EFFECTS OF SALT STRESS ON PIGMENTS, SOLUBLE PROTEIN AND ELECTROLYTIC LEAKAGE CONTENTS OF TWO DIFFERENT MAIZE (*ZEA MAYS* L.) CULTIVARS.

R. DESINGH[1] & G.KANAGARAJ[*2]
[1]Assistant Professor, Department of Botany, Annamalai University, AnnamalaiNagar, Tamil Nadu - 608 002, India.
[*2]Assistant Professor, Department of Botany, Government Arts College for Men, Krishnagiri-635001, Tamil Nadu, India.

Received: February 24, 2019 Accepted: April 01, 2019

ABSTRACT: : *The effect of salinity on photosynthetic pigments, lipid peroxidation and electrolytic leakage in changes were investigated under salinity of different concentrations (0, 50, 100. 150mM). The two maize varieties BC-3033 and BC-3591 were sampling was done in young and fully matured leaves were taken from control and salinity treated plants on 15th Days After Treatment (DAT) and 30th DAT. Treatments were planted in pots. Total chlorophyll, chlorophyll-a, chlorophyll-b were significantly reduced in both varieties in leaves under salinity stressed condition. The reduction in the content of soluble protein was observed. The observations were recorded for the lower rates of membrane lipid peroxidation (MDA) and electrolytic leakage in the leaf of BC-3033 varieties with higher concentration of salt stress. Our data revealed that BC-3033 maintained higher content of photosynthetic pigments as well as accumulated of soluble protein and lower reduction of membrane lipid peroxidation (MDA) and electrolytic leakage content when compared to variety BC-3591 during the adverse effect of salinity stress.*

Key Words: *Salinity Stress, Photosynthetic pigments, Maize, Lipids, Electrolytic leakage*

INTRODUCTION

Salinity is one of the major abiotic stresses in arid and semi-arid regions but salt-affected soils have been recorded in practically all the climatic regions where more than 800 million hectares of agricultural and or over 6% of the world surfaces are salt affected. Sodium chloride is the most soluble, pervasive, and superabundant salt in the world[1]. Effect of salinity stress on carbohydrate, lipid peroxidation and proline contents of two horse gram varieties[2]. The reduction of chlorophyll a and chlorophyll b with NaCl application was reported in many plants such as *Zea mays* this due to increasing the destructive enzymes chlorophyllase[3]. Reduction in the pigments system is attributed to the induced weakening of protein-pigment-lipid complex by salt or to the increase in the chlorophyllase activity [4]. The most common effects in plants are toxicity, diminished CO2 assimilation and enhanced generation of reactive oxygen species[5] . Changes in fundamental processes have also been observed, such as growth, photosynthesis, protein synthesis and lipid metabolism[7]. The metabolic imbalances due to ionic toxicity, osmotic stress and nutritional deficiency may lead to oxidative stress[6] . Salt stress affects all major processes including photosynthesis, protein synthesis, lipid and energy metabolism[7]

Corn, commonly known as maize (*Zea mays* L.), is annual crop that belongs to the family of grass i.e. Poaceae. Maize is also recognized by different synonyms such as zea, corn, silk corn etc.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The certified maize (*Zea mays* L.) seeds (Variety: BC–3033, BC–3591) were procured from Tamilnadu Agriculture University Coimbatore and Paiyur. Seeds with inform size were selected and the plants were raised in pots containing red and clay soil and pH of the soil was 7.2 with EC of 0.2 dsm^{-1}. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. Plants were grown under natural climatic conditions. Plants were watered for the first 20 days after germination.

SALINITY TREATMENTS

The seedlings were divided into four groups. One group of seedlings was maintained under non-salinized conditions which served as control plants. The watering solution for control plants consists of tap water. Other three groups were salinized by irrigation daily to soil capacity (500 ml d^{-1}) with the nutrient medium containing 50 mM, 100 mM and 150 mM NaCl. 50mM consider as a low salinity level, 100mM consider as a medium salinity level and 150mM salinity consider as a high salinity level. All the plants used in this study were of comparable size.

SAMPLING DAYS

Young and fully matured leaves were taken from control and salinity treated plants on 15th Days After Treatment (DAT) and 30th (DAT) for all the experiments described below.

PHOTOSYNTHETIC PIGMENTS

TOTAL CHLOROPHYLL

The total chlorophyll content of the leaves was estimated according to Arnon, (1949).

$$\text{Total chlorophyll} = \frac{(20.2 \times A645) + (8.02 \times A663)}{1000 \times w \times a} \times V \text{ (mg/g fw)}$$

CHLOROPHYLL-A

Chlorophyll-a content was estimated according to [9].

$$\text{Chlorophyll-a} = \frac{(12.7 \times A663) - (2.69 \times A645)}{1000 \times w \times a} \times V \text{ (mg/gfw)}$$

CHLOROPHYLL-B

Chlorophyll-b content of the leaf was also estimated according to[9] using the formula:

$$\text{Chlorophyll-b} = \frac{(22.9 \times A645) - (4.88 \times A663)}{1000 \times w \times a} \times V \text{ (mg/gfw)}$$

PROTEIN CONTENT

Total leaf protein content was estimated by Lowry's method [8]. The mixture was incubated in darkness; the blue color was read at 660 nm in Spectrophotometer. The values are expressed as mg/gfw.

ELECTROLYTE LEAKAGE

The total inorganic ions leaked out in the leaves during salinity stress were measured as described[10].The electrolytic leakage was calculated using the formula,

$$\textbf{Electrolytic leakage (\%)} = \frac{EC_b - EC_a}{EC_c} \times 100$$

Lipid Peroxidation

Lipid peroxidation rates were determined by measuring the malondialdehyde equivalents according[11]. Lipid oxidation rate equivalents (nmol malondialdehyde ml^{-1}).

Statistical Analysis

Data for each parameter analyzed by Two-Way ANOVA and significant differences between treatment mean and varieties were determined by using SPSS (version 15.0, SPSS, Chicago, IL, USA). Data are presented as the mean ± SE of five independent determinations and significance was determined at the 95% confidence (P≤0.05) limits.

RESULTS

TOTAL CHLOROPHYLL

Effect of salinity on total chlorophyll content was studied in two maize varieties and it was decreased with increasing salinity levels on all the sampling days as shown in **Figure 1.** On 30th DAT highest total chlorophyll content was recorded in the variety BC-3033 (0.64 mg/gfw) over to control plants (1.29 mg/gfw, respectively) under 150mM salinity stress, whereas low total chlorophyll content was observed in the variety BC-3591 (0.57 mg/gfw) relative to controls (1.20 mg/gfw, respectively).

CHLOROPHYLL 'a' and 'b'

On all the sampling days, Chlorophyll 'a' and 'b' content was decreased with increasing salinity levels in all the maize varieties **(Figure 2 & 3).** On 30th DAT, with 150mM salinity treatment, lowest chl 'a' and chl 'b' content was observed in the variety BC-3591 and it was 0.23 mg/gfw and 0.41 mg/gfw, respectively, over the controls (0.40 mg/gfw and 0.79 mg/gfw, respectively), while highest chl 'a' and chl 'b' content was recorded in the variety BC-3033 and it was 0.27 mg/gfw, 0.49 mg/gfw, respectively, compared to control plants (0.45 mg/gfw and 0.85 mg/gfw, respectively).

PROTEIN CONTENT

Protein content was decreased in leaves of all the two maize varieties with increasing salinity level on all the sampling days **(Figure 4).** Under 150mM salinity stress, on 30th DAT, protein content was highly decreased in BC-3591 by 50% (28.67 mg/gfw) over the control plants (55.58 mg/gfw, respectively), while low decrease of protein content was recorded in the variety BC-3033 by 26% (32.67 mg/gfw) relative to control plants (57.51 mg/gfw, respectively).

ELECTROLYTIC LEAKAGE

Electrolytic leakages were measured in control and salinity treated plants (Figure, 5). In both the varieties (BC-3033 and BC-3591) electrolytic leakage percentage increased with increasing salinity concentrations. In BC-3033 at 150 mM salinity, on 15th Days After Treatment (DAT) and 30th DAT, electrolytic leakage percentage was 2.28 when compared to control plants which showed percentage of 1.83, while in BC-3591 at 150 mM salinity electrolytic leakage percentage was 3.26 when compared to the control plants which showed percentage of 2.05.

LIPID PEROXIDATION

Malondialdehyde content was measured in the leaves of both the maize varieties BC-3033 and BC-3591 (Figure, 6). Salinity stress has resulted increased content of malondialdehyde with increasing salinity. In BC-3033 at 150 mM salinity, on 15th Days After Treatment (DAT) and 30th DAT, malondialdehyde content was increased by 36% compared to control plants. At 150 mM salinity in BC-3591 melondialdehyde content was increased by 48% (3.83 nmol/ml) when compared to control plants (2.12 nmol/ml).

DISCUSSION

TOTAL CHLOROPHYLL, CHLOROPHYLL-a AND CHLOROPHYLL-b

[12]Reported chlorophyll content as one of the parameters of salt tolerance in crop plants. [13]Observed higher chlorophyll degradation in sodium chloride-sensitive pea cultivar as compared to tolerant one. In the current study, the total chlorophyll content of leaves averaged over two varieties indicated that it decreased significantly with the increase in salt concentration (**Figure 1. Figure 2, Figure 3).** Varieties differed significantly under salt stress treatments. However, highest total chlorophyll content under salinity stress was recorded in BC-3033 on all the sampling days (15th DAT and 30th DAT) even at high salinity concentrations, whereas low level of total chlorophyll content was observed in BC-3591 under salinity stress. The decrease in chlorophylls in salinized plants could be attributed to increased activity of the chlorophyll-degrading enzyme, chlorophyllase and ion accumulation in leaves[14].

It is generally known that photosynthetic efficiency depends on photosynthetic pigments such as chlorophylls '*a*' and '*b*', which play an important role in photochemical reactions of photosynthesis[15]. In our study, salinity stress led to a decrease in chlorophyll 'a' and 'b' on all the sampling days (15th DAT and 30th DAT) and this effect increased consistently with increasing salinity levels as compared to non- stressed treatment. However, higher reduction of chlorophyll 'a' and 'b' was observed in BC-3591 and lowest reduction was noticed in the variety BC-3033 on all the sampling days with varying salinity levels. The present study on the pigment composition clearly showed that the variety BC-3033 maintained high pigment content on all the sampling days than other maize varieties when subjected to salt stress.

SOLUBLE PROTEIN CONTENT

Plant species and even different cultivars within the same species differ greatly in their response to salinity[16]. Under saline conditions, there is a change in the pattern of gene expression and both qualitative and quantitative changes in protein synthesis[17]. In this investigation, when NaCl concentration increased, a soluble protein in two maize varieties was significantly changed **(Figure 4).** Comparatively, lower decrease of soluble protein content was observed in the leaves of maize variety BC-3033 on all the sampling days even with high salinity levels as compared to controls. More decrease of soluble protein content was observed in the variety BC-3591 on all the sampling days compared to control plants. High temperature, salinity and drought stress can cause denaturation and dysfunction of many proteins[18]. The results in this study clearly indicates that even though the protein synthetic machinery of the maize varieties are affected by the salinity stress, variety BC-3033 maintained higher protein content on all the sampling days compared to other varieties.

ELECTROLYTIC LEAKAGE AND LIPID PEROXIDATION

The electrolytic leakage and lipid peroxidation rates were observed in two maize varieties such as BC–3033, BC–3591 in control and treated plants of different salinity concentrations (50mM, 100mM, and 150mM). Electrolytic leakage and lipid peroxidation was more in varities BC-3591 compared to BC-3033

(Figure. 5, Figure 6). Lipid perexidation is a destructive chain reaction and it can directly damage the structure of membrane[19]. Lipid peroxidation of biological membranes might lead to structural alterations in salinity stressed plants. Experimental evidence suggests that lipid peroxidation reactions of cellular membranes may play an important role in radical mediated cell injury[20]. Our results suggest that salinity stress can induce membrane lipid peroxidation resulting membrane fluidity leading to enhanced electrolytic leakage. Our data also indicate that the degree of cell membrane injury and levels of membrane lipid were relatively less in BC–3033 than BC–3591 under salinity stress conditions.

CONCLUSIONS

In conclusion, based on the relative tolerance results provided, it seems that BC-3033 is highly salt tolerant in comparison with the other maize varieties. In fact, for most parameters recorded, better performance was observed in BC-3033 under salt stress on all the sampling days at all salinity levels. This variety probably maintains the photosynthetic pigments with protein and osmotic adjustment with accumulation of electrolytic leakage and lipid peroxidation and prevents oxidative and other stresses induced by Na^+ with well regulation of biochemical constituents and these traits would be useful as selection criteria during breeding for salt tolerance in maize.

Acknowledgements

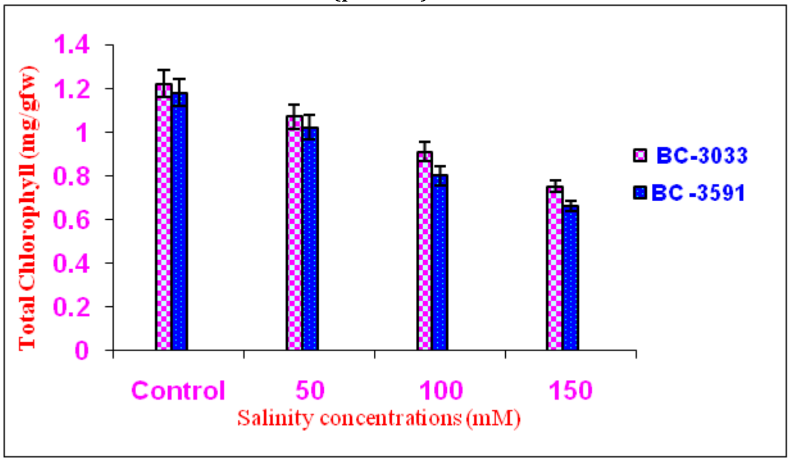
The authors highly thankful to Dr.V.Ravi, Prof and Head, Department of Botany, for providing necessary Lab facilities.

REFERENCES

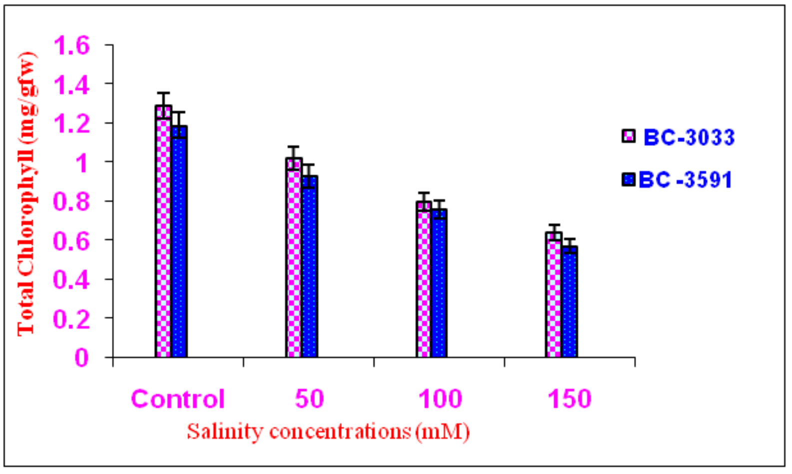
1. 1.FAO. (2008). Global network on integrated soil management for sustainable use of salt-affected soils. http://www.fao.org/ag/agl/agll/spush.
2. 2.Kanagaraj G, Sathish C, (2017). Effect of salinity stress on carbohydrate, lipid \peroxidation and proline contents of two horse gram varieties. Journal of Scientific Agriculture 2017, 1: 37-45.
3. 3.Parvaneh Rahdari, Shahrokh Tavakoli, Seyed Meysam Hosseini. (2012). Studying of Salinity Stress Effect on Germination, Proline, Sugar, Protein, Lipid and Chlorophyll Content in Purslane (Portulaca oleracea L.) Leaves. Journal of Stress Physiology & Biochemistry, Vol. 8 No. 1, pp. 182-193.
4. 4.Turan, M.A., Kalkat, V. and Taban, S. (2007). Salinity-induced stomatal resistance, proline, chlorophyll and Ion concentrations of bean. Int. J. Agric. Res. 2 (5), 483–488.
5. 5.Chawla S, Jain S, Jain V. (2013). Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (Oryza sativa L.). Journal of Plant Biochemistry and Biotechnology, 22(1), 27-34.
6. 6.Kholova J, Sairam RK, Meena RC Srivastava GC. (2009). Response of maize genotypes to salinity stress in relation to osmolytes and metal-ions contents, oxidative stress and antioxidant enzymes activity. Biol. Plant. 53; 249-256.
7. 7.Desingh R, Kanagaraj G. (2007). Influence of salinity stress in photosynthesis and antioxidative systems in two cotton varieties. Gen. Appl. Plant Physiol. 33; 221-234.
8. 8.Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. (1951). Protein measurement with Folin-Phenol reagent. Journal of Biological Chemistry 193, 265-275.
9. 9.Arnon DI. (1949). Copper enzymes in intact chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol. 24; 1-15.
10. 10.Sullivan CY, Ross WM. (1979). Selecting the drought and heat resistance in grain sorghum. In. M. Mussel and R.C. Stapes (eds). Stress physiology in crop plants. 263 – 281. John Wiley and sons, New York.
11. 11.Hodges MD, Delong JM, Forney C.F Prange PK.(1999). Improving the thiobarbituric acid-reactive substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta. 207; 604-611.
12. 12.Srivastava TP, Gupta SC, Lal P, Muralia PN, Kumar A. (1988). Effect of salt stress on physiological and biochemical parameters of wheat. Ann. Arid Zone. 27; 197-204.
13. 13.Hernandez JA, Olmos E, Corpas FJ. Sevilla F, Del Rio, LA. (1995). Salt induced oxidative stress in chloroplasts of pea plants. Plant Sci. 105; 151-167.
14. 14.Sultana N, Ikeda T, Itoh R. (1999). Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Env. Exp. Bot. 42; 211-220.
15. 15.Taiz, L, Zeiger, E. (2006). Plant physiology (Fourth Edition). Sinauer Associates, Inc., Publishers, Sunderland, USA. P 764.
16. 16.Borsani O, Valpuesta V, Botella M A. (2003). Developing salt tolerant plants in a new century: molecular biology approach. Plant Cell. Tiss. Org. Cult. 73; 101-115.
17. 17.Xu C, Sibicky T, Huang B. (2010). Protein profile analysis of salt-responsive proteins in leaves and roots in two cultivars of creeping bentgrass differing in salinity tolerance. Plant Cell Rep. 29; 595-615.

18. 18.Vinocur B, Altman A. (2005). Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr. Opin. Biotechnol. 16; 123-132.
19. 19.Desingh R, Ramachandra Reddy A. (2005). Differential responses to salinity stress in seedlings of three Eucalyptus species. Indian J. Plant physiol. 10; 307 – 314.
20. 20.Bhaunik G, Srivatsanes KK. Selvamurthy W. (1995). The role of free radicals in cold injuries. Int. J. Biomet. 38; 171-175.

Figure 1. Changes of total chlorophyll content in leaves of maize varieties on 15th DAT (a), 30th DAT (b) under varying levels of salinity. Each value represents mean ± SE of five independent determinations ($p<0.05$).

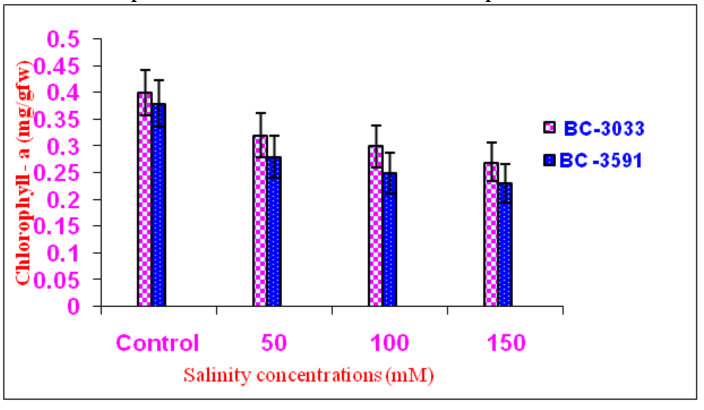


a

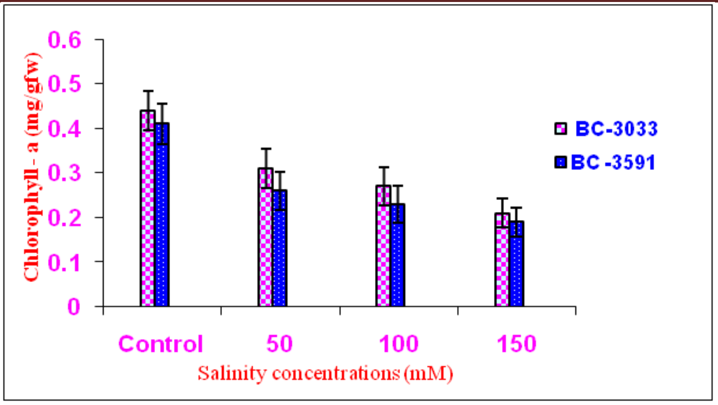


b

Figure 2. Influence of varying levels of salinity on chlorophyll 'a' content in leaves of maize varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

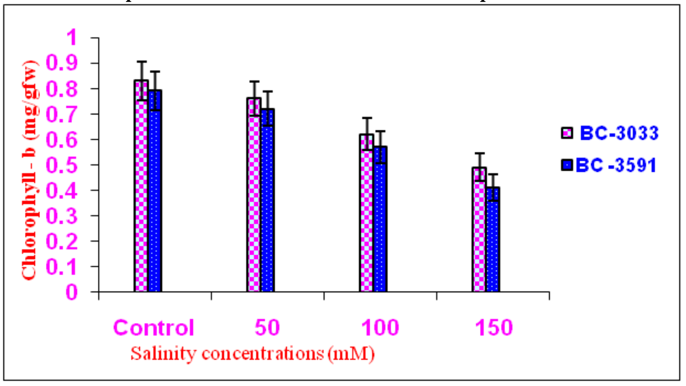


a

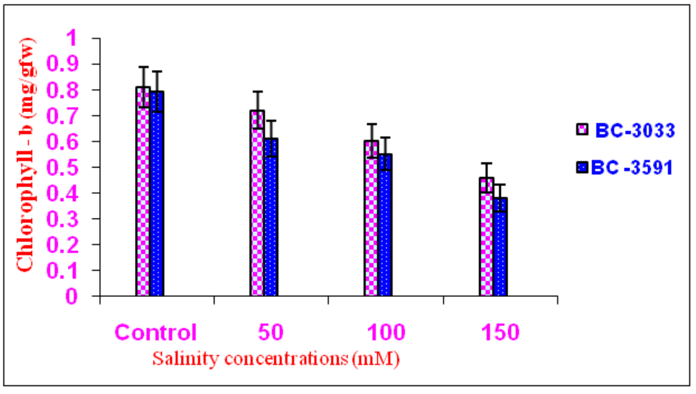


b

Figure 3. Effect of varying levels of salinity on chlorophyll 'b' content in leaves of maize varieties on 15th DAT (a 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

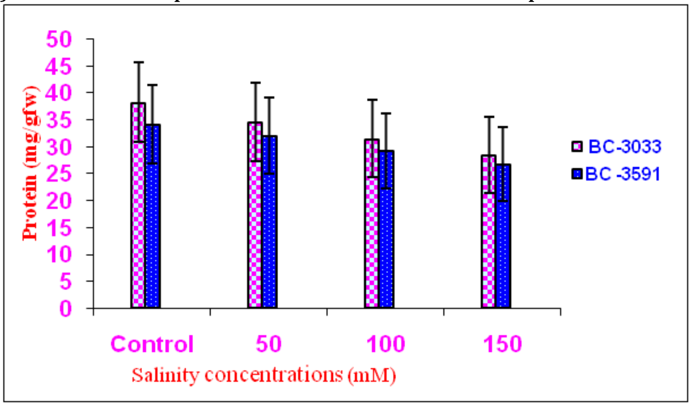


a

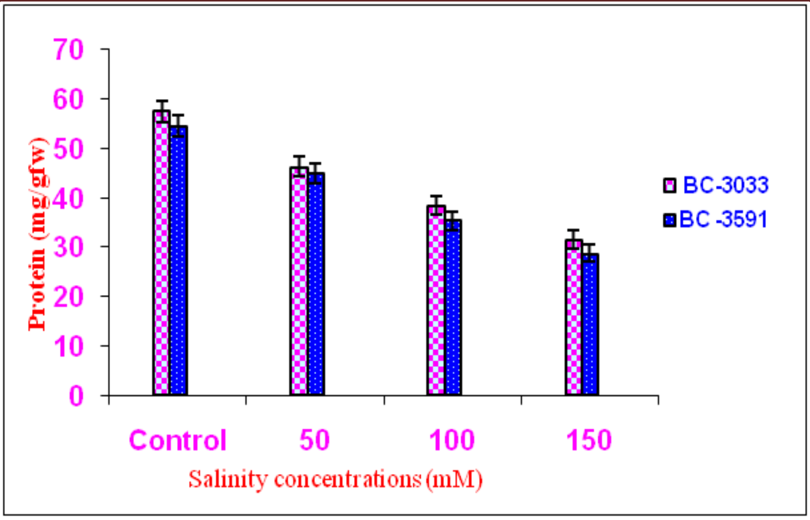


b

Figure 4. Effect of different salinity levels on soluble protein content in the leaf extracts of maize varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

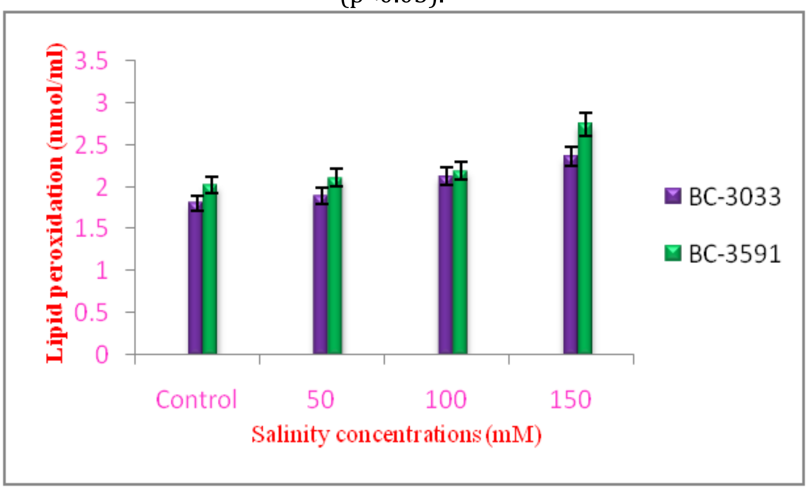


a

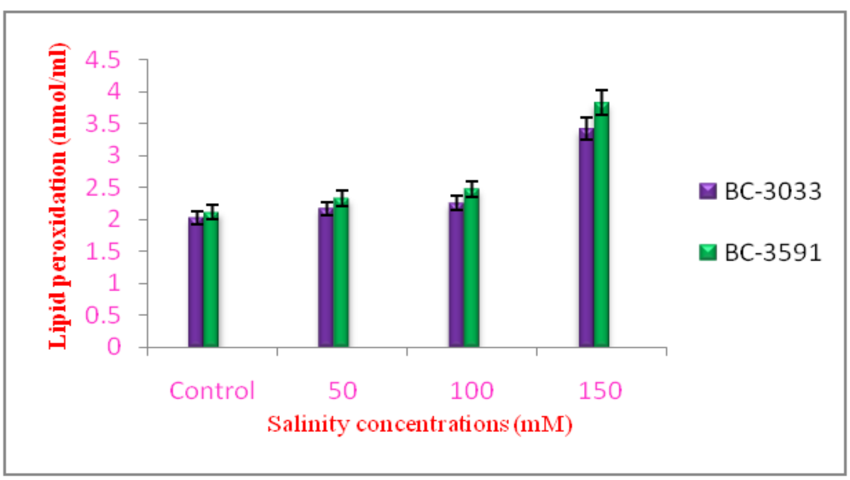


b

Figure 5. Effect of different salinity levels on lipids peroxidation content in the leaf extracts of maize varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).

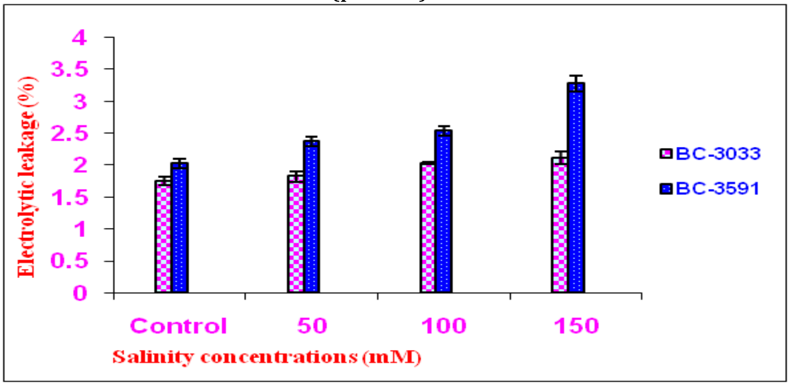


a

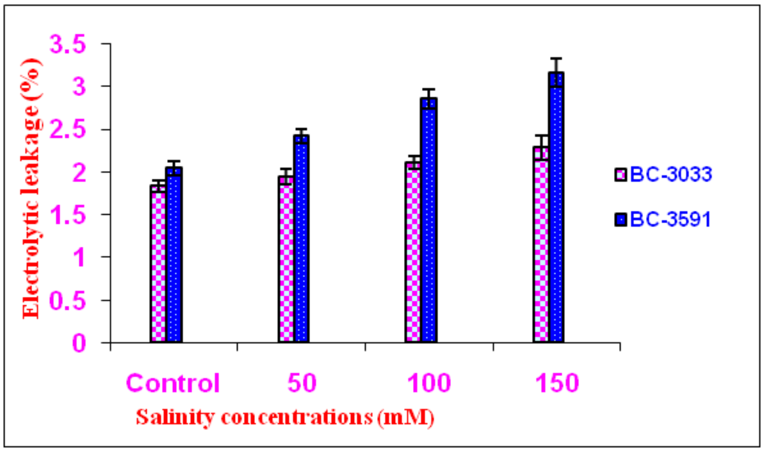


b

Figure 6. Effect of different salinity levels on electrolytic leakage content in the leaf extracts of maize varieties on 15th DAT (a), 30th DAT (b). Each value represents mean ± SE of five independent determinations ($p<0.05$).



a



b