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EFFECT OF SALT STRESS ON GROWTH AND ANTIOXIDANT ENZYMES IN TWO CULTIVARS OF MAIZE (*ZEA MAYS* L.)

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Abstract

The effect of various concentrations of NaCl (50, 75, 100, 125, 150 mM) was determined on the growth and biochemistry of two maize (*Zea mays* L.) cultivars (Pioneer X8F932 and DK -C61-42). Seed germination under salt stress conditions was more affected in cv. Pioneer X8F932 than cv. DK-C61-42. A significant reduction ($p<0.05$) in root and shoot growth was observed at 100, 125 and 150 mM salt concentrations in both the cultivars. Salt stress also caused a decrease in fresh weight of seedlings in a dose dependant manner ($p>0.05$). Among the two cultivars DK-C61-42 showed better tolerance towards salt stress (tolerance index = 105.4 at 75 mM) compared to Pioneer X8F932 (tolerance index = 76 at 50 mM). Total soluble protein content increased in both the cultivars under salt stress in a dose dependant manner with maximum protein content at 150 mM (6.004 mg/g tissue in DK-C61-42 and 7.375 mg/g tissue in cv. Pioneer X8F932). In DK-C61-42 highest peroxidase activity was at 125 mM (0.017 mg/g tissue) while in Pioneer X8F932 highest peroxidase activity was at 50 mM (0.006 mg/g tissue). The difference in enzyme activity between control and salt treated seedlings was significant ($p<0.05$). The catalase activity decreased under salt stress conditions in case of DK-C61-42 while an increase in activity of the enzyme was observed in Pioneer X8F932 at high salt concentrations. Among the two cultivars DK-C61-42 was better adapted towards salinity stress.

Key words: *Zea mays*, Salt stress, Peroxidase, Catalase, Phytotoxicity.

Introduction

Soil salinity is an important environmental factor that may inhibit crop growth causing a decrease in productivity (Hamdia & Shaddad, 2010). In areas where irrigation is necessary for assistance to agriculture, salinity is always a threat to crop productivity since most of the water on earth is salty. The water normally used for irrigation naturally contains 30 g of NaCl/L that is continuously impacting the land used for crop growth (Flower, 2004). In arid and semi-arid areas of the world where land degradation, water shortage and population growth are already a major concern, the most widespread environmental menace to crop production is the soil salinity (Munns & Tester, 2008; Geissler *et al*., 2010). Over 6% of the land area all around the world (More than 800 million ha of land) is salt-affected (Rengasamy, 2006). To meet the exponential population growth, more and more land is being utilized and irrigated for agricultural production which is causing an increase in salt-affected areas (Lambers, 2003; Munns, 2005). Moreover, about 1.6 million ha/year of irrigated lands are becoming saline due to unmanaged irrigation practices (secondary salinization) (Tanji, 2002). The global annual cost for management of salinity is probably well over US$12 billion (Qadir *et al*., 2008).

In Pakistan, salinity is an important environmental issue, mainly caused by soil erosion and long-term mismanagement in irrigation system caused by man. Almost 25% of the irrigated land in the country is affected by salinity at various levels, about 1.4 million hectares of the total agricultural land has now become barren (Anon., 2006). The crop losses in Pakistan due to salinity amount to a total annual cost of 15 - 55 billion rupees (Rs) ($340 million to $1.2 billion) per year. This does not include the Rs 15 billion ($340 million) estimated to have been lost due to unproductive lands. With an average cost of Rs 35 billion ($790 million) per year from reduction in yields, the costs due to salinity in Pakistan has reached to 0.6% of gross production in 2004 (Anon., 2006). Under the prevailing conditions of salinity the future of agricultural production depends on our ability to grow plants on salt-affected lands (Rozema & Flowers, 2008). Most of the crop species can tolerate salinity to a certain level above which a decrease in crop yield is observed. The salinity tolerance level of a given plant species depends upon its genetic variability. Therefore an important strategy to overcome salinity is to identify crop varieties that may successfully grow on saline soils without any significant decrease in yield (Ashraf *et al*., 2006). Plants have developed different biochemical and physiological mechanisms to survive under salt stress conditions. One of these mechanisms involves activation of antioxidant enzymes and synthesis of antioxidant compounds (Rahnama *et al*., 2010). Peroxidases and catalases are the two most important antioxidant enzymes which are involved in tolerance against salt stress (Gondim *et al*., 2012; Kachout *et al*., 2013).

Maize (*Zea mays* L.) is an important crop of Pakistan ranking third for its grain production after wheat and rice. It is cultivated on an area of 1.02 million hectares in all the four provinces, but NWFP and Punjab are the main areas of production. The annual grain production of the crop is 2.96 million tons while the average grain yield is 2893 kg ha^{-1} (GOP, 2007). It has become highly polymorphic and possesses high genetic variability. Being cross pollinated, the plant is believed to be tolerant to salt stress (Paterniani, 1990). A number of maize varieties are being cultivated in Punjab and the main aim of the present study was to determine the effect of various concentrations of salt on physical growth and activity of two antioxidant enzymes peroxidase and catalase of two selected maize cultivars (Pioneer X8F932 and DK-C61-42) commonly grown in fields of Punjab.

Materials and Methods

Plant material: The authenticated seeds of both the cultivars were collected from Punjab Seed Corporation. The seeds were stored at room temperature in air tight containers. All the seeds were surface sterilized to avoid fungal contamination by soaking for 30 min in 1% solution of mercuric chloride and were thoroughly washed several times with sterilized distilled water.

Imposition of salt stress and growth conditions: The stock solutions of NaCl at 50, 75, 100, 125, 150 mM concentrations were prepared using water. Seeds of approximately equal size were selected and were allowed to germinate in soil supplemented with 20 ml of respective salt concentration. Distilled water served as control. The soil was kept moist by the addition of 20 ml of respective salt solution at regular interval and plates were kept at 25 ± 2°C. The experiment was performed in triplicates.

Growth parameters

Seed germination: The seeds with radicles more than 2 mm were considered as germinated. The rate of germination was determined by counting the total number of germinating seeds for three days at 24 h intervals.

Seedling growth: The seedling growth was monitored for 15 days at three days interval. The root and shoot lengths were measured by using a centimeter scale. The biomass of the seedlings was determined by fresh weight.

Percentage phytotoxicity: The phytotoxic effect of the salt on the growth of root and shoot of maize seedlings was determined after fifteen days of growth applying the formula given by Chou & Lin (1976).

$$\text{Phytotoxicity of root/shoot (\%)} = \frac{\text{Root/shoot length in control} - \text{Root/shoot length in treatment}}{\text{Root/shoot length in control}} \times 100$$

Seedling vigor index: Vigor index (VI) was determined by the following formula of Iqbal & Rahmati (1992):

$$VI = (\text{mean shoot length} + \text{mean root length}) \times \% \text{ germination}$$

Tolerance index: To determine the tolerance of seedlings against tested concentrations of NaCl Wilkinson tolerance index (WTI) was calculated (Koornneef *et al*., 1997):

$$I_t = (I_{me}/I_c) \times 100$$

where:
I_{me} = increase in root length in NaCl solution and
I_c = increase in root length in the control after 15 days

Biochemical parameters: To assess the biochemical changes in the seedlings of the two cultivars under different salt concentrations the total content of soluble proteins and two antioxidant enzymes, peroxidase and catalase was determined at weekly intervals for two weeks.

Extraction of proteins: For protein extraction five seedlings of both the cultivars randomly selected for each concentration were crushed using 0.1 M phosphate buffer (pH 7.2) and 0.1 g of PVP keeping the ratio of plant material and buffer 1:5. The slurry was centrifuged at 12,000 rpm for 20 min at 4°C. The supernatant was filtered through four layers of muslin cloth and used to estimate the protein contents and activity of peroxidase and catalase.

Determination of total soluble protein content: The total content of soluble proteins was determined by using the Biuret method of Racusen & Johnstone (1961). The assay mixture contained protein extract (0.1 mL) and Biuret reagent (1.0 mL). In the control set up distilled water (0.1 mL) was used instead of protein extract. The absorbance was measured at 545 nm using spectrophotometer. Total protein content was calculated from the standard protein curve prepared using bovine serum albumin and was expressed as mg g^{-1} of tissue (Fig. 18).

Estimation of peroxidase activity: Peroxidase activity was determined using the method of Racusen & Foote (1965). The reaction mixture contained crude enzyme extract (0.1 mL), 1% guaiacol solution (0.2 mL), 0.1 M phosphate buffer (pH 7.2) (2.5 mL), and 0.3% H_2O_2 solution (0.2 mL). The control mixture contained distilled water instead of guaiacol. The absorbance was measured at 470 nm and the enzyme activity was expressed as mg g^{-1} of tissue.

Estimation of catalase activity: Catalase activity was determined by using the method of Aebi (1974). The reaction mixture contained 10 mM H_2O_2, enzyme extract and 25 mM potassium phosphate buffer (pH 7.0). Catalase activity was calculated by measuring the absorbance at 240 nm.

Statistical analysis: The experiment was carried out in triplicates. The data was presented as mean ± standard deviation (SD). For statistical analysis Microsoft Excel 2007 was used and differences between means were determined by using Student's t-test at $P = 0.05$.

Results and Discussion

Effect of NaCl on % seed germination: It has been observed in a number of studies that tolerance at the seedling stage in crop plants reflects the high salt tolerance at the mature plant level. This observation has been used in different researches as a means for selecting salt tolerant varieties in many crop species including maize (Ali *et al*., 2014; Bafeel, 2014). In our study the results showed that salt concentration did not affect seed

germination significantly in cv. DK-C61-42 at the tested concentrations ($p>0.05$) except at 75 mM ($p<0.05$) with 70% seed germination compared to control with 85% germination (Fig. 1). In cv. Pioneer X8F932 a significant decrease in % seed germination was observed at all the tested concentrations of salt ($p<0.05$) except 75 mM ($p>0.05$). Water plays the most important role in the germination of seeds, but high salt concentration prevents the seeds from absorbing adequate water required for normal growth. This decrease in water absorbance may be due to a decrease in the rate of water uptake due to osmoeffects, due to toxic effects of specific ions, or due to a nutritional imbalance as a result of inter-element antagonism (Hamdia & Shaddad, 1996; Najafi *et al*., 2010 and Hamdia *et al.*, 2012).

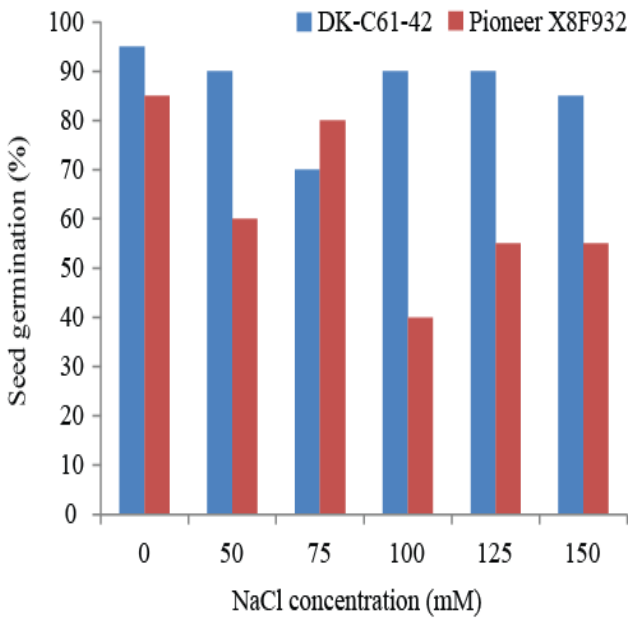

Fig. 1. Effect of NaCl concentration on seed germination in two *Z. mays* cultivars.

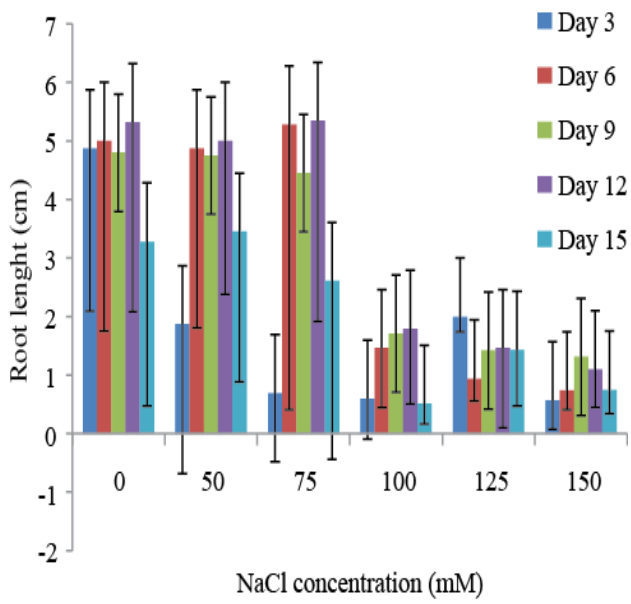

Fig.2. Effect of NaCl concentration on root length of *Z. mays* (cv.DK-C61-42).

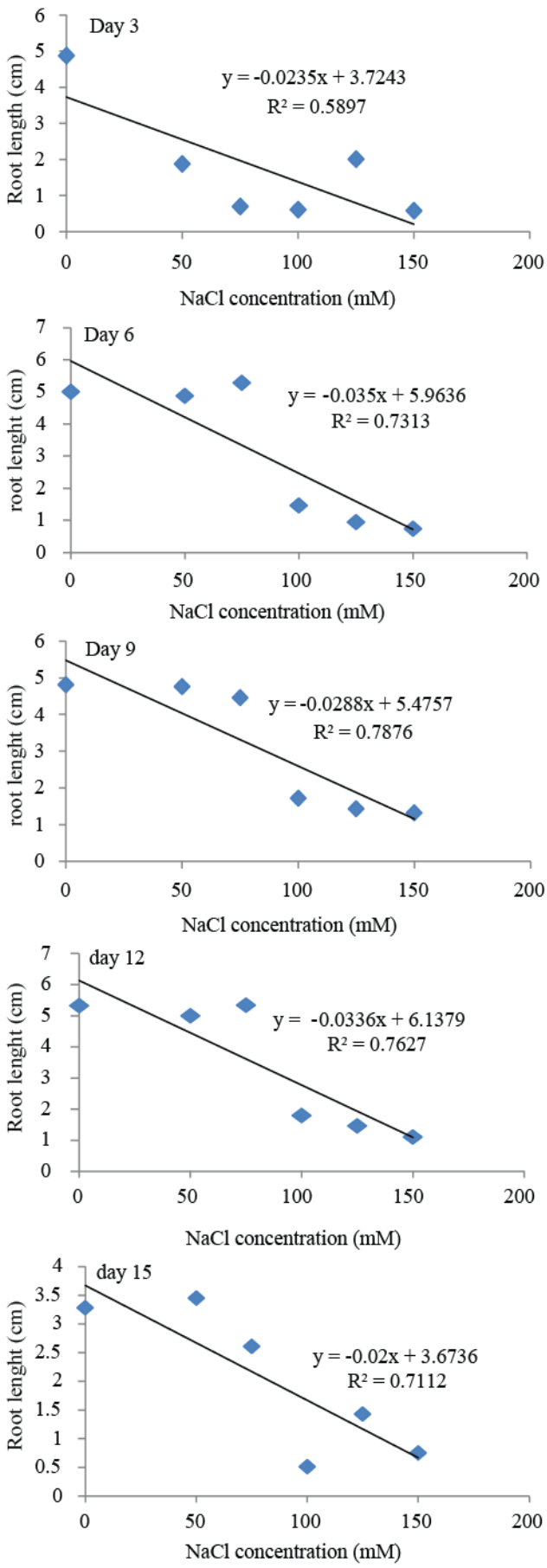

Fig. 3. Correlation between NaCl concentration and root length in *Z. mays* (cv. DK-C61-42).

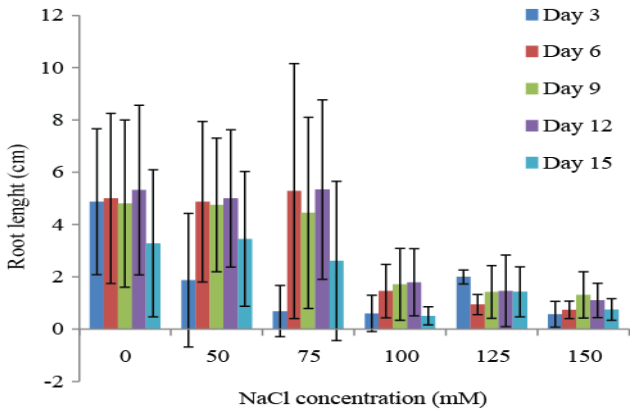

Fig. 4. Effect of NaCl concentration on root length of *Z. mays* (cv. Pioneer X8F932).

Effect of salt stress on root growth: The response of roots towards salinity stress indicates the salt tolerant potential of the plants. At high salt concentrations, O_2 content decreases affecting the rate of respiration thus depriving the plant of its primary energy source. Under these conditions high levels of ethylene get accumulated inside the plant that inhibit root elongation (Koning & Jakson, 1979) by reducing root growth. Studies have shown that root growth is sensitive to high salt concentrations in the medium and there is a reduction in root growth under high saline conditions (Mohammad *et al.*, 1998; Cramer *et al.*, 1988; Ashraf *et al.*, 2005). In the present study the root length was significantly decreased with increase in NaCl concentration in both the cultivars (Figs. 2, 4, 16 and 17). Significant reduction in root growth was observed at 100, 125 and 150 mM concentration ($p<0.05$) in both the cultivars throughout the study period. The decrease in root length at low concentrations (50 and 75 mM) was not significant ($p>0.05$). In case of Pioneer X8F932 seedling growth ceased on day 12 at 100 mM NaCl and on day 15 at 75 and 150 mM NaCl. Pioneer X8F932 was more sensitive towards salt stress than DK-C61-42 regarding root growth. A significantly negative correlation between NaCl concentration and root length was observed in both the cultivars throughout the study period (R^2 = 0.7112 and 0.6769 on day 15 for cv. DK-C61-42 and Pioneer X8F932 respectively) (Figs. 3 and 5).

Effect of salt stress on shoot growth: In both the cultivars high salt concentration (75, 100, 125 and 150 mM) significantly inhibited shoot growth indicated by reduction in shoot length. In DK-C61-42 shooting was delayed at 75 and 150 mM salt concentration (Fig. 6). At the end of study period normal seedling growth was observed only at 50 mM salt concentration while a significant reduction in shoot length was observed at all the other tested concentrations ($p<0.05$) (Figs. 16 and 17). In cv. Pioneer X8F932 seedling growth, in terms of increase in shoot length, seized after 12 days (Fig. 8). Increase in shoot length was comparable to control at 50 and 75 mM salt concentrations ($p>0.05$) while at all the other concentrations a significant reduction in shoot length was observed ($p<0.05$). Statistically significant negative correlation between NaCl concentration and shoot length was observed in both the cultivars throughout the study period (R^2 = 0.7921 and 0.8359 on day 15 and day 12 for cv. DK-C61-42 and Pioneer X8F932 respectively) (Figs. 7 and 9). A comparison of seedlings of the two cultivars growing under

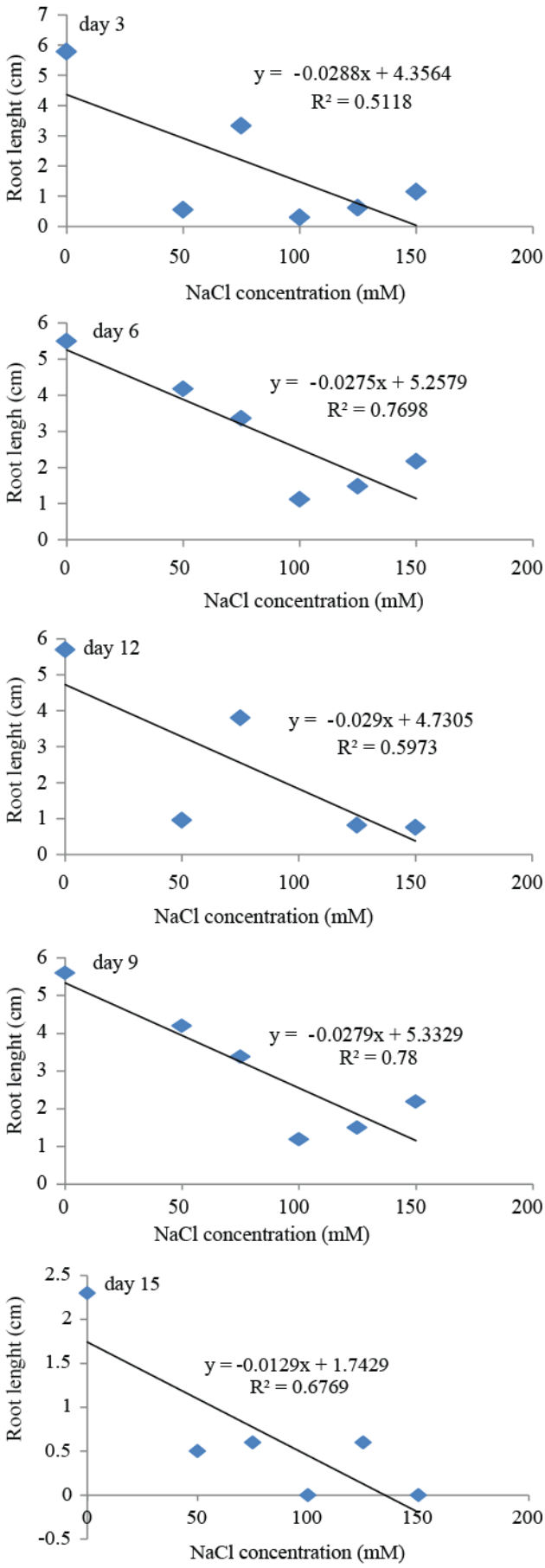

Fig. 5. Correlation between NaCl concentration and root length in *Z. mays* (cv. Pioneer X8F932).

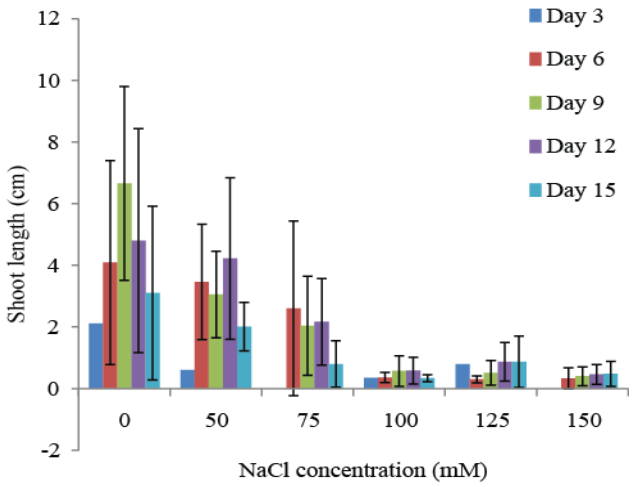

Fig. 6. Effect of NaCl concentration on shoot length of *Z. mays* (cv. DK-C61-42).

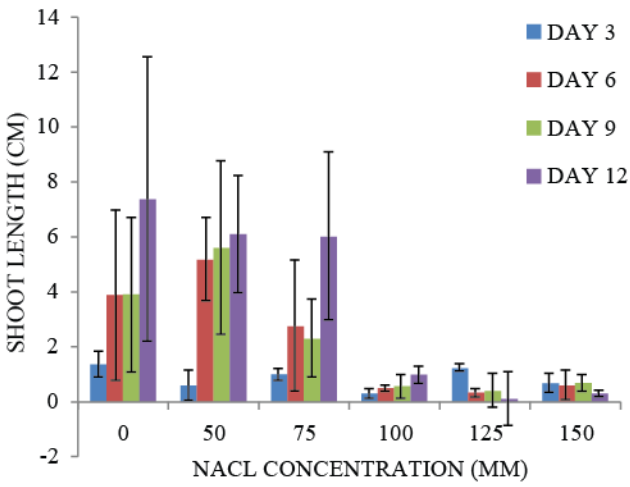

Fig. 8. Effect of NaCl concentration on shoot length of *Z. mays* (cv. Pioneer X8F932).

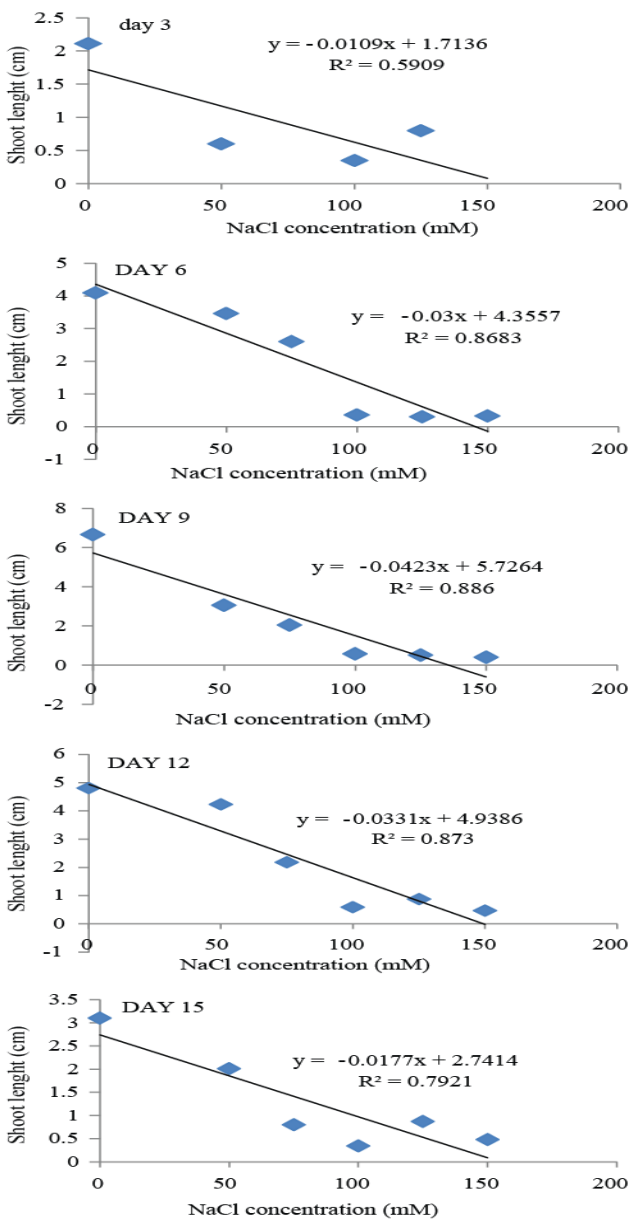

Fig. 7. Correlation between NaCl concentration and shoot length in *Z. mays* (cv. DK-C61-42).

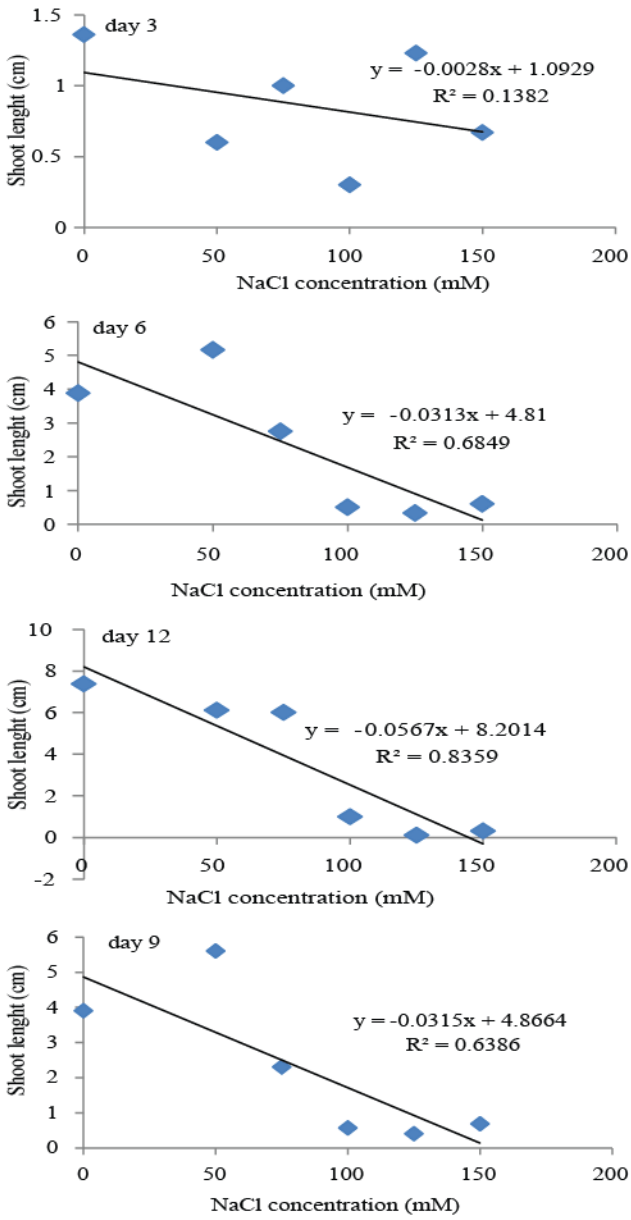

Fig. 9. Correlation between NaCl concentration and shoot length in *Z. mays* (cv. Pioneer X8F932).

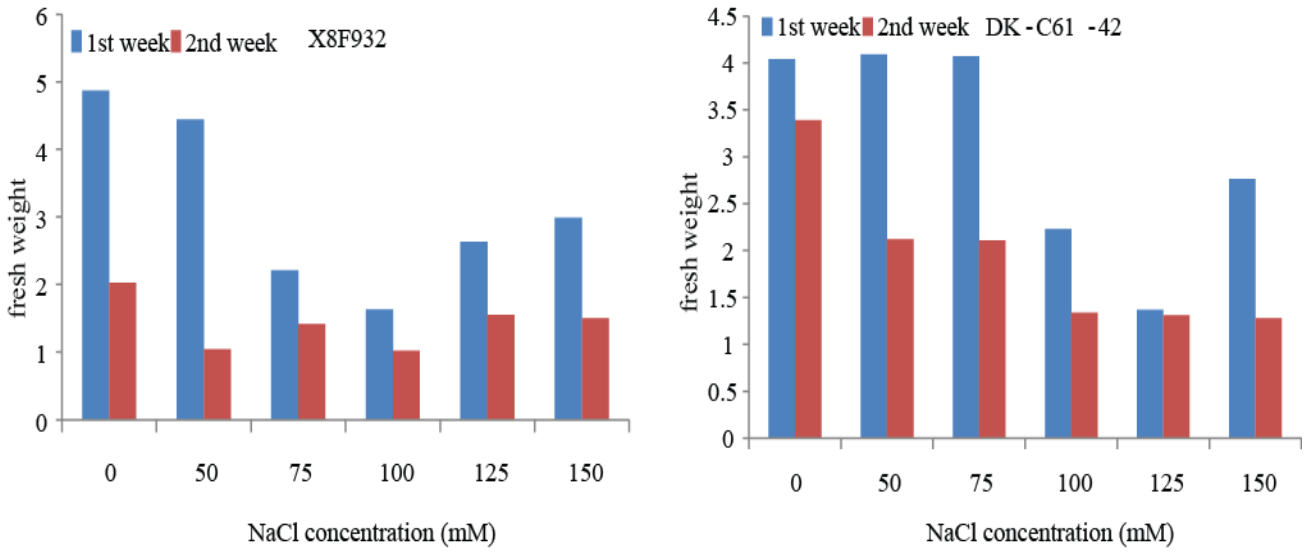

Fig. 10. Effect of NaCl concentration on fresh weight of two *Z. mays* cultivars.

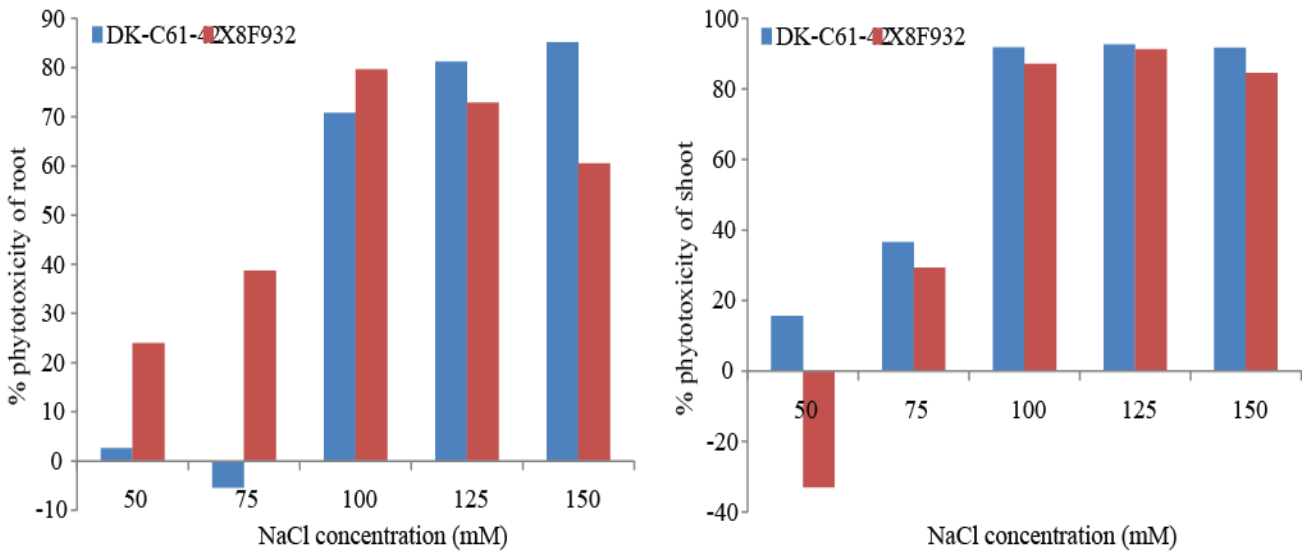

Fig. 11. Effect of NaCl concentrations on phytotoxicity of root and shoot (%) in *Z. mays* L. cultivars.

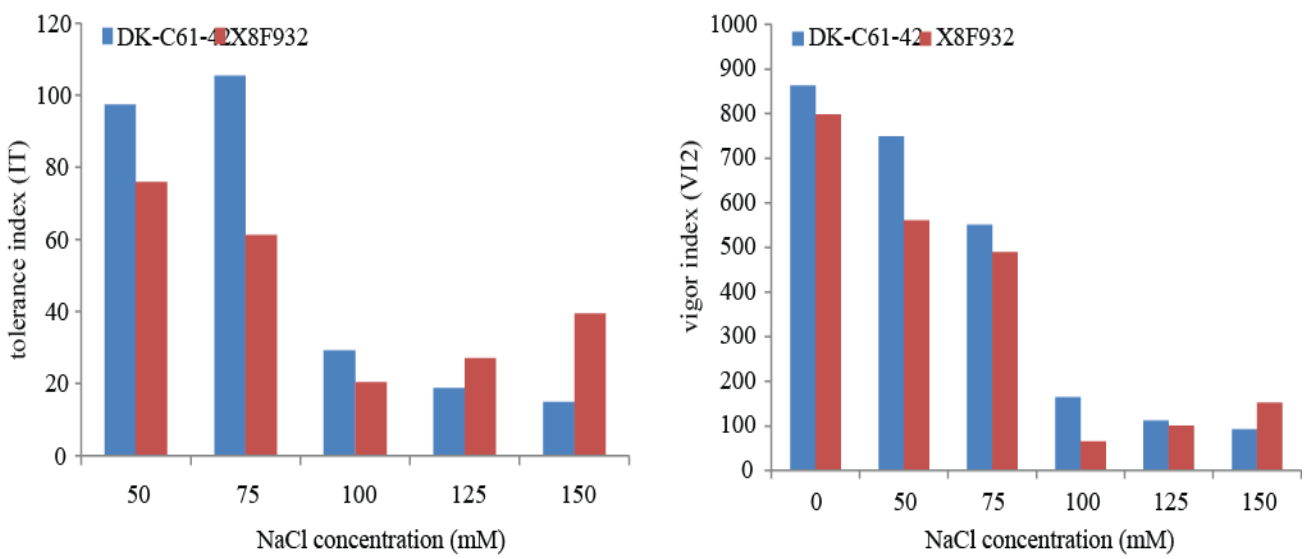

Fig. 12. Seedling vigor indices and tolerance indices of two maize cultivars at different salt concentrations.

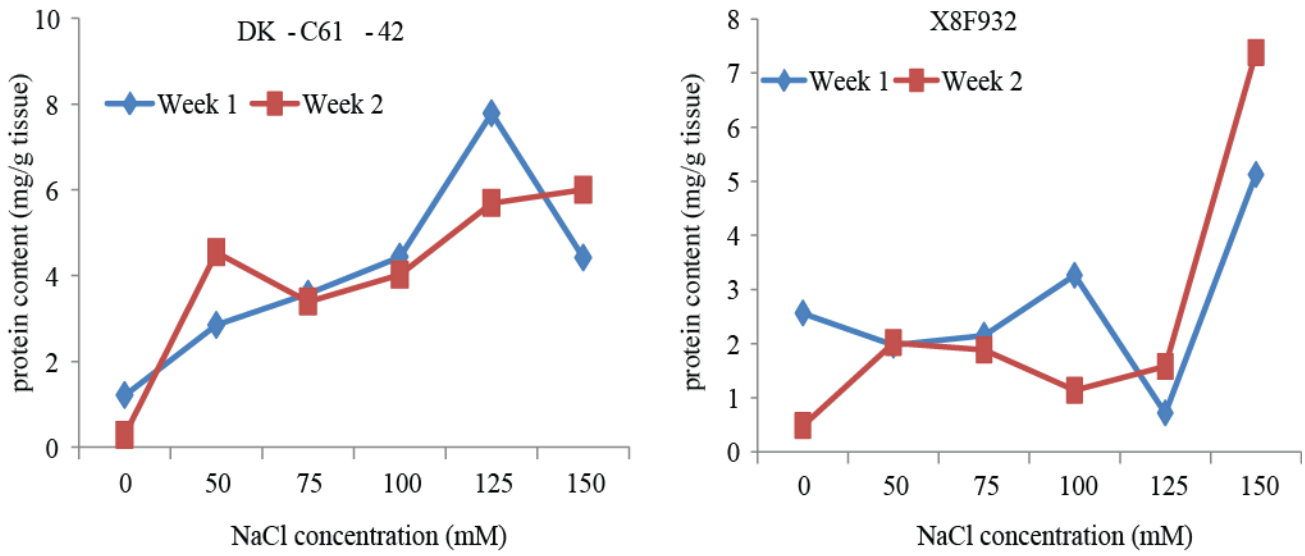

Fig. 13. Effect of NaCl concentration on protein content of two *Z. mays* cultivars.

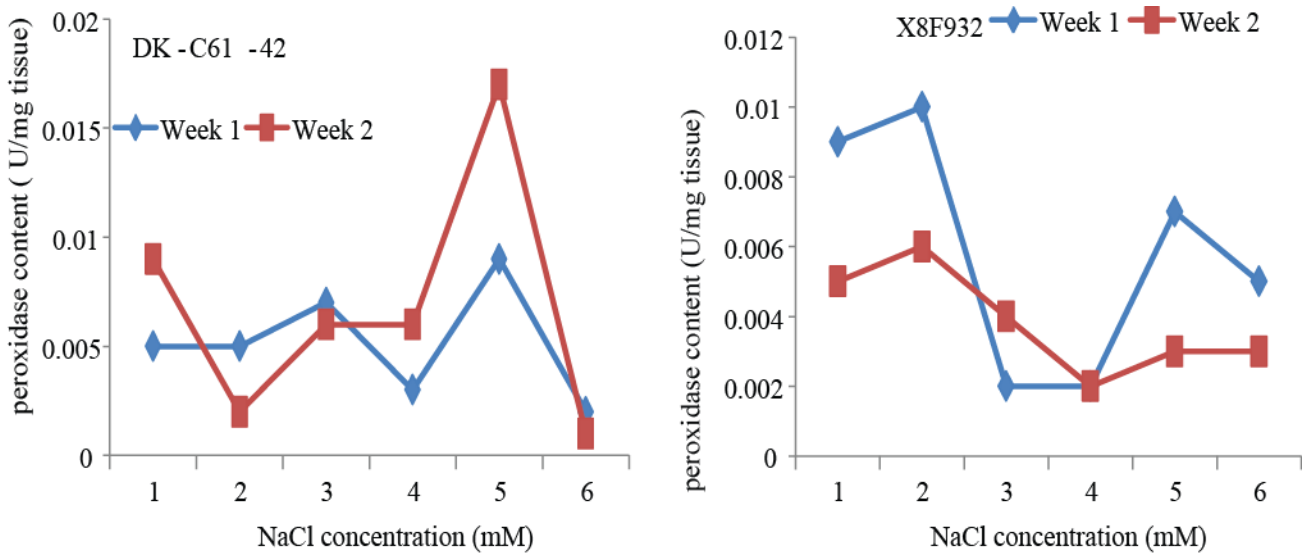

Fig. 14. Effect of NaCl concentration on peroxidase content of two *Z. mays* cultivars.

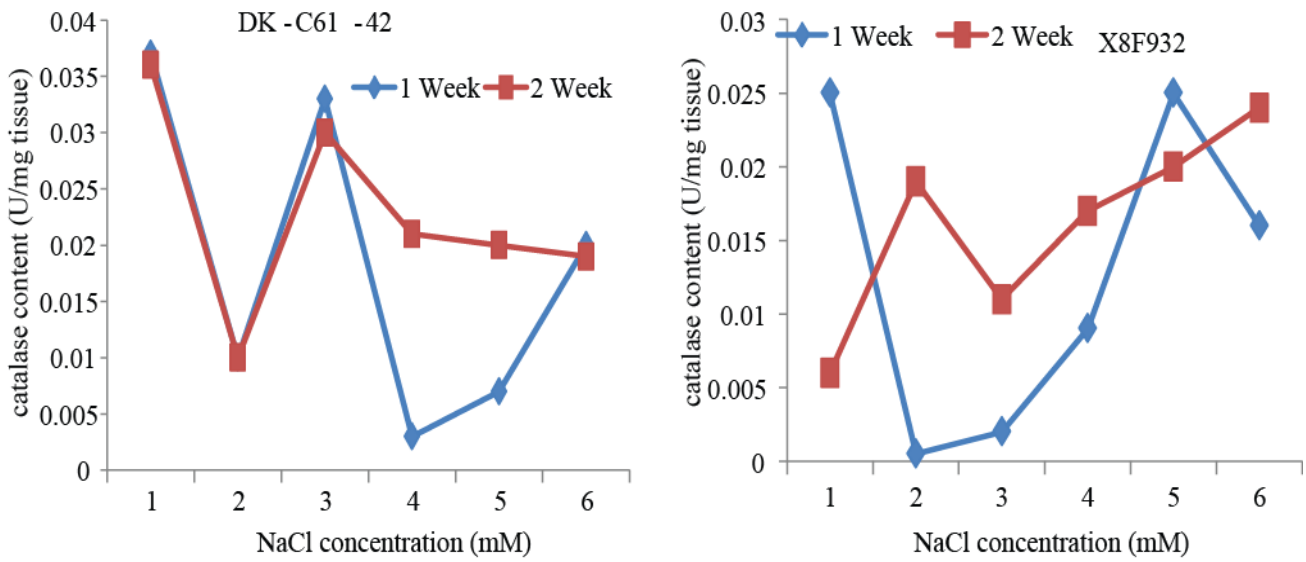

Fig. 15. Effect of NaCl concentration on catalase content of two *Z. mays* cultivars.

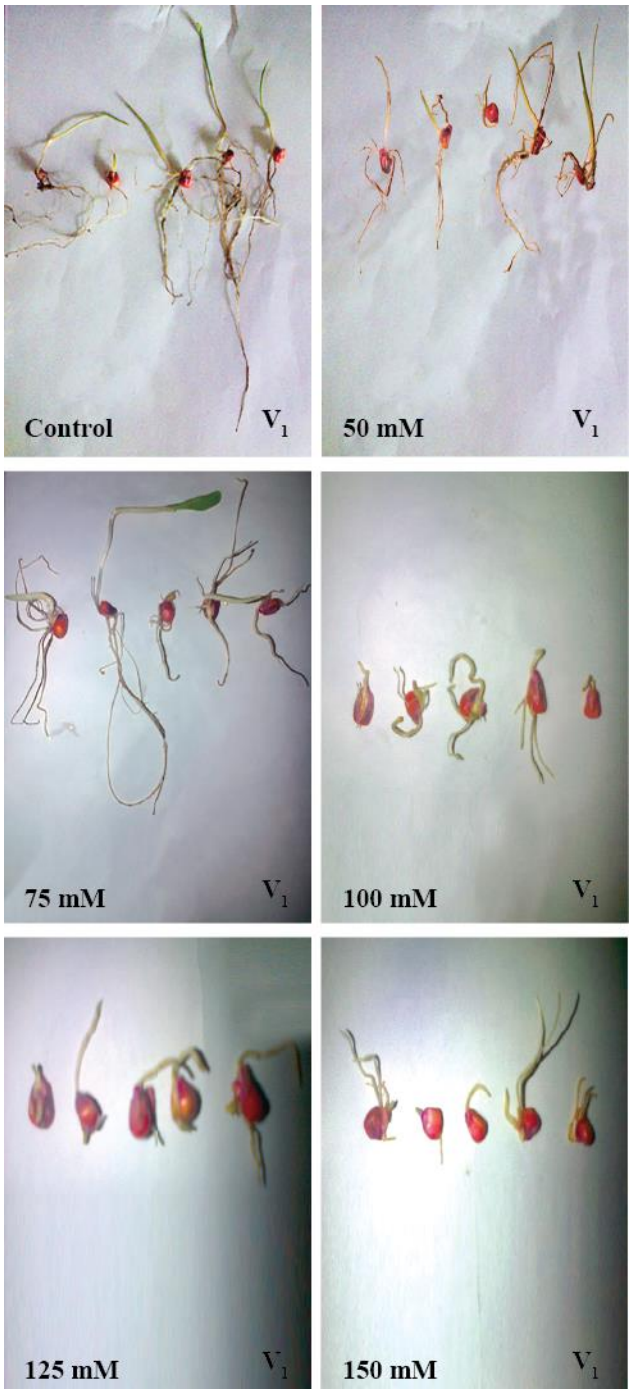

Fig. 16. Root and shoot length of maize seedlings (cv. DK-C61-42) growing under different salt concentrations after two weeks.

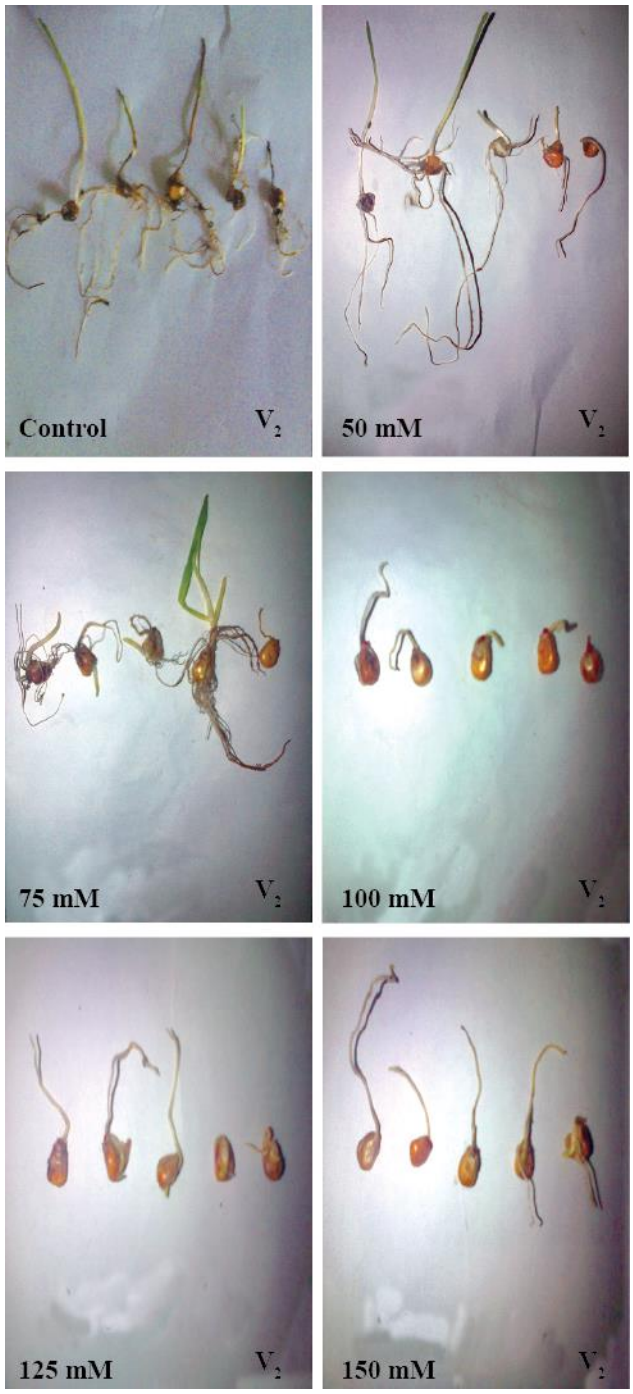

Fig. 17. Root and shoot length of maize seedlings (cv. Pioneer X8F932) growing under different salt concentrations after two weeks.

Effect on fresh weight: High salt concentration in nutrient medium causes reduction in plant growth (Cherian *et al.*, 1999; Takemura *et al.*, 2000). This reduction in plant growth is indicated by a reduction in fresh weights of seeds, stem and roots (Ali Denar *et al.*, 1999; Chartzoulakis & Klapaki, 2000). In the present study both the cultivars responded differently towards NaCl stress. A significant decrease in fresh weight of seedlings with increase in NaCl concentration was observed in DK-C61-42 at 125 mM (1.31 g) and 150 mM (1.28 g) concentration compared to control (3.39 g). In case of Pioneer X8F932 the average fresh weight of seedlings slightly decreased with increase in NaCl concentration and was highest in control (2.03 g) and minimum at 100 mM (1.02 g). A significant difference was observed between fresh seedling weight in case of control and tested concentrations ($p > 0.05$) (Fig. 10).

Phytotoxicity of root and shoot: Salinity stress showed a strong phytotoxic effect on root and shoot growth in both the cultivars at high salt concentrations (100, 125 and 150 mM). In cv. Pioneer X8F932 root growth was more affected at low salt concentration than cv. DK-C61-42 (Fig. 11).

Seedling vigor index: A significant reduction in seedling vigor index was observed in both the cultivars in a dose dependant manner. Seedling vigor index in cv. DK-C61-42 was higher in control (863.55) than in salt treated seedlings. In experimental set up vigor index significantly decreased from 749.7 at 50 mM to 92.22 at 150 mM. In Pioneer X8F932 highest vigor index was for control (798.15) and decreased under salt treatment in a dose dependant manner from 561 at 50 mM to 64.8 at 100 mM (Fig. 12).

Effect on tolerance index: Both the maize varieties showed a significant decrease in tolerance index with increase in salt concentration. High tolerance index was observed for both the cultivars at 50 and 75 mM which significantly reduced at 100, 125 and 150 mM. At low salt concentration high tolerance index was observed for cv. DK-C61-42 while at high concentration cv. Pioneer X8F932 showed high tolerance (Fig. 12).

Effect of NaCl on total soluble protein content: Effects on photosynthesis, protein synthesis and energy and lipid metabolisms in plant during the onset and development of salt stress have been observed (Parida & Das, 2005). In the present study total soluble protein content increased in both the cultivars under salt stress in a dose dependant manner (Fig. 13). In DK-C61-42 maximum protein content was at 125 mM (7.784 mg/g tissue) during first week and at 150 mM during second week of growth (6.004 mg/g tissue). In cv. Pioneer X8F932 highest protein content during first and second week was determined for seedlings growing at 150 mM of salt (5.124 and 7.375 mg/g tissue respectively). Among the two cultivars the protein content during the study period was higher in DK-C61-42 than Pioneer X8F932.

Effect of NaCl on peroxidase activity: Under salinity stress the concentration of toxic compounds is increased including reactive oxygen species (ROS) and excess ions that induce metabolic changes. These ROS are either removed from the cell by the action of antioxidant enzymes including catalase and peroxidases (POX) by which plant cells respond to the environment by changing gene expression, metabolism and physiology (Suzuki & Mittler, 2006). In the present study a significant decrease in peroxidase activity was observed in DK-C61-42 at 50 mM (0.002) and 150 mM concentration (0.001) as compared to control (0.009) after two weeks. Highest peroxidase activity was at 125 mM (0.017 mg/g tissue) ($p<0.05$). In case of Pioneer X8F932 highest peroxidase activity was observed at 50 mM (0.006 mg/g tissue) while in control group the enzyme activity was 0.009 mg/g tissue (Fig. 14). The difference in enzyme activity between control and experimental was significant ($p<0.05$).

Effect of NaCl on catalase activity: During first week of growth catalase activity in cv. DK-C61-42 was maximum in control (0.037 mg/g tissue) and minimum at 100 mM (0.003 mg/g tissue) concentration. In second week maximum enzyme activity was in control (0.036) and minimum at 50 mM (0.01). The activity of catalase at 75 mM NaCl was comparable to control in both first and second week (0.033 and 0.3 mg/g tissue respectively). In cv. Pioneer X8F932 catalase activity in first week under salt stress conditions comparable to control (0.025 mg/g tissue) was at 125 mM (0.025 mg/g tissue) and minimum at 50 mM (0.0005). In second week enzyme activity was maximum at 150 mM (0.024 mg/g tissue) and minimum in control (0.006 mg/g tissue) (Fig. 15). Increase in antioxidant enzymes in plants growing under salt stress has been reported in number of studies (Abd-Allah *et al*., 2015; Hend *et al*., 2015) supporting the results of present study.

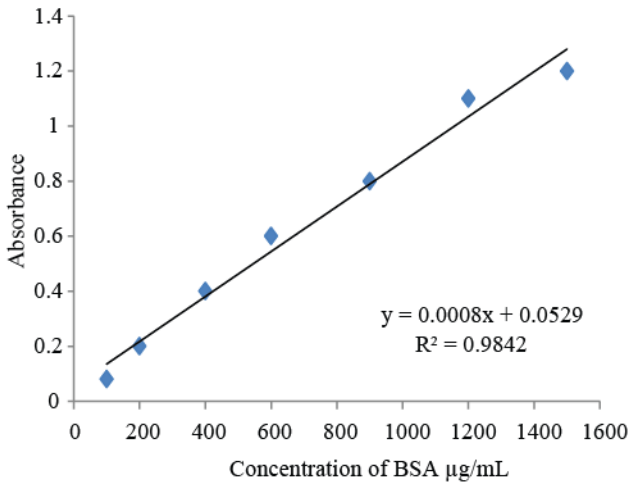

Fig. 18. Standard protein curve.

Conclusion

In the present study, both the maize cultivars responded differently to different concentrations of NaCl. Maize variety, DK-C61-42 showed better growth under salt stress as indicated by high root and shoot growth and proved to be tolerant to 50 and 75 mM concentration of salinity. Further research regarding field studies is suggested to determine the effect of salt stress on this cultivar under natural environmental conditions.

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