EFFECT OF SALT STRESS ON GROWTH, CARBOHYDRATES, LIPID PEROXIDATION, ELECROLYTIC LEAKAGE OF TWO COTTON VARIETIES

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ABSTRACT: *A study was conducted to determine the growth and physiological characteristics of two cotton varieties, Arya-Anubam and LRA-5166 subjected to salinity stress (NaCl) of different concentrations (0, 50, 100, 150mM). Sampling was done after 30 days of salinity treatments in leaves. Plant height, leaf area, fresh and dry weight of whole plant decreased marginally by salinity stress in Arya-Anubam while LRA-5166 showed more reduction. Arya-Anubam maintained higher total sugar content under salinity stress compared to LRA-5166 while reverse trend was observed in starch content. Lower rates of lipid peroxidtion and electrolytic leakage were noticed in the leaves of Arya-Anubam under salinity stress. The results indicate that maintenance of higher growth rate and carbohydrate level under salinity stress in cotton variety Arya-Anubam contributed to its salt tolerant characteristics.*

Key Words: *Cotton, electrolytic leakage, growth, lipid peroxidation, salinity stress, sugar.*

INTRODUCTION

Salinity is an environmental stress that limits growth and development in plants (Greenway and Munns, 1980; Meloni et al., 2003; Jian et al., 2006; Sharifi et al., 2007; Shao et al., 2008; Negrao et al., 2019). The effects of saline soils on plant growth have been a focus of research for nearly 100 years because salt stress is a major stress, limiting crop productivity (Fougere et al., 1991). Salt tolerance of plants is a complex phenomenon that involves morphological and developmental changes as well as physiological and biochemical processes. Primary metabolites such as soluble sugars including glucose and sucrose help regulate many developmental and physiological processes in plants (Koch, 1996; Gibson, 2000; Sotiropoulos, 2007; Gupta and Huang, 2014). Sugars are also thought to help control key metabolic processes such as photosynthesis (Krapp et al., 1993) and starch synthesis and breakdown (Koch, 1996). Lipids play an important role in determining the physiological properties of biological membranes. Membrane disruption leads to a series of events namely the decrease of photosynthetic activity (Lauriana et al., 2000). Lipid peroxidation is considered as an indicator of the extent of oxidative damage under stress (Bor et al., 2003)

Cotton varieties are commercially important crops worldwide and their practical utilization ranges from fibres of textile industry and cotton seed oil. In India, cotton has a wide geographic distribution extending over a range of environmental conditions. However, as other major crops in India, cotton is also subjected to environmental stresses, particularly salinity stress. Because of inherent sensitivity of cotton plants to salt stress, the salinity has become a serious production constraint for cotton. The yield level can be consolidated by developing high yielding varieties with high level of tolerance to salinity stress. The present investigation therefore, attempts to analyse the growth and physiological basis of salt tolerance in two cotton varieties (Arya-Anubam and LRA-5166). Such an understanding will be useful in future breeding programme for developing salt tolerant cotton varieties.

MATERIALS AND METHODS

Plant material and growth conditions

The certified delinted cottons seeds (Variety: Arya Anubam and LRA-5166) were procured from PASIC, Pondicherry. Seeds with uniform size were selected and the plants were raised in pots containing red and clay soil. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. The maximum irradiance (PAR, 400-700nm) available during growth was. 1800-2000 µmol $m^{-2}s^{-1}$ on a clear day. Daily maximum and minimum temperatures were 29-33^{0}C and 20-22^{0}C, respectively. 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 and one-fourth strength of Hoagland nutrients. Other three group 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. All the plants used in this study were of comparable size. Young and fully matured leaves were taken at 30 days after salinity treatments for all the experiments described below.

Plant height, leaf area, fresh and dry weight of the whole plant

The height of the plants was measured with a measuring tap after 30 days of salinity treatments. The leaf area was calculated multiplying the length and breadth of the broadest regions of the leaf. For fresh weights, mature plants were carefully uprooted and the roots were washed, blotted and whole plant was weighed. For dry weights, the whole plant was dried in an oven at 75-80^0 for 40 hours until a constant weight was obtained.

Alcoholic Extraction

Leaf sample was macerated and 25 mg of the dried powder was boiled in water bath with 10 ml of ethyl alcohol (80%). The homogenate was centrifuged at 1500 g for 15 minutes and the supernatant was made upto 20 ml with 80% ethyl alcohol. This alcoholic extract was used for quantitative estimation of total sugars and the residue was saved for starch estimation.

Total Sugar and starch

Total sugar content was estimated using the method of Dubois et al., (1956). To 1 ml of ethanolic extract, 4 ml cold anthrone reagent was added. This was heated for 10 minutes in boiling water bath and test tubes were closed with glass marbles to prevent evaporation. After cooling, absorbance was recorded at 620 nm in spectrophotometer. A standard graph was prepared using glucose and the amount of total sugars was calculated using standard graph. Starch content of the leaf was estimated according to Mc Cready et al., (1950). To the residue left after alcoholic extraction, 5 ml of distilled water and 5 ml of perchloric acid (52%) were added. The mixture was incubated for 30 minutes and filtered and was made upto 100 ml with glass distilled water. 2 ml of distilled water was added to 0.5 ml of the above extract and 5 ml of cold anthrone reagent was also added. The contents of the tube were heated for 7.5 minutes in boiling water bath and cooled using running tap water. The bluish green color was read at 630 nm in spectrophotometer against blank.

Lipid peroxidation and Electrolyte leakage

Lipid peroxidation rates were determined by measuring the malondialdehyde equivalents according to Hodges et al., (1999). The leaf tissue (0.5g) was homogenized in a mortar with 80% ethanol. The homogenate was centrifuged at 3000xg for 10min at 4^{0}C. The pellet was extracted twice with the same solvent. The supernatant were pooled and 1ml of these sample was added to a test tube with an equal volume of either the solution comprised of 20% TCA and 0.01% butylated hydroxy toluene (BHT) or solution of 2% TCA, 0.01% BHT and 0.65% TBA. Samples were heated at 25^{0}C for 25min and cooled to room temperature. Absorbances were read at 440, 532 and 600nm. Lipid oxidation rate equivalents (nmol malondialdehyde ml^{-1}) were calculated by using the formula given by Hodges et al., (1999). The total inorganic ions leaked out in the leaves during salinity stress were measured as described by Sullivan and Ross (1979). 20 leaf discs were taken in a boiling tube containing 10ml of deionized water and electrical conductivity (EC) was measured (EC_a). The contents were heated at 45 and 55^{0}C for 30min each in a water bath and EC was measured (EC_b). Later, the contents were boiled at 100^{0}C for 10min and the EC again recorded (EC_c). The electrolytic leakage was calculated using the formula

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

Statistical analysis

Five independent determinants from individual plants were used for statistical analysis. Student's t-test and analysis of variance (ANOVA) were used for analyzing significant differences between the control and treated plants ($p<0.05$).

RESULTS

Height of the plants and leaf area were measured at 30 days after salinity treatments of different concentrations (0, 50,100, 150mM). Results were compared with the control plants (0 mM) as shown in Figs. 1 and 2. In Arya-Anubam, the reduction of plant height and leaf area at 150mM salinity was to the tune of 45% (22.78 cm) and 46% (63.40 cm^2) respectively, compared to control plants (41.15 cm, 115.68 cm^2

respectively). At the same time, plant height and leaf area was reduced in the tune of 54% (16.61 cm) and 60% (45.74 cm^2) respectively at 150mM salinity in LRA-5166 when compared to control plants (35.82 cm, 110.72 cm^2 respectively).

Figs. 3 and 4 show the effect of salinity stress on the fresh and dry weight of the whole plants. There was progressive decreasing in the plant fresh and dry weights with increasing salinity concentrations. At 150mM salinity, the reduction in the fresh and dry weight of the whole plants in Arya-Anubam was to the tune of 54% (16.62 grams) and 56% (8.19 grams) respectively, compared to control plants (35.44 grams, 18.63 grams respectively), while in LRA-5166 at 150mM salinity the reduction in fresh and dry weight of the whole plant was to the tune of 63% (10.18 grams) and 67% (5.31 grams) respectively compared to control plants (27.15 grams, 15.36 grams respectively).

The effect of salinity stress on the amount of total sugars was depicted in Fig. 5. The data clearly show that salinity stressed plants exhibited greater reduction in the total sugars content. At 150 mM salinity the reduction in total sugars in Arya-Anubam was 37% (12.09 mg/gdw) compared to control plants (19.74 mg/gdw). In LRA-5166, at 150 mM salinity reduction of total sugars was 53% (8.12 mg/gdw) compared to control plants (17.91 mg/gdw.). The data in Fig. 6 show the amount of leaf starch in relation to salinity stress. It is significant to notice that with increasing salinity concentration, there was a significant accumulation of starch in all treatments. In Arya-Anubam, 150 mM salinity stressed plants accumulated more starch (58.92 mg/gdw) compared to control plants (40.63 mg/gdw). Similarly in 150 mM salinity stressed plants of LRA-5166, the starch content was 75.34 mg/gdw. when compared to control plants (48.73 mg/gdw).

Salinity stress has resulted increased content of malondialdehyde with increasing salinity (Fig. 7). In Arya-Anubam at 150 mM salinity malondialdehyde content was increased by 35% compared to control plants. At 150 mM salinity in LRA-5166 melondialdehyde content was increased by 47% (3.93 nmol/ml) when compared to control plants (2.12 nmol/ml). In both the varieties (Arya-Anubam, LRA-5166) elelctrolytic leakage percentage increased with increasing salinity concentrations (fig. 8) In Arya-Anubam at 150 mM salinity, electrolytic leakage percentage was 3.46 when compared to control plants which showed percentage of 2.02, while in LRA-5166 at 150 mM salinity electrolytic leakage percentage was 6.15 when compared to the control plants which showed percentage of 3.11.

DISCUSSION

Physiological and biochemical studies of salinity in higher plants have greatly increased our understanding of salinity stress. However, there are several constraints to elucidate the mechanism of salinity stress effects on plant growth and metabolism. This is because of the limitations to understand the physiology of salt uptake, its transport, distribution with in the plant and the complete metabolic sequences. The main objectives of this study is to understand the plant growth and its associated physiological changes in cotton under salinity stressed conditions.

The growth parameters (Figs. 1-4) were taken at 30 days after salinity treatments and the reduction in cotton growth associated with salinity stress is the main observation in the present study. It is believed to be due to its partial closure of stomata which leads to limit in the photosynthetic capacity of the treated plants. Salt accumulation in leaves might first inhibit photosynthesis by increasing stomatal and mesophyll conductance to $C0_2$ diffusion and is known impair RuBP carboxylase [Bongi and Loreto, 1989; Delfine et al., 1998; Querghi et al., 2000]. Salinity has also affected the leaf area development as well as the leaf elongation rate in cotton varieties both Arya-Anubam and LRA-5166. This is believed to the due to the reduced cell enlargement as well as the modifications in the cellular physiology in the cotton plant. The cotton variety LRA-5166 showed more reduction of plant height (Fig. 1) and leaf area (Fig. 2) expansion than the Arya-Anubam. The reduced plant height and leaf area in salinity treated plant was due to the reduction in photosystem II activity in salt stressed plants might be a crucial factor in determining the photosynthetic productivity in cotton plants (Meloni et al., 2003). The total fresh weight (Fig. 3) of the salinity treated plant was positively related to the leaf expansion rate and the plant height, which was significantly decreased under salinity stressed conditions in both the cotton varieties Arya-Anubam and LRA-5166. The reduction in plant dry weight (Fig. 4), can be attributed to the reduced photosynthetic capacity of the leaves under salinity stressed conditions [Sanchez-Rodriguez et al., 1999; Querghi et al., 2000]. It is predict that decreased photosynthetic rates under salinity condition could have reduced the shoot growth and development, thus finally yielding lower biomass production compared to control plants. In the present study, the fresh and dry weight of the whole plant was reduced more in LRA-5166 than the other cotton variety Arya-Anubam.

It was hypothesized that due to limitation supply of structural and non structural carbohydrate in cotton leaves, plant growth will be significantly reduced due to limited supply of energy and carbon skeletons during various stages of growth. In the present study, high content of sugar was maintained in the Arya-Anubam than the LRA-5166 under salinity stressed conditions (Fig. 5). The sink systems of the plant compete for the limited carbon supplies under salinity which affect the overall plant growth and yield [Munns and Termaat, 1986; Daie, 1996]. As a consequence, the different growth responses to salinity can be presumed and interpreted as resulting from changes in the allocation and partitioning of photoassimilates [poljakolf-Mayber and Lerner, 1994]. It is surprising to observe that starch content was inversely related to salinity (Fig. 6). The reducing levels of starch in control and low salinity concentration (50mM) treated plants indicate that the export of carbohydrates to various organs is at a faster rate compared to those with high salinity treated plants. The study also suggest that salinity stress causes a significant accumulation of starch in the leaves which might ultimately reduce the CO_2 assimilation patterns in the intact leaves. Salinity stress might alter the export of photoassimilates to the growing regions, thus affecting the overall growth and development of cotton. The regulation of carbon allocation and partitioning would have an important influence in the maintenance of growth rate and yield [Sacher and Stables, 1985; Balibrea et al., 1999]. Our study on carbohydrates clearly indicates that Arya-Anubam had on effective carbohydrate partitioning mechanism than LRA-5166 which might contributed for efficient photosynthesis.

Lipid peroxidation is a destructive chain reaction and it can directly damage the structure of membrane [shah et al., 2002; Desingh and Reddy, 2005; Koca et al., 2006). Induction of oxidative stress in salinity stressed plants is known to increase the accumulation of metal ions available for the Haber-Weiss reaction, enhance the oxidative damage of lipids and cell membrane proteins [Mano, 2002; Bor et al., 2003]. Cell membrane are the first target of many plant stresses and maintenance of their integrity and stability under stress conditions (Salinity/water) is a major component of tolerance in plants [Bajji et al., 2002; Meloni et al., 2003; Fazeli et al., 2007]. 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 Arya-Anubam than LRA-5166 under salinity stress conditions (Figs. 7-8).

CONCLUSION

Salinity tolerance of cotton variety Arya-Anubam is associated with higher growth with carbohydrates and lower level of lipid peroxidation than in LRA-5166, which leads to higher biomass production.

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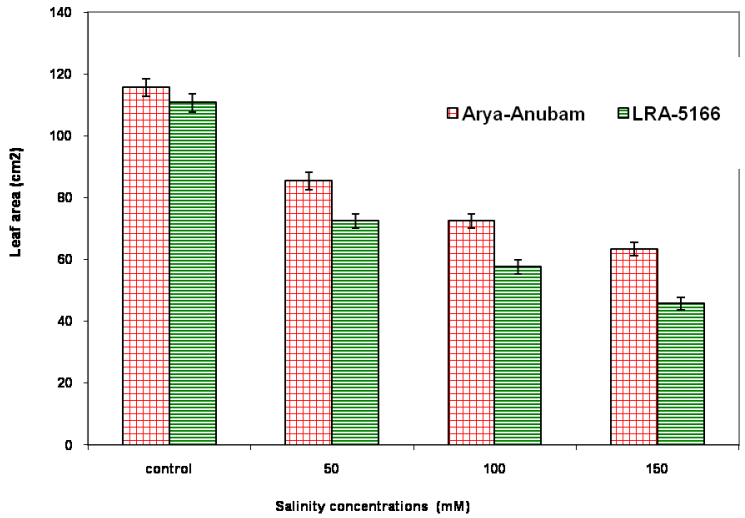

Fig. 2. Influence of salinity stress on the leaf area of the plants in two cotton varieties. Each value represents mean±S.E. of five independent determinations, $p<0.05$.

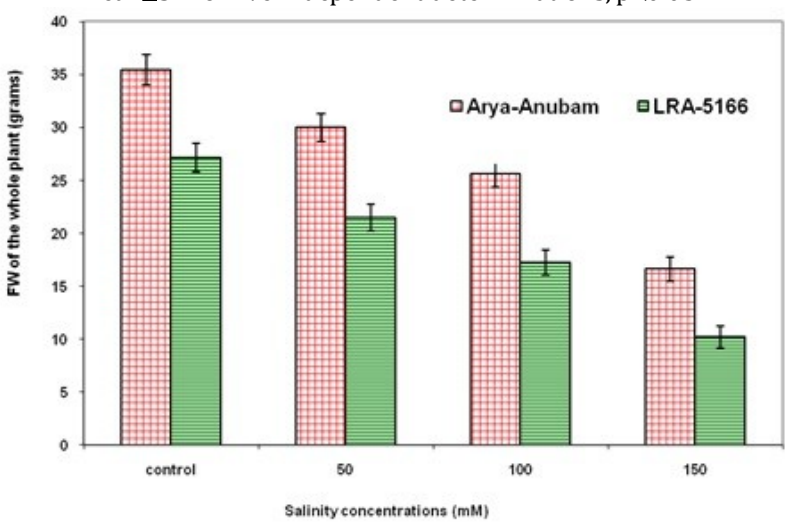

Fig. 3. The effect of salinity stress on the fresh weight of the whole plants in two cotton varieties. Each value represents mean±S.E. of five independent determinations, $p<0.05$.

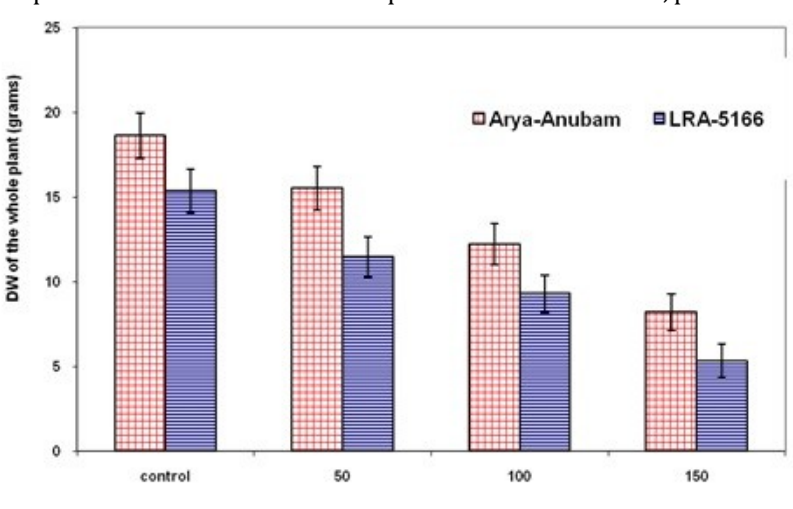

Fig. 4. The effect of salinity stress on the dry weight of the whole plants in two cotton varieties. Each value represents mean±S.E. of five independent determinations, $p<0.05$.

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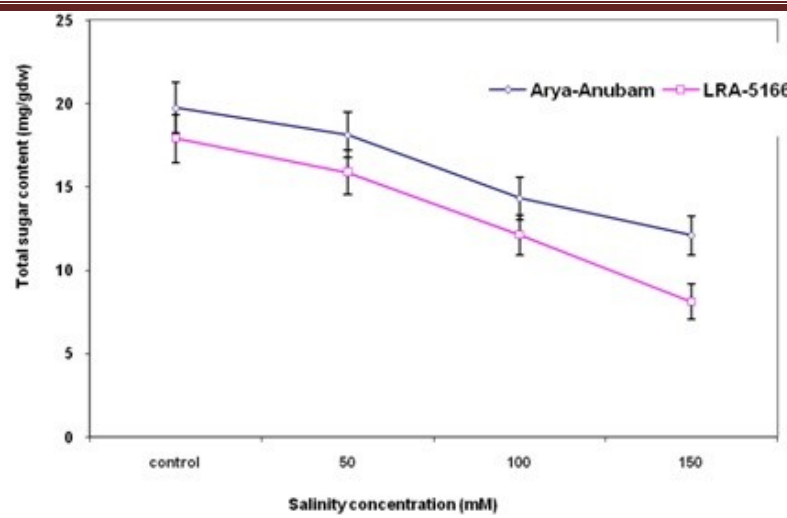

Fig. 5. Salinity stress effects on content of total sugars in the leaves of two cotton varieties. Each value represents mean±S.E. of five independent determinations, $p<0.05$.

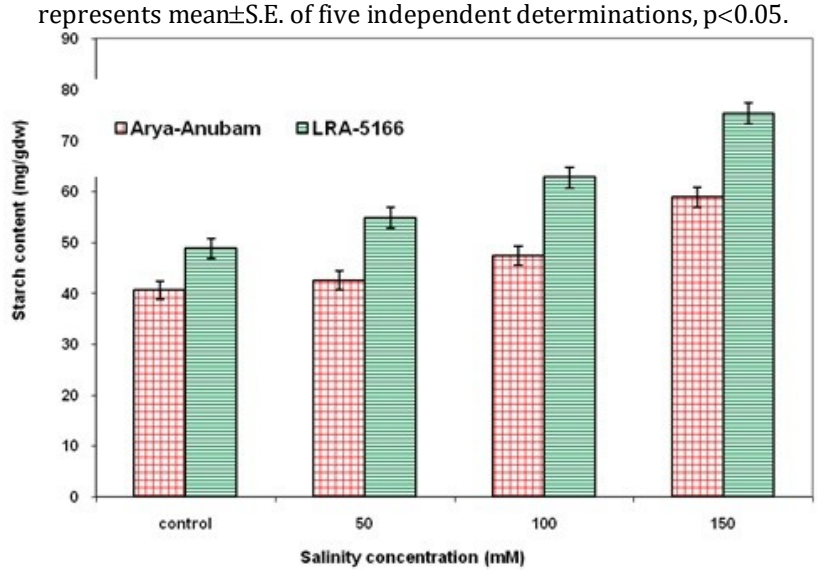

Fig. 6. Changes of starch content in the leaves of two cotton varieties subjected to salinity stress. Each value represents mean±S.E. of five independent determinations, $p<0.05$.

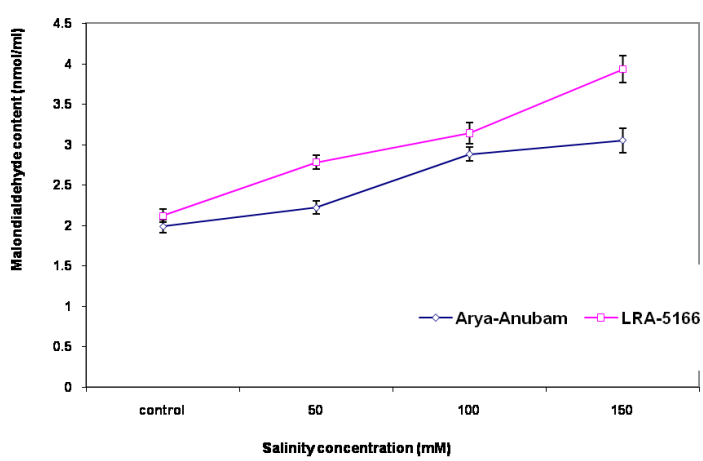

Fig. 7. Lipid peroxidation in the leaves of two cotton varieties under salinity stress. Each value represents mean±S.E. of five independent determinations, $p<0.05$.

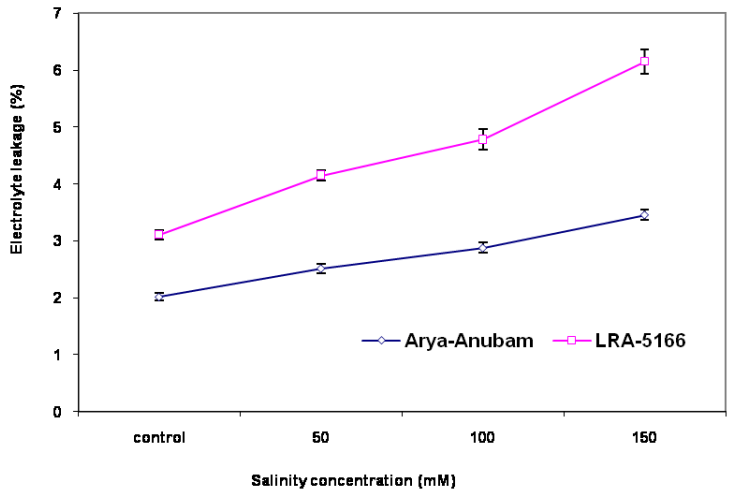

Fig. 8. Salinity stress effects on electrolytic leakage in leaves of two cotton varieties. Each value represents mean±S.E. of five independent determinations, $p<0.05$.