of standard BSA were simultaneously used in the experiment. The volume of each tube was made to 1.0 ml with distilled water. The blank contained only 1.0 ml distilled water. The reagents in the test tube were thoroughly mixed and allowed to stand for 10 minutes at room temperature. Then 0.5 ml of Folin-phenol reagent (1:1 diluted) was added, mixed well and kept for 30 minutes at room temperature. The optical density (O.D) was read at 620 nm on spectrophotometer (Hitachi-220). Total soluble sugars were determined according to the method of Yemm and Willis (1954). Well-grinned seed material (0.1g) was extracted in 80% ethanol solution. Dried material was ground so as to pass through 1mm sieve of millimicro mill (Model Culatti, DFH-48) and it was shaken for 6h at 60^0C. This extract was used for the estimation of total soluble sugars. Plant extract (100 µ l) was taken in 25 ml test tubes and 6 ml anthrone reagent was added, and then heated in boiling water bath for 10 minutes. The test tubes were incubated for 20 minutes at room temperature (25^oC). Optical density was read at 625 nm on spectrophotometer (Hitachi 220). Blank was also run in the same way. The soluble sugars were calculated from standard curve developed by using glucose, following the above-mentioned method of Yemm and Willis (1954).

For oil contents, seed samples of each treatment were dried for 16 h at 50^oC and then ground into a powder. Procedure was adopted as Official method of AOAC. (1984).Weighed accurately 4 to 5 g of the ground sample into a filter paper and enclosed in a second filter paper folded in such a fashion as to prevent escape of meal. The second filter paper was left open at the top like thimble. A piece of absorbent cotton was placed in the top of thimble to distribute the solvent as it dropped on the sample. Wrapped sample was placed in the Butt extraction tube and assembled the apparatus. 25 ml of petroleum ether was added to the tarred extraction flask before attaching to the tube. Extraction tube was heated on the water bath at such a rate that the solvent would drop from the condenser on the center of the thimble at the rate of 150 drops per minute. Extraction was continued for 4 hours. Repeated the heating until constant weight was obtained.

$$\text{Moisture in ground sample, \%} = \frac{\text{Loss in weight}}{\text{Weight of sample}} \times 100$$

Calculation

$$\text{Oil in ground sample, \%} = \frac{\text{Weight of oil}}{\text{Weight of sample}} \times 100$$

Crude fiber (%) was determined by the method of American Oil Chemists Society-Association of Official Analytical Chemists Method (AOAC, 1995). 2g ground (grain) material was extracted with petroleum ether. Transferred the material to 600 ml beaker, avoiding fiber contamination from paper or brush. Then added 1.0 g freshly prepared asbestos, 200 ml 1.25% boiling H_2SO_4, and one drop of diluted antifoam. The beaker

was placed on the hot plate and boiled exactly for 30 min and filtered the contents. Dried the residues 2 hr at 130 ± 2°C. Cooled in desiccator and weighed. Ignited for 30 min at 600±15°C. Cooled in desiccator and reweighed. % Crude fiber in ground sample = (Loss in wt. on ignition – loss in wt. of asbestos blank) × 100 / wt. of sample.

The data were subjected to statistical analysis using computer software MSTAT-C (Steel and Torrie, 1983). The standard error (±) were used to compare means.

RESULTS AND DISCUSSIONS

The result showed that the two maize cultivars, were able to survive under natural saline conditions but the survival rate of tolerant cultivars (S-2002) was more prominent than that of the salt sensitive (Akbar). Salts present in the natural salt affected field caused significant reduction in the oil content of both cultivars but the reduction was more in salt sensitive than salt tolerant cultivar while by the application of potassium fertilizer the increase was more significant in tolerant cultivar than the sensitive one. As for as the protein contents concerned, tolerant cultivar performed better under salinity stress and natural potassium level of the soil while the potassium fertilizer improved the contents more as compared to the sensitive cultivar (Table 2). Production of total soluble sugars (%) under salt stress and potassium fertilizer application in tolerant cultivar was significant as compared to the sensitive cultivar.

Table 2. Protein contents (%) of maize genotypes under saline field with and with out potassium

Genotypes	Control (EC 10 dS m^{-1})	Salinity × K
S-2002	3.43±0.07	3.87±0.12 (112.8)
Akbar	2.13±0.05	2.31±0.07 (108.4)

Protein (%) and total soluble sugars accumulation in both cultivars is significantly different. Several reports indicate that decrease in protein accumulation is associated with salt stress. Change in protein composition is associated with salinity and increase in exposure to temperature (Panozzo and Eagles, 2000). The salt tolerant genotype had the highest amount of total soluble sugars at salinity and potash levels (Table 3). The salt sensitive genotype Akbar had lower amount at salinity and potash levels.

Table 3. Total soluble sugars (%) of maize genotypes under saline field with and with out potassium supply

Genotypes	Control (EC 10 dS m^{-1})	Salinity × K
S-2002	1.29±0.04	1.48±0.05 (114.4)
Akbar	1.03±0.04	1.11±0.01 (107.7)

Imposition of salinity in the growth medium reduced the total soluble sugars while under the potassium nutrition the contents were increased. S- 2002 had higher while Akbar had lower total soluble sugars at both salinity and K levels. Added K had a significant effect that reduced the leaf and root Na^+ levels that were much higher than control and the K-application played a role in the transfer of nitrate from roots to shoots and leaves. Without K-application, nitrate accumulates in the roots and feedback mechanism to the root cells stops further nitrate uptake. Therefore, the K-application, consequently increased the rapid N-metabolism and the nitrate in the plant reduced to amines and then incorporated into amino acids to ultimately from proteins. Both factors, nitrate and proteins not only increased the yield but also contributed to crop quality in terms of oil contents, total soluble sugars and crop yield. These results are in accordance with the findings of Cakmak (2002) and Shirazi *et al.*, (2005).

Saline growth medium caused the significant reduction in oil contents of salt sensitive genotype as compared to salt tolerant while the supplementation of potassium nutrition enhanced the oil contents (Table 4). The genotype S-2002 produced the higher amount of oil contents as compared to the genotype Akbar, under both the treatments. The oil contents and crude fiber also quantified along with other chemical and biochemical variables. The oil contents differed significantly among the genotypes under saline conditions and K-application increased this variable due to the well maintenance of water potential by the genotypes S-2002 as compared to the Akbar. The same trend was found in case of crude fiber (Table 5).

Table 4. Seed oil contents (%) of maize genotypes under saline field with and with out potassium

Genotypes	Control (EC 10 dS m^{-1})	Salinity × K
S-2002	3.11±0.03	3.51±0.10 (112.2)
Akbar	2.24±0.06	2.41±0.05 (107.8)

Table 5. Crude fiber (%) of maize genotypes under saline field with and with out potassium

Genotypes	Control (EC 10 dS m^{-1})	Salinity × K
S-2002	1.10±0.03	1.21±0.03 (110)
Akbar	0.85±0.02	0.91±0.01 (107)

These results are in accordance to the findings of Mangel (1980) and Sarwar *et al.,* (2003). These results are also in accordance with the findings of Francois (1994) who reported that oil contents and protein content in oil free seed meal of canola were not affected by salinity. Yermanos *et al.,* (1964) and Irving

(1988) predicted the same findings in case of safflower. Added K increased the oil contents that were higher than control values but significantly lower in saline treatment.

A large number of plant species accumulate glycinebetaine and proline in response to salinity stress and their accumulation may play a role in combating salinity stress (Ashraf, 1989; Ashraf, 1994; Hanson and Burnet, 1994; Mansour, 2000; Ashraf and Harris, 2004). These studies reported that NaCl increased proline accumulation more in S-2002 than Akbar (Table: 6), which is supposed to correlate with the adaptation to salinity. Our results implicate that NaCl stress increases proline accumulation in shoot of two maize cultivars, strongly in S-2002. We infer that proline accumulation in S-2002 might have a role in salt tolerance. High proline levels, total soluble sugars and protein contents are suggestive of their involvement in osmotic adjustment, since it has proven that high concentrations of proline or protein or soluble sugars are not required for their protective effects under salinity (Mansour *et al.*, 2005). Some researchers have reported that it is a sign of stress (Rai *et al.*, 2003), while; others suggest that at a high concentration it acts as a solute for intercellular osmotic adjustment (Silveira *et al.*, 2003). According to our results, proline concentration was higher and detected earlier at high salinity concentration in the salt tolerant genotype S-2002 compared to the salt sensitive Akabr. There was a gradual production of proline in S-2002 as compared to Akabr, while; the addition of potassium fertilization had non-significant effect on both the genotypes (Table 6). Salinity had a significant effect on the 1000-grain weight of both the genotypes and Akbar had the lowest weight among both the genotypes. Under both treatments the genotype S-2002 produced the higher, while, Akbar produced the lower 1000 seed weight (Table 7).

Table 6. Free proline contents (µ mol g^{-1}) of maize genotypes under saline field with and with out potassium supply

Genotypes	Control (EC 10 dS m^{-1})	Salinity × K
S-2002	4.1±2.45	4.1±1.94 (97.8)
Akbar	1.7±1.07	1.6±077 (88.8)

Table 7. 1000 seed weight (g) of maize genotypes under saline field

Genotypes	Control (EC 10 dS m^{-1})	Salinity × K
S-2002	273.9±5.18	334.9±5.61(122)
Akbar	152.7±4.92	191.1±7.63 (125)

Each value is an average of 3 replications ± S.E.
Values in ( ) are % of respective control

Based on all the biochemical parameters measured in this study, it can be concluded that maize genotypes differed in salt tolerance showed a differing response to salt stress and potassium supply with respect to these variables.

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