PHYSIOLOGICAL AND MOLECULAR STUDIES OF SALINITY TOLERANCE IN COTTON (*Gossypium herbaceum* L.)

Thesis submitted to the
University of Agricultural Sciences, Dharwad
In partial fulfillment of the requirements for the
Degree of

MASTER OF SCIENCE (AGRICULTURE)

IN

CROP PHYSIOLOGY

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I. INTRODUCTION

Cotton is one of the most important economic fibre crops in the world. Cotton known as the 'King of fibre' and called as the 'White Gold' is the most vital crop of commerce to many countries including India. Cotton plays a major role in India's economy both in terms of providing employment directly or indirectly to about 60 million people and in earning foreign exchange for the country. There is no single country in the world to compare the cotton cultivation as it has all the four commercial genotypes under cultivation and encompasses various ecosystems.

Cotton is cultivated in 70 countries of the world with the total coverage of 34 million ha. A production of 24 million metric tonnes and a productivity of above 621 kg lint per ha. India being the traditional home for cotton and cotton textiles, ranks first in the cultivated area occupying about 9.2 million ha producing 138 lakh bales with the productivity of 403 kg lint per ha (Anon., 2005). In Karnataka, it is grown in an area of 5.5 lakh ha with a production of 8 lakh bales and productivity of 246 kg lint per ha (Anon., 2004).

Though, India has the largest area under cotton, it ranks third in production due to low productivity. The major reasons for low yield in India are climatic, biotic, abiotic, institutional and technological problems. One of the major abiotic stresses affecting plant productivity is water stress resulting either through drought or salinity.

Salinity is one of the major hazards, usually confined to arid and semi-arid regions of the world (Ashraf, 1994 and Lin *et al*., 1997). It has been estimated that 20 per cent of world's cultivated land and nearly 50 per cent of all irrigated land is adversely affected by soil salinity.

In India, out of total geographical area of 329 million ha nearly 8 million ha is affected by salinity. In Karnataka, it is estimated to be 179 thousand ha. This vast occurrence of salt affected lands in arid and semi-arid regions of India is because, the annual rainfall is not sufficient to leach down salts to deeper layers. High evaporation leads to accumulation of large amounts of salts in the root zone and this intensity of soil salinization increases with the increase in dryness of climate and in command areas is mainly due to seepage, use of poor quality underground water/or irrigation, indiscriminate use of water with inadequate drainage.

Most crop plants are glycophytes, plants whose growth is reduced even in very low levels of salinity. In these plants, excess salt (Nacl) in the soil solution interferes with germination of seeds, inhibit plant growth and metabolism (Kingsbury and Epstein, 1986). Plants growing under salinity stress show non-availability of water due to reduction in solute potential of soil solution, ion toxicity, nutritional imbalances (Hasegawa *et al*., 2000) stunted growth, excess amounts of reactive oxygen species (ROS) resulting oxidative damage to many cellular components, including membrane lipids and altered levels of enzymes of various metabolic pathways. In general, the crop plants growing under saline environments are shown to have a lowered rate of metabolism affecting biochemical, physiological and biophysical processes.

Under salt stress, plants have evolved complex mechanisms in order to cope with the changes taking place in their environment. The mechanisms include osmotic adjustment by accumulation of compatible solutes (proline, sugar *etc*.), lowering the toxic concentrations of ions in the cytoplasm by restriction of Na^+ influx or its sequestration into vacuole and/or its extrusion (Hajibagheri *et al*., 1987) and by employing antioxidants and antioxidant enzymes (SOD, POX, CAT *etc*.) for effective scavenging of reactive oxygen species which are produced in excess amounts under stress conditions (Asada, 1992).

Although, cotton is classified as salt tolerant crop (Maas and Hoffman, 1977) yet, it is sensitive at germination stage (Saghir Ahmad *et al*., 2002). Therefore, there is a need to focus efforts for developing salt tolerant varieties of cotton. A prior information regarding the physiological mechanisms or characters and molecular mechanisms conferring tolerance towards salinity stress at various growth stages is a pre-requisite for successful breeding of this crop for salt tolerance. The present investigation therefore attempts to analyse the physiological, biochemical and molecular basis of salt tolerance in cotton genotypes differing in their sensitivity to salinity stress.

With this background in view, the present investigation was planned with the following objectives.

1. To study the effect of different levels of salinity on seed germination and seedling vigour of tolerant and susceptible cotton genotypes.
2. To study the antioxidant enzyme activities in saline tolerant and susceptible cotton genotypes.
3. To study the cell membrane integrity of tolerant and susceptible cotton genotypes under different levels of salinity stress and
4. To study the isozyme profiles of antioxidant enzymes in saline tolerant and susceptible cotton genotypes.

II. REVIEW OF LITERATURE

Salinity like drought remains as one of the world's most serious environmental problems. Excess amount of salt in the soil adversely affects plant growth and development processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set, consequences of all these leads to growth arrest and molecular damage and ultimately lead to plant death.

However, the trends and magnitudes of these consequences varied according to the level and duration of salinization as well as the plant species used and show different responses to combat the stressful condition. In this chapter reviews have been made on the following aspects.

2.1 GERMINATION AND SEEDLING GROWTH

Salinity imposes a major setback in increasing the yield of cotton due to reduction in germination, leaf area, stem thickness, shoot and root length and weight, biomass production and ultimately brings about decrease in seed cotton yield. A threshold salinity level at which initial cotton declines is 7.7 dS m^{-1} with a 50 per cent reduction in yield at 17.0 dS m^{-1} (Saghir Ahmed *et al*., 2002).

Qadir and Shams (1997) has reported that the germination of cotton seed, emergence of seedling, vegetative shoot growth and root development is generally delayed due to salinity, while increase in shoot growth with lower concentration of salts has also been observed (Pessarakli, 1995).

According to Javid *et al.* (2001), significantly higher germination, seedling length and weight of cotton was found at 1.5 and 5 dS m^{-1} salinity levels and significant reduction in seed germination (84%), seedling length and weight (44%) was observed with increase in salinity level upto 30 dS m^{-1}. The reduction in germination with increase in salinity was in line with the results of Gupta *et al.* (1995).

Cotton appears to be more sensitive for salinity at germination stage. It can tolerate salinity level of 4 mmhos cm^{-1} at 25^0C in saturation extracts during germination but can tolerate three times this salinity level once the seedlings are well established (Ayers and Hayward, 1948).

Mehta and Desai (1958) reported that under increased salinity created by either Nacl or $CaCl_2$, germination was delayed and per cent emergence decreased. The order of tolerance for salt was tobacco > bajra > cotton > bean > tomato > pea.

Emergence of cotton cultivar H-777 was not affected at soil salinity levels upto 5.8 dS m^{-1} but progressively declined beyond this value (Sharma *et al*., 1991). Similarly, Ahmad *et al.* (1991) and Khan *et al.* (1995) reported that cotton seed germination significantly declined at 21 dS m^{-1} and cultivar NIAB-78 was more tolerant at germination.

Ghoulam *et al.* (2002) found that a high Nacl concentration caused reduction in growth parameters such as leaf area, root and shoot length, germination, fresh weight and dry weight and more of solute leakage in case of sugar beet.

Germination and seedling growth of four groundnut cultivars were suppressed by different salt concentrations and in general root length was affected more than the hypocotyl length. Sodium carbonate was found to be more toxic for germination than sodium sulphate and magnesium sulphate (Nautiyal *et al*., 1989).

Janardhan *et al.* (1976b) observed that at salinity level of 12 mmhos cm^{-1}, per cent germination on 30^{th} day was 40 and 25 per cent in cotton cultivars Varalakshmi and Bhagya and 15 per cent in Hampi. They have further stated that these differences may have greater impact, if cotton is grown on salt affected land/or under saline water irrigation.

According to Zidan and Elewa (1995), germination was unaffected at 160 mM Nacl, but at higher salinity level (280 mM) completely inhibited the germination of anise and corriander but not in cumin and caraway, where 35 and 60 per cent germination was found

respectively and also gradual decrease in root length and shoot length with increase in Nacl concentration, above 80 mM. Lower salinity levels (40-80 mM Nacl) stimulated shoot and root length and production of dry matter in caraway and cumin.

Anuradha and Seetaram Rao (2002) found that around 50 per cent reduction in germination, root length, shoot length and seedling dry weight in rice was observed at 150 mM Nacl when compared with control. Brassinolide was found to alleviate the inhibitory effect of germination and seedling growth may be because of enhanced levels of nucleic acids, soluble proteins and free proline.

Eleven cultivars of chickpea were tested for their relative salt tolerance. At different levels of salt stress (2, 4, 6 and 8 dS m^{-1}), germination relative index (GRI) was significantly reduced in sensitive cultivars than tolerant ones. Similar results were observed with regard to the early seedling growth measured as root/shoot length, as well as their dry weights, in addition a gradual accumulation of total soluble sugars was observed in all the cultivars, but accumulation was maximum in tolerant cultivars (Neera Garg and Jasleen, 2004).

Karadge and Gaikwad (2003) reported that Nacl induced salinity reduced the plant height, leaf area, number of buds, flowers, pods and dry weight per plant of the *Catheranthus roseus* G. DON. However, the shoot:root ratio was increased with increasing salinity level indicating the adverse effect of Nacl on the root growth suggesting the salt sensitive nature of plant. This effect was more severe at higher salinity level of 200 mM.

Rema Devi and Gopalkrishnan (1997) in an experiment to study the seedling growth of cowpea in distilled water and in various solution of NaCl and $CaCl_2$ (1000, 3000 and 5000 ppm) after 96 hrs of sowing noticed that the root length, shoot length as well as total seedling length and fresh and dry weights of these decreased significantly at various salinity levels of both the salts.

A pot trial was conducted to study different levels of salinity irrigation water in cumin. Salinity levels at 8 dS m^{-1} significantly reduced seed yield, nutrient uptake and levels of most of leaf metabolites such as total chlorophyll, starch and soluble protein (Garg *et al*., 2002).

Malik and Makhdum (1987) studied the effect of four different levels of salinity (4, 8, 12 and 16 dS m^{-1}) on four cotton cultivars and found that significant decline in seed germination in all the cultivars with increasing salinity.

Subbarao *et al.* (1991) found that there was 80 to 100 per cent germination in the saline tolerant wild pigeonpea genotypes at 6 dS m^{-1} EC while in susceptible genotypes more than 70 per cent mortality was observed.

In a controlled environment when the cotton plants were grown in sand culture with nutrient solution and different levels of Nacl and $CaCl_2$, the plant growth decreased with increasing salinity especially at four and eight weeks after imposing salinity treatments. It was further noticed that the seedling and early vegetative growth stages were found to be more sensitive to salinity (Lashin and Atanasiu, 1972).

Gossypium barbadense varieties exhibited more tolerance than *Gossypium hirsutum* or *Gossypium arboreum* cottons and the seedlings growth was more sensitive than germination to the salt stress (Abul-Nass and Omran, 1974).

Hampson and Simpson (1990a) reported that salt stress decreased germination, root and shoot lengths and growth of wheat plant. Seedlings in magnesium salt treatments had shorter shoots and roots than seedlings in other Na and K salts. Hypocotyl elongation increased with increasing temperature and at high osmotic stress there was reduction in hypocotyl elongation.

Bhumbla *et al.* (1968) while studying the salt tolerance of some important crops such as sesbania, cotton and rice observed 50 per cent reduction in germination at EC of 4, 8 and 12 mmhos cm^{-1} for sesbania, cotton and rice respectively.

Salinity of 5 mmhos cm^{-1} greatly increased the growth of sunflower seedling in terms of root and hypocotyl length while, salinity of 10 mmhos cm^{-1} reduced the growth of seedling

only in terms of root and hypocotyl length, further, at higher salinity of 15, 20 and 25 mmhos cm^{-1} were inhibitory in all aspects (Saha and Gupta, 1997).

Cotton is classified as salt tolerant crop which can withstand salinity of 8 to 12 mmhos cm^{-1} (Vanden Berg, 1950). The reduction in germination under saline conditions could be due to increased osmotic pressure of the soil solution. Consequently, the absorption rate diminishes causing moisture stress in seed. It could be also due to influx of ions in quantities large enough to make them toxic to seed embryo (Ayers, 1952).

According to the findings of Silberbush and Ben-Asher (1987), the growth of root was very poor at higher salinity levels (>287 mM Nacl). In an another study, cotton cultivars, Acala and SJ-2 when treated with 75, 150, 225, 300 and 375 mM Nacl, showed delayed primary root growth and reduced peak elongation rates.

The salinity tolerance of different genotypes of mustard was assessed at three artificially created salinity levels *i.e.,* 2, 6 and 10 dS m^{-1}, it was found that germination, root and shoot length and speed of germination were reduced significantly with increasing salinity levels (Lallu and Dixit, 2003). Similar results were reported by Nanja Reddy *et al.* (2003) in groundnut.

In an in vitro and in vivo study, 23 cotton genotypes have been screened for germination, root and shoot length and seedling vigour index and found huge genotypic variability for salt tolerance. In general all the parameters decreased with increase in the salinity levels and maximum reduction was seen at 250 mM Nacl salinity level and per cent reduction was more under in vivo condition compared to in vitro (Janagoudar *et al*., 2004).

Rajgopal (1999) has reported that cotton genotypes differed widely in their response to salinity, the genotype AK-235, LRA 5166 and AK 84635 would be better suited for salinity stress which had lower reduction in germination percentage, root and shoot vigour index, leaf area, total dry matter, seed cotton yield and catalase activity. In contrast, there was higher accumulation of the total soluble sugars and proline at higher salinity level.

2.2 ANTIOXIDANT ENZYMES ACTIVITIES

When plants are subjected to environmental stress, the balance between the production of O_2 species and the quenching activity of antioxidants is upset, often resulting in oxidative damage (Fridovich, 1986 and Davies, 1987). Since internal O_2 concentrations are high during photosynthesis, chloroplasts are especially prone to generate activated oxygen species (O_2^-). Once produced, O_2^- will rapidly dismutated either enzymatically or non-enzymatically, to yield $H_2O_2 + O_2$. Conversion of O_2^- to H_2O_2 poses a problem for plants, since H_2O_2 is a powerful inhibitor of calvin cycle (Kaiser, 1976).

In varying degrees, plants possess antioxidant defense mechanism that protect against the potentially cytotoxic species of activated oxygen and H_2O_2. A large body of evidence has shown that antitoxidant enzyme systems are enhanced under abiotic stresses including salinity. Superoxide dismutase (SOD) react with the superoxide radical to produce hydrogen peroxide. Hydrogen peroxide is scavenged by catalase (CAT) and peroxidase (POX) (Chang *et al*., 1984).

In wheat, comparative study was conducted between salinity tolerant and susceptible genotypes. In tolerant genotype Kharachia-65 had lowest accumulation of H_2O_2 and Malondialdehyde (MDA) content and highest membrane stability. SOD, catalse and glutathione reductase activity were more in Kharachia-65 than HD-2009 (susceptible genotype). From the results, it is apparent that salinity stress tolerance of Kharachia-65 is associated with its ability to retain higher RWC, pigment content, osmolyte content, lower sodium content and sodium/potassium ratio, higher antioxidant activity resulting in lower hydrogen peroxide and higher membrane stability (Veerabhadra Rao, 2001).

Hernandez *et al*. (1993) found increased release of active oxygen species in mitochondria of *Pisum sativum* L. and further observed that mitochondrial Mn-SOD appears to have a function in the molecular mechanism of plant tolerance to salt stress in pea in conjugation with other enzymes.

Sairam and Tyagi (2004) reported that the activity of enzymes like SOD, catalse, ascorbate peroxidase and glutathione reductase increased under different kinds of stress condition to detoxify the effects of active oxygen species and also observed the increased activities of all these enzymes in salt tolerant cultivars of wheat.

Salinity stress decreased marginally, the rate of photosynthesis, RUBP carboxylase activity and chlorophyll content in salt tolerant rice cultivar CSR-13 and pokkali (salt tolerant check) but MI-48 (Salt susceptible) showed greater reduction. SOD and ascrobate peroxidase activities were highly induced under salt stress in pokkali but CAT activity decreased. In CSR-13 induction of SOD, ascorbate peroxidase (APX) and CAT activities was higher than that of MJ-48, this shows that induction of SOD, APX and catalase activities under salt stress in CSR-13 contributed to its salt tolerance characteristics (Madanpal *et al*., 2004).

Ebru *et al.* (2004) studied salt stress on antioxidant responses in shoots and roots of 14 day old lentil seedlings and observed significant enhancement of SOD activity (88%) in roots along with ascorbate peroxidase and catalse activities when compared to shoot tissues and also suggested that root tissues of lentil are protected better from Nacl stress induced oxidative damage due to enhanced total SOD activity together with a higher level of ascorbate peroxidase activity.

Ganesan and Jaybalan (2005) developed salt tolerant cotton plants through direct organogenesis. In these tolerant plants, increased activities of antioxidant enzymes resulted with increasing salinity levels to the extent of 147, 290 and 362 per cent increase in catalase, peroxidase and superoxide dismutase activities, respectively in tolerant plants, when compared with the control plants.

Gossett *et al*. (1996) reported that growth was not reduced in Nacl tolerant cotton cultivar as against 96 per cent reduction in control. The Nacl tolerant line had significantly higher catalse, peroxidase, glutathione reductase, SOD and ascorbate peroxidase activities than the susceptible cultivar.

Sen Gupta *et al.* (1993) reported that transgenic tobacco plants which over expresses pea Cu/Zn SOD can increase oxidative stress protection, when compared to tobacco plants with no over expression and also with increased levels of SOD, increased expression of the H_2O_2-scavenging enzyme ascorbate peroxidase.

Fadzilla *et al.* (1997) studied the shoot cultures derived from salt sensitive *Oryzae sativa* var. Taipei-309 and observed that overall levels of Mn-SOD, Cu/Zn SOD activity and H_2O_2 activity were significantly elevated. After one day there was notable decline in tissue concentrations of glutanione and corresponding increase in GSSG (oxidized glutathione). Activities of ascorbate peroxidase and catalse were similar in presence or absence of Nacl.

In cultivars of millet (*Setaria italica* L.) under differential Nacl concentrations, the salt tolerant cultivar (cv. Prasad) exhibited increased total SOD activity, ascorbate peroxidase activity and contained a lower amount of Na+ ions and showed a lower electrolyte leakage than sensitive seedlings (Sreenivasulu *et al*., 2000).

Sumesh (2003) reported that transgenic tobacco grown at 100 mM and 200 mM Nacl was more tolerant to salt stress than wild progenitor. The transgenic tobacco had higher activity of antioxidant enzymes such as SOD, ascrobate peroxidase and catalase with lower membrane damage, higher RWC and chlorophyll content.

Gossett *et al.* (1994) stated although cotton (*Gossypium hirsutum* L.) is classified as a salt tolerant plant, variation in salt tolerance has been observed among different cultivars. He reported that the 150 mM Nacl treatment resulted in more than 40 per cent reduction in growth in salt sensitive cultivars, while less than 30 per cent reduction in tolerant ones. The salt-tolerant cultivars had higher constitutive levels of catalase (121-125%), α-tocopherol (312-420%), peroxidase (38-72%) and glutathione reductase (85-101%) activity.

Yeonghod *et al.* (2004) studied and reported that comparatively salt tolerant *Setaria viridis* showed higher growth rate, lower Na^+ accumulation, higher photosynthetic rate and induced more SOD, CAT, APX and glutathione reducatse activity and lower increase of

Malondialdehyde content as compared to the salt sensitive *Oryza sativa*, while *Echinochloa oryzicola* showed higher growth rate, lower Na^+ accumulation and no observable increase of MDA content, CAT and APX activities.

Shim *et al.* (2003) have noticed decrease in chlorophyll fluorescence ($\Delta F/F_m$) and catalase activity, following the treatment with Nacl and low temperature conditions in rice, wheat and cucumber seedlings tested and also observed that the content of salicylic acid increased in rice seedlings stressed by Nacl treatment.

Gueta-Dahan *et al.* (1997) has studied the activity of SOD and ascorbate peroxidase and their isoforms in leaf and root tissues of control and salt irrigated citrus plants. Salt treatment markedly enhanced the activity of cytosolic Cu/Zn SOD isoform in leaf but activity was reduced slightly in roots along with the Mn-SOD isoform. Salt induced a large increase in the activity of ascorbate peroxidase in leaves, while only a slight increase was observed in roots.

Seeds of four chickpea genotypes were subjected to varied levels of salinity stress during seedling establishment. Higher activities of peroxidase, catalase, amylase and protease were observed conferring tolerance in genotypes SG-11 and DHG-84-11 while lower activities of these enzymes were observed in susceptible genotypes (Pusa-256 and Phule-G-5) (Singh *et al*., 2001).

Singh *et al.* (2004) reported that the upper most leaf of sunflower had smaller amounts of chlorophyll and carotenoids and lower activities of SOD and its isoforms, ascrobate peroxidase and peroxidase than the third leaf at flower opening stage whereas they increased significantly at seed filling stage. The antioxidant enzyme activities were very low in bracts.

Bowler *et al.* (1991) reported that protection of sensitive metabolic reactions by stabilizing protein complexes or membrane structures and by increasing capacity for free radical scavenging is an important potential strategy to engineer for abiotic stress tolerance and a number of cellular antioxidant enzymes which detoxify the reactive oxygen species. Similarly, in tomato and rice salt tolerance is attributed to the increased activities of SOD, APX and CAT (Mittova *et al*., 2004 and Dionisio-Sese and Tobita, 1998).

Sudha *et al.* (2003) reported that salt drastically reduced the callus growth in mothbean cultivars. The tolerance in three cultivars of mothbean was found to be associated with enhanced level of proline content, whereas the activities of scavenger enzymes like SOD, POX and ascrobate peroxidase increased with the increase in concentration of salt on 7^{th} and 14^{th} day.

Cultured ovules of cotton cultivars that has been shown to exhibit salt tolerance showed increase in the activity of SOD, glutathione reductase and catalase than the sensitive ones. Whereas, the enzymes peroxidase activity increased in tolerant genotypes and decreased in the susceptible ones (Rajguru *et al*., 1999).

Janagoudar *et al.* (2001) reported that in cultured cells of cotton cultivars exposed to 200 mM Nacl, mean fruit weight decreases by 52 per cent in tolerant cultivar (Dhumad), 89 per cent in medium tolerant cultivar (H-14) and 91 per cent in sensitive cultivar (RAHS-2) and antioxidant enzymes, SOD and glutathione reductase activities increased with increasing salinity in the tolerant cultivar. In contrast, the mean catalase activity decreased progressively with increasing salt concentration in all cultivars, except for sensitive with 100 mM Nacl, where mean catalase activity was greater than control.

2.3 MEMBRANE INTEGRITY AND TOTAL SOLUBLE SUGAR CONTENT

Rina Basu *et al.* (1988) found that rice seedlings which were treated with 150 mM Nacl solution showed leakage of metabolites like amino acids and sugars and total electrolyte leakage were high when compared with the control and also leakage of amino acids and sugar increased with increase in the concentration of Nacl indicating the damage to membrane of cell. Similarly, Madanpal *et al.* (2004) reported membrane lipid peroxidation was

higher in susceptible genotype of rice MI-48, when compared to the salt tolerant check 'Pokkali'.

Walker *et al.* (1988) reported that stressed plants accumulated more sucrose and starch in the stem and also higher concentrations of sucrose, reducing sugars and starch was observed in the main root of pistachio plants.

In *Catharanthus roseus* G. Don, there was considerable accumulation of total soluble sugars in leaves upto 50 mM NaCl and accumulation in the stem and roots at all concentrations of salt were observed. It appears that accumulation of soluble sugars is an adaptive feature of this species to cope up with adverse saline conditions (Karadge and Gaikwad, 2003).

When salinity stress imposed on different crops, leaf sucrose level increased most in bushbean (sensitive) but less in rice (moderately tolerant), whereas, it decreased slightly in soybean (moderately tolerant) and more in cotton (tolerant), indicating sucrose and starch content may assist in the selection for salt tolerant within species (Rathert, 1984).

In salt tolerant calli raised from H-75-3S genotype of Cicer aerientinum L. there was an overall increase in soluble sugar content with increasing number of days. In case of L-144 (salt sensitive) root calli sugar content remained almost equal to the control at 30 days and 45 days, but increase was observed at 15 days (Vinod Sangwan, 1997). Accumulation of sugars may be due to their less utilization in biosynthesis leading to reduction in growth of the calli and may be used for osmotic adjustment (Yeo, 1981 and Yang *et al*., 1990).

Mansour (1994) has found in the resistant genotypes of *Triticum aestivum* and *Hardeum vulgare*, permeability of membrane did not differ significantly between control and treatment but in susceptible genotypes, there was increase in membrane permeability.

Ebru *et al.* (2004) has observed significant increase in MDA content and electrolytes in leaf tissues under 200 mM Nacl stress, whereas, in root tissues the amount of electrolyte leakage was not significant.

Hampson and Simpson (1990b) has studied the effect of different salts of Na, K and Mg on relative leakage ratio of wheat roots, generally, Mg salts produced the highest and Na salts, the lowest membrane leakage among the two salts. All salt solutions induced greater membrane leakage than distilled water and PEG treatment.

Uday Burman *et al.* (2001) found that starch content declined with increasing sodicity, which was generally associated with an increase in the levels of total soluble sugars. However, changes in starch and soluble sugars were greater in magnitude in sensitive genotypes and much lower in tolerant genotypes.

Garg *et al.* (1997) has reported that with increasing Nacl levels reduced the starch content reduced significantly with an associated increase in reducing sugars. A similar observation was made earlier in case of grape vine (Downton, 1977), cotton (Rathert, 1983), clusterbean (Lahiri *et al*., 1987).

Zidan and Elewa (1995) has reported that soluble carbohydrate (sugars) content in anise and coriander seedlings remained unaffected at 80 mM Nacl. Whereas, in case of caraway and cumin seedlings, all salinity levels resulted in higher content of soluble carbohydrates than in control. Moreover, this increase was more pronounced under the moderate levels of salinity.

Ghoulam *et al.* (2002) observed that the presence of Nacl in the rooting medium induced a significant increase in the electrolyte leakage in young leaves of all cultivars of sugar beet and also noticed leakage was greater as salt concentration increased. On the other hand, relative leakage values were high in Top cultivar at higher Nacl concentrations when compared with the NJM cultivar, suggesting that membrane permeability/integrity was more affected for the former cultivar than the latter.

Rush and Epstein (1976) reported that sugar levels tended to be lower in tomato under salt stress when compared with the unsalinized control, except fructose which is not

affected by the salt treatment. Dominant sugars found are glucose and fructose, small amounts of xylose, ribose and mannoheptulose were also present.

Neera Garg and Jasleen (2004) observed a gradual and progressive accumulation of total soluble sugars in chickpea cultivars. However, the quantum of accumulation was maximum in the tolerant cultivars than sensitive ones. Similar results have been noticed in cumin (Garg *et al*., 2002).

Isozyme profiles of antitoxidant enzymes

Salt stress like many other environmental stresses appear to elicit an oxidative stress in cotton. A strong correlation is shown between antioxidant enzyme activity and tolerance to salt stress in cotton (Gossett *et al*., 1994) and Ganesan and Jayabalan (2005).

The increase in the total enzyme activities may be related to the variation in the expression of isoforms of different antioxidant enzymes.

Kim *et al.* (2005) reported significant increase in the activities of antioxidant enzymes like SOD, CAT, POX, APX in the Nacl stressed barley roots and it was highly correlated with the increased expression of the constitutive isoforms as well as the induced ones. The specific, SOD activity catalase activity and peroxidase activity was increased and it coincided with a variable increase in the individual isoform expression. At least five or six isoforms of SOD, 2 or 3 isoforms of catalase and 10 isoforms of peroxidase were detected in root and shoots of the Nacl stressed barley plants. Gueta-Dhana *et al.* (1997) showed that in citrus leaves five isoforms of SOD were resolved under salt induced oxidative stress.

At high Nacl treatment (400 mM) in *Bruguiera parviflora,* activity of SOD and POX were increased and activity of SOD showed 5 bands and peroxidase showed six bands. Among six bands of peroxidase major being the POX-6 (~73%), but at high salt concentration loss in catalase activity was observed with four isoforms. However, the extent of decrease among the catalase isoforms was not the same for all the isoforms. This differential increase in some of the isomers of enzymes indicated specific up regulation of antioxidative defense system in *Bruguiera parviflora* (Parida *et al*., 2003). Similarly, Hernandez *et al.* (1993) noticed that Nacl tolerant plants of pea increased the induction of the mitochondrial Mn-SOD isoform activity compared to the Nacl sensitive plants. It implies that mitochondrial Mn-SOD function in the molecular mechanisms of plant tolerance to Nacl.

Ebru *et al.* (2004) found that salt treatment differentially affected the SOD isozyme activities, the isozymes were identified as Cu/Zn –SOD (I and II), Fe-SOD and Mn-SOD. At higher stress a significant decrease was observed in Fe-SOD activity. Whereas, Cu/Zn-SOD activity was enhanced in leaf and in root tissue of lentil and the activity change in Cu/Zn-SOD isozymes was considerable. The catalase, peroxidase and SOD activity is inferred to be more in tolerant genotypes of rice. The tolerant genotypes (TRY-I, TR-20, ADT-39) produced more isoforms when compared to susceptible genotypes (white ponni and ADT-38) and intensity of band also varied with the degree of tolerance (Djanaguiraman *et al*., 2003).

III. MATERIAL AND METHODS

The laboratory experiment was conducted at the Department of Crop Physiology and Seed Research Laboratory (NSP), College of Agriculture, University of Agricultural Sciences, Dharwad. The materials used and the methods followed during the study are presented here.

3.1 MATERIALS

3.1.1 Genotypes

The plant material used in the study comprised of four genotypes *viz.,* RAHS-14, Jayadhar, Dhumad and Kumta and were obtained from the Agricultural Research Station, Dharwad, University of Agricultural Sciences, Dharwad.

3.1.2 Chemicals and supplies

All the chemicals used for the study were of analytical grade supplied by Himedia and SD Fine chemicals Limited, Mumbai.

3.1.3 Equipment

a. Pestle and mortar

b. Centrifuge : Table top micro-centrifuge model Spin-Win Supplied by Remi Motors Pvt. Ltd., Mumbai.

c. Electrophoresis unit

   i. Electrophoresis unit : Model GTV 100 of Ultra Violet Products (UVP) London, UK

   ii. Power pack : PSU 400/600 of UVP, London, UK

   iii. Transilluminator : UV/visible transilluminator of UVP, London, UK

d. Spectrophotometer : Model SL 159, UV-VIS spectrophotometer supplied by ELICO, India

e. pH meter : Digital pH meter model DI-707, supplied of Pigisun Electronics, Hyderabad, India.

3.2 LABORATORY STUDIES

3.2.1 Germination percentage

The germination test was conducted as per the ISTA procedure using rolled paper method. The rolled paper towels were placed at slanting position in a cabinet seed germinator at constant temperature of 25 $\pm$ 1^0C and 95 $\pm$ 1 per cent relative humidity. The number of normal seedlings were counted at the 1^{st} count on 4^{th} day and end of 10^{th} day as final count of germination. Seeds showing minimum of 2 mm radical length were considered as germinated and expressed as percentage.

3.2.2 Root length (cm)

Five normal seedlings were selected at random from each of the replication of the germination test on 10^{th} day (final count day) and used for measuring the root length. The root length was measured from the tip of primary root to base of hypocotyl and mean root length was expressed in centimeter.

3.2.3 Shoot length (cm)

The ten seedlings used for measuring root length were also used for measuring shoot length. The shoot length was measured from the base of the primary leaf to the base of hypocotyl and mean shoot length was expressed in centimeters.

3.2.4 Root:shoot ratio

The ratio of root length to the shoot length of each seedling selected above was calculated and the average for five seedlings was expressed as the root:shoot ratio.

3.2.5 Seedling vigour index (SVI)

Seedling vigour index was calculated by following the formula as suggested by Abdul-Baki and Anderson (1973).

SVI = (Root length + Shoot length) × Germination percentage

3.2.6 Seedling dry weight (mg/seedling)

Five normal seedlings used for measuring root and shoot length were kept in butter paper bag and dried in hot air oven maintained at $70^0 \pm 1^0$C temperature for 24 hours. Then the seedlings were weighed after allowing them to cool at ambient temperature in a dessicator and expressed in milligrams per seedling.

3.3 POT CULTURE STUDY

3.3.1 Experimental site

The pot culture experiment was conducted during 2004-05 at the greenhouse of the Department of Crop Physiology, College of Agriculture, Dharwad.

3.3.2 Experimental design

The experiment was laid out in completely randomized block design with four replications.

Methodology

The growth medium consisted of sieved river sand, washed with the running tap water to remove the clay and silt and soaked in acid solution (HCl 17% w/v) for 24 hours and then thoroughly washed with the distilled water. The sand was dried and placed in plastic cups (300 ml capacity), which were subsequently steam sterilized.

A modified Arnon and Hoagland nutrient solution (Subbarao *et al*., 1991) with 3.6 mM NH_4NO_3 was used as the growth medium. It was amended with Nacl for creation of different levels of salinity. The composition of nutrient solution was : 0.46 mM K_2HPO_4, 1.04 mM KCl, 0.50 mM $MgSO_4$, 0.74 mM $CaCl_2$, 0.003 mM $MnSO_4$, 0.00046 mM $ZnSO_4$, 0.0005 mM $CuSO_4$, 0.002 mM H_3BO_3, 0.001 mM $Na_2M_0O_4$ and 0.08 mM NaFe EDTA. The control treatment, in which no salt was added to the nutrient solution had an electrical conductivity (EC) of 0.52 dS m^{-1} and also the corresponding EC values for 50, 100, 150 and 200 mM are 6.62, 12.3, 18.5 and 23.0 dS m^{-1} respectively.

The amount of half strength hoagland's solution required to maintain field capacity was measured gravimetrically. The pots containing sand were weighted at two days intervals during the experimental period and based on the loss of weight of the pots, nutrient solution with different salinity levels was added to pots, to maintain required moisture level and salinity levels in the respective pots. After imposing the salinity treatments in the pots 3 or 4 seeds of each genotypes were sown in each pot. The pots were irrigated with nutrient solutions once in two days based on the evapotranspiration from the pots. After germination, one or two seedlings were allowed to grow in pots and others were thinned-off. The salt concentration in each cup is maintained as per the treatments by adding the salt at regular intervals based on EC readings.

3.4 BIOCHEMICAL PARAMETERS

3.4.1 Estimation of antioxidant enzymes

Extraction buffer

Solutions of sodium dihydrogen orthophosphate (NaH_2PO_4) 0.1 M and di-sodium hydrogen orthophosphate (Na_2HPO_4) were prepared by dissolving 13.6 and 17.4 g separately

in distilled water and volumes made upto 1 litre in each case. Solutions of NaH_2PO_4 and Na_2HPO_4 were mixed in the ratio of 16:84 and the pH was adjusted to 7.5 with pH meter. To 1000 ml of the solution 0.186 g of ethylene diamine tetra acetic acid (EDTA) 0.5 mM was added and used for enzyme extraction.

Twenty one days old seedlings were collected and washed with distilled water and surface moisture was wiped out. Samples (1 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 mM EDTA in pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4^0C in refrigerated centrifuge for 15 minutes at 15000 rpm. The supernatant was transferred to 30 ml tubes and referred as enzyme extract.

Superoxide dismulatse activity

Speroxide dismutase activity was estimated according to the method of Dhindsa *et al.* (1981), 3 ml of the mixture containing 0.1 ml of 1.5 M sodium carbonate, 0.2 ml of 200 mM methionine, 0.1 ml of 2.25 mM NBT, 0.1 ml of 3 mM EDTA, 1.5 ml of 100 mM phosphate buffer, 1 ml of distilled water and 0.05 ml of enzyme was taken in test tubes in duplicate from each enzyme sample. Two tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 ml of riboflavin (60 µm) and placing the tubes below a light source of two 15 W fluorescent lamps for 15 minutes. Reaction was stopped by switching-off the light and covering the tubes with black cloth. Tubes without enzyme developed maximal colour. A non-irradiated complete reaction mixture, which did not develop colour, served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme, which reduced the absorbance reading samples to 50 per cent in comparison with tubes lacking enzymes.

Catalase activity

Catalase activity was determined according to the method of Aebi (1984). H_2O_2-PO_4 buffer was prepared by diluting 0.16 ml of H_2O_2 (10% w/v) to 100 ml of phosphate buffer (pH 7.3). Three ml of H_2O_2-PO_4 buffer and 0.01 – 0.04 ml of sample was added and mixed well. The time required for a definite decrease in absorbance at 240 nm in a spectrophotometer was noted. The enzyme activity was expressed as units per gram fresh weight per minute.

Peroxidase activity

Peroxidase activity was estimated following the method of Mahadevan and Sridhar (1986). Three ml of phosphate buffer (pH 7.0), 0.05 ml guaiacol solution, 0.1 ml enzyme extract and 0.03 ml hydrogen peroxide solution were pipetted into a cuvette. The absorbance was adjusted to zero at 460 nm in a UV-VIS spectrophotometer. The change in absorbance was noted at an interval of 20 seconds after adding 0.5 ml of 2 per cent H_2O_2 and inverting the cuvette. The enzyme activity was expressed as units per g fresh weight per minute.

3.4.2 Estimation of sugars by anthrone method

Sugar content was estimated in oven dried samples of 21 days old seedlings by anthrone method (Dobois *et al*., 1951 and Yoshida, 1972).

Dry sample extraction

Hundred mg of dried sample powder was taken in a conical flask and 10 ml of 80 per cent ethanol was added. Contents were boiled on hot water bath for 10 minutes, allowed to settle down and the supernatant was transferred to another dry flask. To the residue in the flask, 10 ml of 80 per cent ethanol was added and extracted as before. Extraction was repeated again and the final volume was made up to 25 ml with 80 per cent alcohol.

From this, 5 ml of the extract was taken in a beaker and evaporated on hot water bath (until alcohol smell lost) and made up the volume of the extract to 10 ml with distilled water. This was used for estimating sugar content as follows.

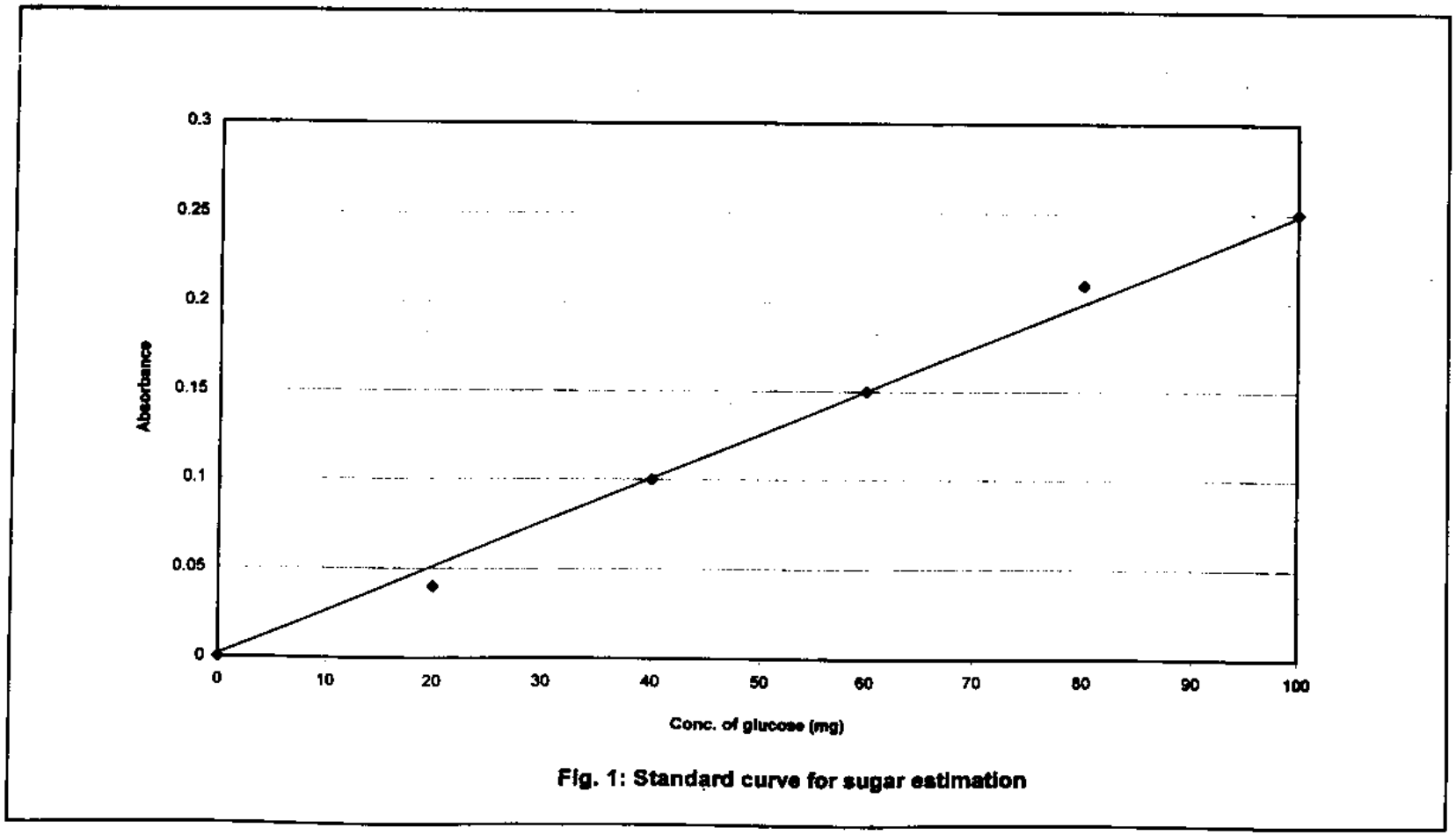

Fig. 1: Standard curve for sugar estimation

Fig 1: Standard curve for sugar estimation

Anthrone method

0.2 g of anthrone was dissolved in 100 ml of concentrated sulphuric acid. Fresh solution was prepared just before use.

Procedure

One ml of the aliquot was taken in a test tube. The volume was made upto 2.5 ml with distilled water. All the test tubers were kept in the ice bath and to which 5 ml of anthrone reagent was added slowly. Contents were stirred gently with a glass rod and heated on boiling water bath exactly for 7.5 minutes and cooled immediately on ice bath. After cooling, the absorbance of solutions were measured at 630 nm against the blank in a spctrophotometer (ELICO-SL-159) and the sugar content was calculated through the standard curve (Fig. 1).

Standard curve

Hundred mg of glucose was dissolved in little quantity of water and the volume was made up to 100 ml to get a stock solution. From this, different concentrations were made from 10 to 100 mg per ml by diluting and used for standard curve. The other procedure followed was similar to that used for plant samples.

3.4.3 Membrane integrity

Membrane integrity was measured based on solute leakage and relative leakage ratio from the cell. This parameter gives the information on the relative ion content in the apoplastic space. Electrolyte leakage was assessed as descried by Lutts *et al.* (1996b).

Solute leakage

Young leaf discs were collected from each treatment and washed three times with deionized water to remove surface adhered electrolytes. Leaf discs were placed in closed vials containing 10 ml of deionized water and incubated at 25^0C on a rotary shaker for 24 hours. After incubation, a 3 ml aliquot of bathing solution was removed from the vials and the absorbance was determined spectrophotometrically at 280 nm (A_{280}).

Relative leakage ratio (membrane permeability)

Similar procedure used for solute leakage is followed. The aliquot taken for spectrophotometric observation was then added back to its original solution and vials were frozen at -20^0C for 12 h to break squeeze the cells. The final absorbance (A'_{280}) was measured after thawing and relative leakage ratio (RLR) of the UVAS was defined as follows.

$$RLR\ (\%) = (A_{280}/A'_{280}) \times 100$$

3.4.4 Screening of genotypes for electrophoretic profiles of isozymes

In the present study, four cotton genotypes were considered for the analysis of isozymes, superoxide dismutase (SOD EC : 1.15.1.7), catalase (CAT EC : 1.11.1.6) and peroxidase (POD EC : 1.11.1.7) by following the poly acrylamide gel electrophoresis (PAGE) technique (Davis, 1964).

The following stock solutions were prepared by using different required chemicals.

1. Monomer solution

Acrylamide	- 30.00 g
Bisacrylamide	- 0.80 g
H_2O	- to 100 ml

2. Polymerisation catalysts

a. Ammonium per sulphate (APS : 0.14%). This solution was prepared freshly on the day of use.

b. TEMED : Used as supplied, stored at 4^0C in an amber coloured bottle.

3. Resolving gel buffer (3 M Tris-HCl, pH 8.8)

Tris - 36.6 g

Adjusted to pH 8.8 with 1 N HCl - 48 ml (approximate)

H_2O - to 100 ml

4. Tank buffer (0.05 M Tris, 0.0384 M glycine, pH 8.3)

Tris - 0.60 g

Glycine - 2.88 g

H_2O - to 1000 ml

5. Extraction buffer

Tris-buffer (pH 7.4)

[0.1 M Tris-HCl, pH 7.4)

Tris - 1.21 g

Sucrose - 17.00 g

Cysteine - 0.10 g

Ascrobic acid - 0.10 g

Adjusted to pH 7.4 with 1 N HCl

6. Staining solutions

a. Superoxide dismutase (SOD)

50 mM tris-HCl, pH 8.0 - 50 ml

Riboflavin - 2 mg

EDTA - 1 mg

NBT - 10 mg

Ingredients were poured over gel and incubated for 30 min later, gel was illuminated on light box. Zones of SOD activity were revealed as achromatic regions on a dark blue background. Gels were rinsed with water and fixed in 2 per cent acetic acid.

b. Catalase

H_2O_2 (0.01%) - 50 ml

H_2O - 50 ml

Ferric chloride - 500 mg

Potassium ferricyanide - 500 mg

H_2O_2 was poured on gel slice and left for 5 min, meanwhile, the remaining above mentioned ingredients were mixed separately. Hydrogen peroxide poured off and the mixture stain solution was added. The gel was swirled gently, until bands were developed. Catalase activity was revealed as achromatic bands on green background. Gel was rinsed with water and fixed in 2 per cent acetic acid till it is immersed.

c. Peroxidase

Na-Acetate buffer (0.2 M pH 5.6) - 100 ml

Benzidine	- 100 mg
H_2O_2	- 0.10 ml

Benzidine and buffer mixture were boiled and solution was cooled down to room temperature. The solution was filtered and H_2O_2 was added to it just before incubation. Gel was incubated till brown bands appeared (in dark) and rinsed in 7 per cent acetic acid. Gel was photographed and stored in 2 per cent acetic acid.

Methods

Extraction of enzymes

Twenty days old seedling leaves from pot sown plants were used for extraction of enzymes. The enzymes were extracted from 0.5 g of leaf material macerating with one ml of extraction buffer (0.1 M Tris-HCl, pH 7.4) in a pre-chilled pestle and mortar in an ice bucket. The paste was transferred to a pre-chilled centrifuge tube. The contents were centrifuged at 3000 rpm for 20 minutes. The supernatant was used for electrophoresis.

Electrophoresis

The recipe for gel preparation using non-dissociating discontinuous buffer system is tabulated below to yield 7.7 per cent acrylamide concentration in resolving gel.

Recipe for gel preparation

Stock solution	Resolving gel (7.5%)
Manomer solution	5.0 ml
Resolving gel buffer	4.0 ml
Distilled water	11.0 ml
Ammonium per sulphate (APS)	20 mg
TEMED	0.050 ml
Total volume	20 ml

Preparation of the resolving gel

1. The glass plates of size 18 cm $\times$ 16 cm, thoroughly cleaned were sandwiched together with 3 mm spacers in between. The glass sandwich was arranged in a gel casting unit.
2. In a 50 ml beaker 20 ml resolving gel solution was mixed according to recipe leaving ammonium per sulphate and TEMED, which were added just before use.
3. The resolving gel mixture was poured into the space between the glass plates and the comb was inserted avoiding air bubbles.

Sample loading and gel running

1. The comb from the gel was removed slowly and carefully.
2. Wells were washed with water.
3. The water was removed from the wells using filter paper strips, without disturbing the wells.
4. The sandwiches were then set in the electrophoresis gel running unit.
5. Both lower and upper tanks were filled with tank buffer (pH 8.3) slowly avoiding bubbles.
6. 50-70 µl of sample along with 5 µl of Bromophenol blue (tracking dye) was mixed well and loaded into the well.

7. After loading of samples was complete, voltage clamps were attached and the gel apparatus was connected to power pack set at 70 volts.

8. The gel was run till the tracking dye was ½-1 cm away from bottom of the gel.

9. Once the run was over, gel sandwich was detached and placed in distilled water to avoid exposure to air. Right hand side was marked by cutting a piece of gel at the end.

10. Later, the gel was transferred to suitable staining solution.

11. After developing bands, gels were fixed in acetic acid and photographed.

Mobility determination

Measured the distance traveled by each protein/enzyme and calculated the mobility relative to the tracking dye (bromophenol blue).

$$\text{Mobility} = \frac{\text{Distance moved by protein (cm)}}{\text{Distance moved by tracking dye (cm)}}$$

STATISTICAL ANALYSIS

Data from the experiment was subjected to completely randomized design (factorial) using Drysoft Statistical Analysis package.

The values of per cent germination and relative leakage ratio were arcsine transformed before subjecting to statistical analysis.

IV. EXPERIMENTAL RESULTS

The experiments were conducted during the year 2004-05 to investigate the influence of different levels of salinity on four cotton genotypes *viz.,* Rahs-14, Dhumad, Jayadhar and Kumta. The genotypes were evaluated for germination, seedling growth parameters and changes in physiological, biochemical and molecular characters under in vitro conditions. The data recorded on these parameters were statistically analysed and experimental results obtained are presented in this chapter.

4.1 GERMINATION PERCENTAGE AND SEEDLING GROWTH PARAMETERS

4.1.1 Germination percentage

The data on seed germination of cotton genotypes as influenced by different salinity levels are recorded and presented in Table 1.

Germination percentage significantly differed among genotypes, salinity levels besides interaction between genotypes and salinity levels.

On an average, over all salinity levels the genotype Rahs-14 had significantly higher germination percentage (85.40) and was followed by Dhumad (82.90). Significantly lowest germination percentage was recorded in Jayadhar (42.20).

Significant reduction in the germination percentage was observed as the salinity levels increased from S1 (control) to S5 (200 mM NaCl). The germination percentage was maximum in S1 (98.30) followed by S2 (90.20), whereas, higher salinity level S5 showed significantly lower germination percentage (15.90). However, at higher salinity levels (S4 and S5) significant variation in germination percentage was observed among the genotypes. The genotype Rahs-14 maintained 72.10 and 34.00 per cent as compared to 16.9 and 2.10 per cent in Jayadhar at S4 and S5 salinity levels, respectively.

The interaction effect of genotypes and salinity levels indicated that Dhumad recorded higher germination percentage under salinity level S1 (100.00) and S2 (100.00) and was on par with Rahs-14 at the same level of salinity S1 (100.00) and S2 (99.53). Whereas, the genotype Jayadhar at S5 (200 mM NaCl) salinity level recorded the lowest germination percentage (2.10) and was on par with the genotype Kumta (4.10).

4.1.2 Root length (cm)

Root length was significantly influenced by genotypes, salinity levels and interaction of genotypes × salinity and is presented in Table 2.

Irrespective of the salinity levels, the root length was maximum in Rahs-14 (6.28) followed by Dhumad (5.82) and these differed significantly with other two genotypes. Lowest root length was observed in the genotype Jayadhar (5.40) and was on par with Kumta (5.43).

Salinity had a negative impact on root length of the seedlings. All the salinity levels differed significantly with each other. It decreased from 7.56 cm at S1 to 4.16 cm at S5.

Interaction was significant, the genotype Rahs-14 showed highest root length at S1 salinity level (8.27) followed by Dhumad (7.90) at the same level. Irrespective of the genotypes root length decreased with increase in the salinity level. Lowest root length was found in Kumta (3.95) at S5 and was on par with the genotype Jayadhar (4.13) at the same level of S5.

The per cent decrease in root length was 49.21 per cent in Rahs-14 from S1 to S5 whereas, it was 44.52 per cent in Kumta.

Table 1: Effect of salinity on germination percentage in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	100.00 (90.00)*	99.53 (86.07)	86.10 (68.09)	72.10 (58.08)	34.00 (35.64)	85.40 (67.58)
Dhumad	100.00 (90.00)	100.00 (90.00)	78.10 (62.10)	54.00 (47.29)	38.50 (38.33)	82.90 (65.54)
Jayadhar	90.40 (71.96)	71.00 (57.42)	42.00 (40.39)	16.90 (24.30)	2.10 (8.38)	42.20 (40.49)
Kumta	95.40 (77.66)	64.60 (53.46)	45.00 (42.13)	22.00 (27.94)	4.10 (11.66)	45.80 (42.57)
Mean	98.30 (82.40)	90.20 (71.73)	64.10 (53.18)	40.30 (39.40)	15.90 (23.50)	65.50 (54.04)
For comparing means	SE m±			CD at 5%		
Variety (V)	0.582			1.642		
Salinity (S)	0.651			1.836		
Interaction (V x S)	1.302			3.671		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl * - Values in parentheses indicate arc sign transformed values

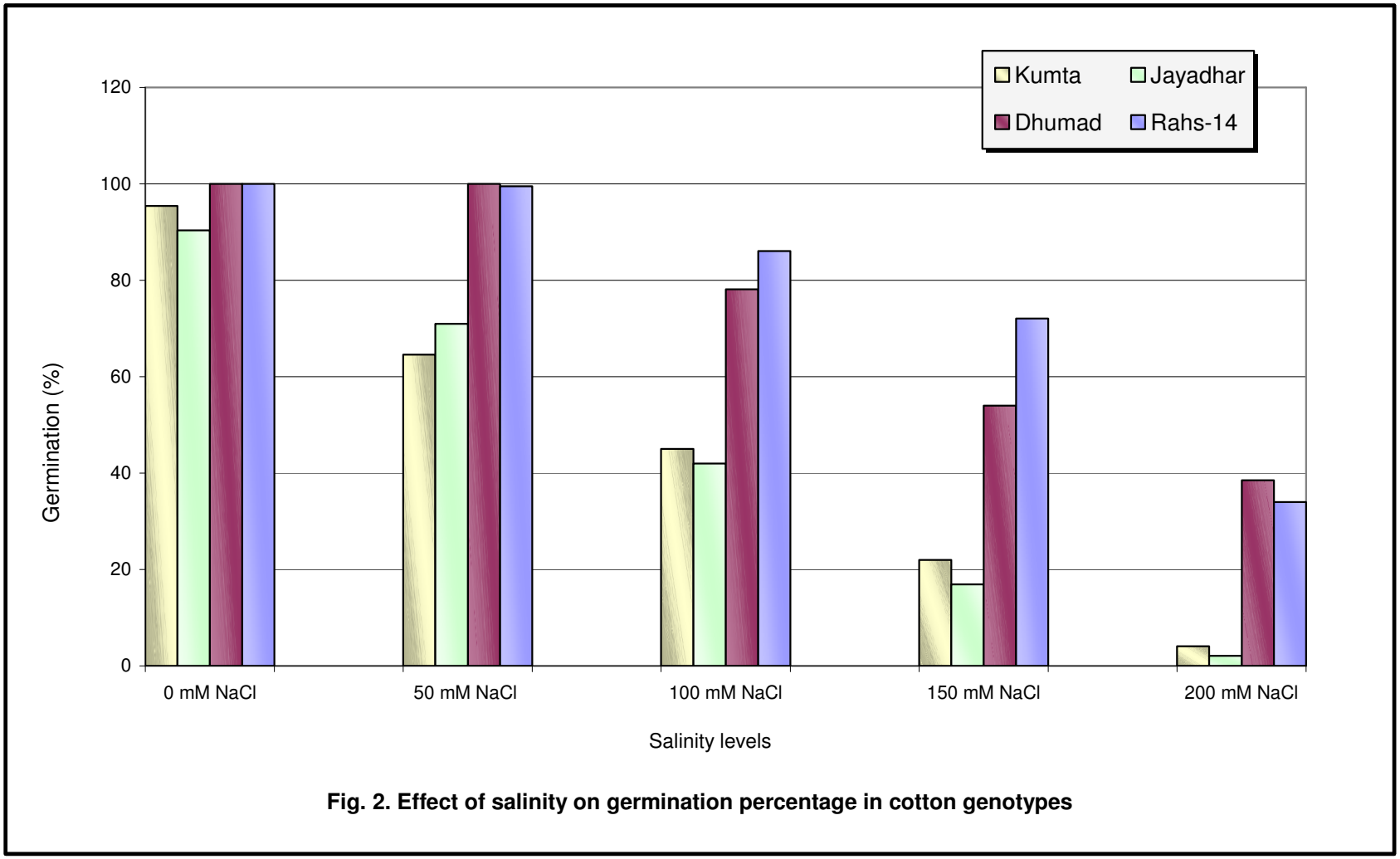

Fig. 2. Effect of salinity on germination percentage in cotton genotypes

Fig. 2. Effect of salinity on germination percentage in cotton genotypes

Table 2: Effect of salinity on root length (cm) in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	8.27	6.52	6.12	5.07	4.20	6.28
Dhumad	7.90	6.80	5.52	4.72	4.17	5.82
Jayadhar	6.97	6.22	5.12	4.75	4.12	5.40
Kumta	7.12	6.60	4.97	4.50	3.95	5.43
Mean	7.56	6.78	5.43	4.71	4.16	5.73
For comparing means	SE m±			CD at 5%		
Variety (V)	0.05			0.14		
Salinity (S)	0.05			0.15		
Interaction (V x S)	0.11			0.30		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

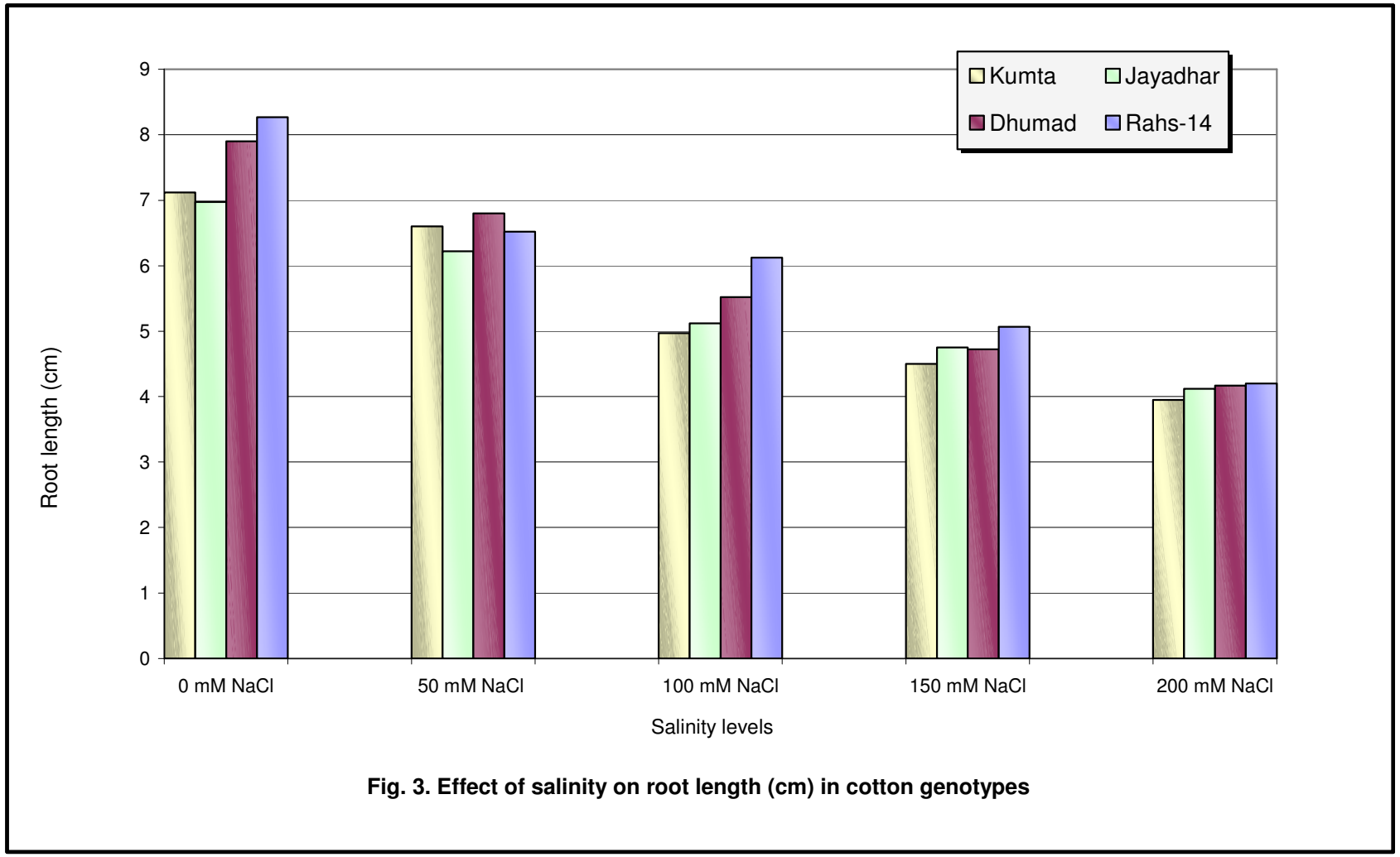

Fig. 3. Effect of salinity on root length (cm) in cotton genotypes

Fig. 3. Effect of salinity on root length (cm) in cotton genotypes

Table 3: Effect of salinity on shoot length (cm) in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	12.92	11.95	9.27	7.52	6.37	9.61
Dhumad	12.50	11.12	8.75	7.30	6.17	9.17
Jayadhar	11.22	9.50	7.30	6.37	5.50	8.07
Kumta	11.05	10.32	7.27	6.15	5.07	7.97
Mean	11.92	10.83	8.15	6.83	5.78	8.70
For comparing means	SE m±			CD at 5%		
Variety (V)	0.07			0.20		
Salinity (S)	0.08			0.22		
Interaction (V x S)	0.16			0.45		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

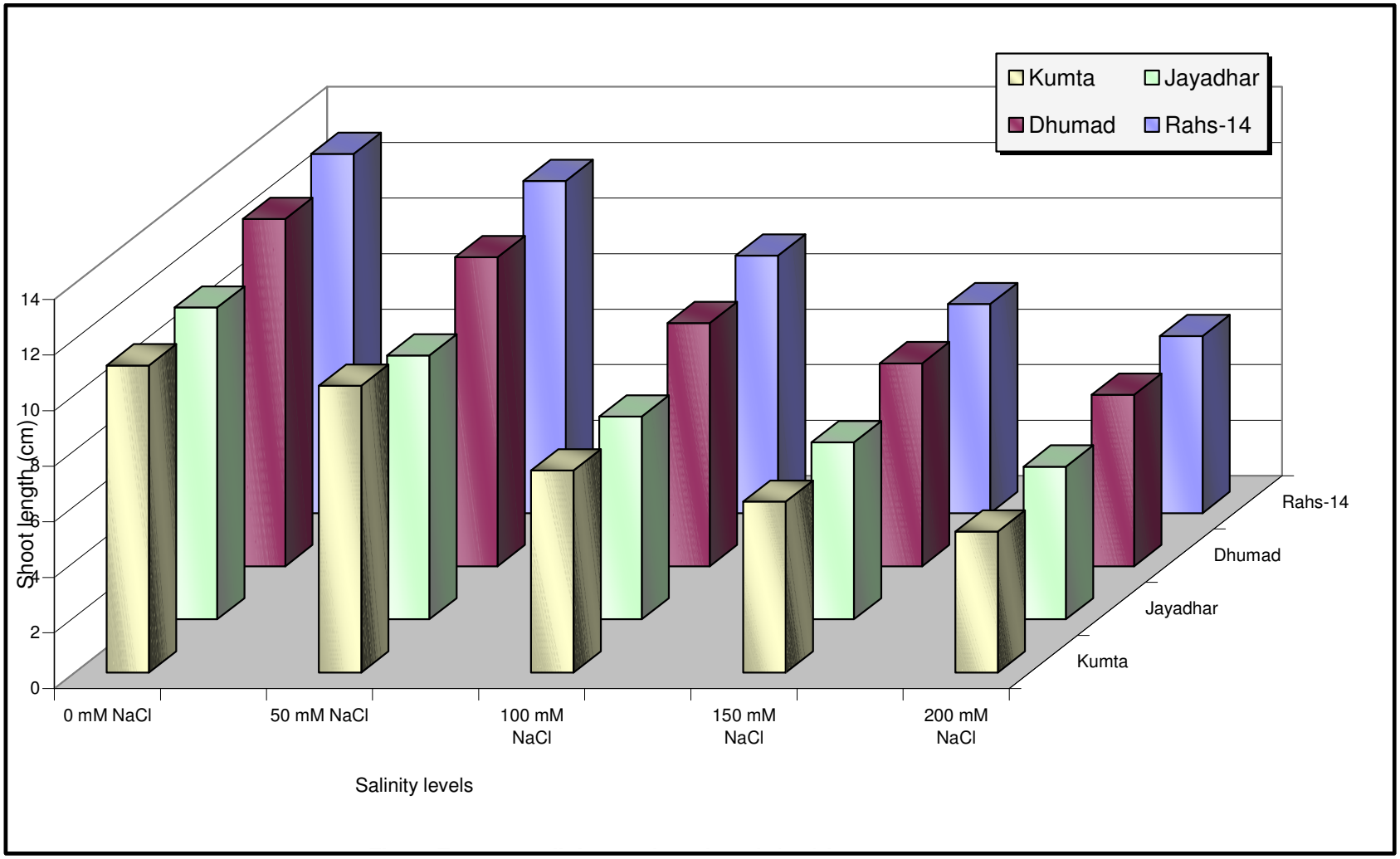

Fig. 4. Effect of salinity on shoot length (cm) in cotton genotypes

4.1.3 Shoot length (cm)

Significant differences for shoot length were observed among cotton genotypes, salinity levels and interactions and data are presented in Table 3.

On an average over salinity levels, Shoot length was significantly influenced by genotypes. The genotype Rahs-14 had the highest shoot length (9.61) followed by Dhumad (9.17). Kumta had the lowest shoot length (7.98) and was on par with Jayadhar (8.07).

With increase in salinity from S1 to S5, shoot length decreased drastically from 11.92 cm to 5.78 cm.

Interaction of genotypes and salinity levels was found to be significant. Irrespective of genotype, with increase in the salinity level, there was significant decrease in the shoot length. The genotype Rahs-14 recorded significantly higher shoot length under S1 (12.92) level and was on par with the genotype Dhumad (12.50) at the same level. Least was found in Kumta (5.07) and it was on par with the Jayadhar (5.50) at S5 level of salinity. The per cent reduction in shoot length was 50.70 per cent from S1 to S5 in Rahs-14, whereas it was 54.12 per cent in Kumta at the same level of salinity.

4.1.4 Root:shoot ratio

Root:shoot ratio varied significantly among genotypes, salinity levels and interaction between genotypes and salinity. The results are presented in Table 4.

The genotype Kumta had significantly higher root:shoot ratio (0.697) and was on par with genotype Jayadhar (0.683). Dhumad recorded lower root:shoot ratio of 0.64 and was on par with Rahs-14 (0.659).

Among the salinity levels, S2 had lower root:shoot ratio (0.624) and was on par with S1 (0.636). Significantly highest root:shoot ratio was observed at S5 (0.725). However, at moderate salinity levels S3 (0.670) and S4 (0.694), the root:shoot ratio was increased over the control (0.636).

Interaction between the genotypes and salinity levels were found to be significant with Kumta having the highest root:shoot ratio at S5 (0.782) and was on par with Jayadhar (0.749) at the same level. Lower root:shoot ratio was observed in Dhumad at S2 (0.610) and it was on par with Rahs-14 (0.628), Jayadhar (0.622) and Kumta (0.638) at S2 level and also similar trend was noticed at S1 salinity level. In general, the root:shoot ratio decreased slightly from S1 to S2, thereafter, it gradually increased upto S5 level in of all the genotypes but in Jayadhar and Kumta relatively more root:shoot ratio was observed.

4.1.5 Seedling vigour index (SVI)

Seedling vigour index was significantly influenced by genotypes, salinity levels and interaction of genotypes $\times$ salinity (Table 5).

The seedling vigour index was maximum in genotype Rahs-14 (1272.4) followed by Dhumad (1252.2) and both were on par with each other. Whereas, least seedling vigour index was observed in Jayadhar (703.3) followed by Kumta (729.3) and both were on par with each other.

Different salinity levels had significant influence on the seedling vigour index. Among all the salinity levels, significantly highest seedling vigour index was observed at S1 (1881.3) and the least was found at S5 (208.4). Irrespective of genotype, the seedling vigour index decreased with increase in salinity level.

The interaction effect of genotypes and salinity levels indicated that Rahs-14 recorded the highest seedling vigour index under salinity level S1 (2120.0). This was followed by Dhumad (2040.0) at S1 only. The genotype Jayadhar at S5 salinity level recorded the lowest seedling vigour index (22.0) and was on par with Kumta (43.5) at the same level of salinity.

Table 4: Effect of salinity on root to shoot ratio in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	0.639	0.628	0.657	0.677	0.695	0.659
Dhumad	0.634	0.610	0.633	0.649	0.676	0.640
Jayadhar	0.624	0.622	0.703	0.717	0.749	0.683
Kumta	0.648	0.638	0.685	0.734	0.782	0.697
Mean	0.636	0.624	0.670	0.694	0.725	0.670
For comparing means	SE m±			CD at 5%		
Variety (V)	0.007			0.020		
Salinity (S)	0.008			0.023		
Interaction (V x S)	0.015			0.05		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

Table 5: Effect of salinity on seedling vigour index in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	2040.0	1774.5	1228.3	865.8	352.5	1252.2
Dhumad	2120.0	1947.5	1198.3	680.5	415.5	1272.4
Jayadhar	1637.5	1148.8	521.5	186.5	22.0	703.3
Kumta	1727.8	1092.0	550.3	232.8	43.5	729.3
Mean	1881.3	1490.7	874.6	491.4	208.4	989.3
For comparing means	SE m±			CD at 5%		
Variety (V)	9.23			26.02		
Salinity (S)	10.31			29.09		
Interaction (V x S)	20.63			58.17		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

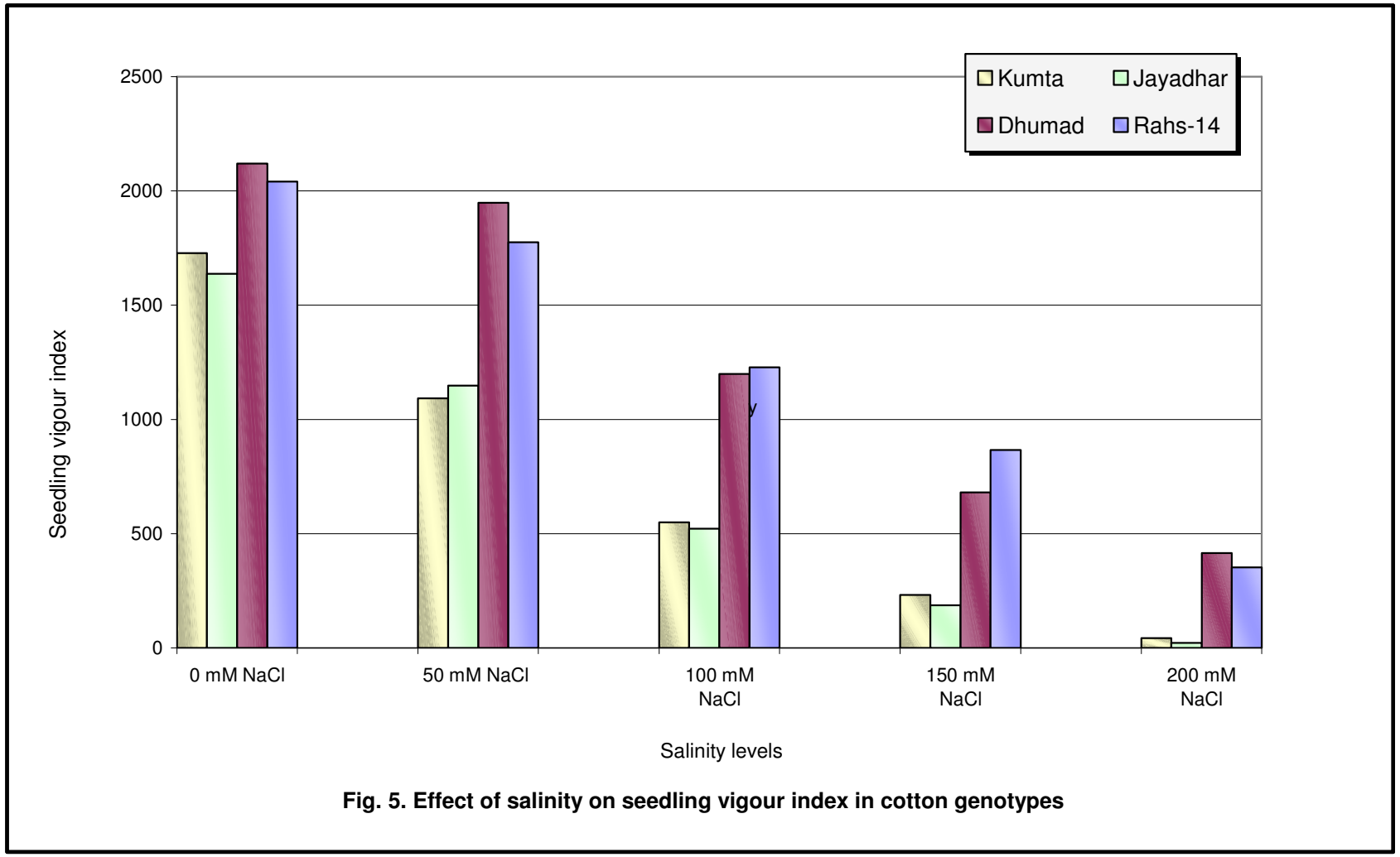

Fig. 5. Effect of salinity on seedling vigour index in cotton genotypes

Fig. 5. Effect of salinity on seedling vigour index in cotton genotypes

Table 6: Effect of salinity on seedling dry weight (mg/seedling) in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	42.70	41.00	35.25	29.00	23.00	34.20
Dhumad	41.00	39.25	34.00	30.00	25.00	33.85
Jayadhar	43.50	38.00	32.25	24.25	19.00	31.40
Kumta	42.00	38.00	33.00	27.50	20.25	32.15
Mean	42.31	39.06	33.62	27.68	21.81	32.90
For comparing means	SE m±			CD at 5%		
Variety (V)	0.317			0.893		
Salinity (S)	0.354			0.999		
Interaction (V x S)	0.708			1.998		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

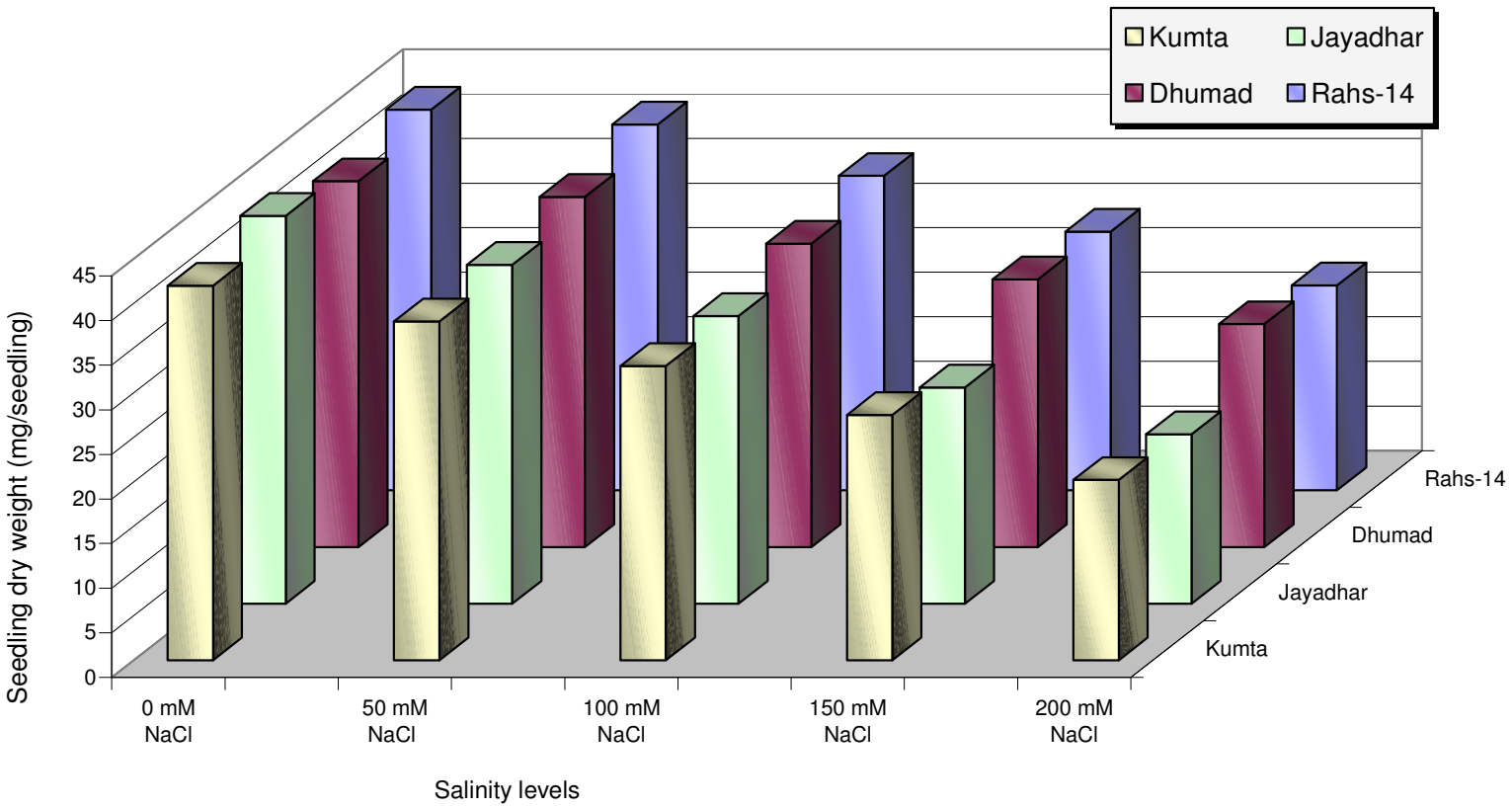

Fig. 6. Effect of salinity on seedling dry weight (mg/seedling) in cotton genotypes

Fig. 6. Effect of salinity on seedling dry weight (mg/seedling) in cotton genotypes

The per cent reduction was 82.74 per cent in Rahs-14 as compared to Dhumad (80.42), Jayadhar (98.66%) and Kumta (97.48%) from S1 to S5 salinity level.

4.1.6 Seedling dry weight (mg/seedling)

The data on seedling dry weight of four cotton genotypes in response to salinity levels are presented in Table 6. The results indicated significant difference among cotton genotype, salinity levels and interaction between genotype and salinity levels.

Irrespective of the salinity levels, on an average, the genotype Rahs-14 had significantly higher seedling dry weight (34.20) and was on par with Dhumad (33.85) as compared to Jayadhar and Kumta. Whereas, Jayadhar recorded lowest seedling dry weight (31.40) followed by Kumta (32.15) and both were on par with each other.

Increase in salinity levels had a adverse effect on the seedlings dry weight. Significantly higher seedling dry weight was observed at S1 (42.31) over all other salinity levels. Lowest seedling dry weight was recorded at S5 (21.81) level.

Interaction effect was significant for seedling dry weight. Accumulation of dry matter in seedlings was significantly more in Jayadhar (43.50) at S1 (control) and was on par with Rahs-14 (42.75). Lower accumulation of dry matter was observed in Jayadhar at S5 (19.00) followed by Kumta (20.25) and both were on par with each other.

4.2 ANTIOXIDANT ENZYME ACTIVITY

Data on effect of salinity on antioxidant enzyme superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) are presented in Table 7, 8 and 9, respectively.

4.2.1 Superoxide dismutase activity (units/g fresh weight)

Genotypes, salinity and interactions were found to be significant for superoxide dismutase activity. The genotype Dhumad had significantly higher superoxide dismutase activity (728.0) over all other genotypes irrespective of all salinity levels followed by genotype. Rahs-14 (616.6). Jayadhar noticed the least enzyme activity (212.2).

Among the genotypes, the only genotype Dhumad had shown the gradual increase in SOD activity from S1 to S5 salinity levels *i.e.,* 530.0, 620.0, 740.0, 859.0 and 891.0 units per g fresh weight, whereas in all other genotypes there was increase upto S3 salinity and later on there was decrease and the extent of decrease varied among the genotypes. The per cent decrease was highest in Kumta at S4 (34.01%) and S5 (50.25%) salinity levels as compared to S3.

The activity of superoxide dismutase did not follow any regular pattern with the salinity, though it differed significantly. The activity of enzyme increased from S1 (383.1) to S3 (574.8) level, thereafter it decreased slightly at S4 (520.6) and S5 (506.1) level. S3 level of salinity had the highest enzyme activity (574.8) and significantly lower enzyme activity (383.1) was observed at S1 (control) salinity level.

Interaction effect indicated that the genotype Dhumad showed significantly more enzyme activity at S5 (891.0) followed by S4 (859.0) in the same genotype. The genotype, Jayadhar showed the least superoxide dismutase activity (159.3) at S5 salinity. The genotype Dhumad showed increasing trend of enzyme activity with increase in salinity level from S1 (control) to S5 (200 mM NaCl). Whereas, in other genotypes, there was a decrease from S3 to S5 level.

4.2.2 Catalase activity (units/g fresh weight)

The catalase activity differed significantly among genotype, salinity levels and interactions of salinity and genotypes.

The mean catalase activity was observed to be higher in Dhumad (383.7) followed by Rahs-14 (311.0) and these two differed significantly. Least catalase activity was found in Jayadhar (126.3) followed by Kumta (265.4).

Table 7: Effect of salinity on superoxide dismutase (units/g fresh weight) activity in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	472.3	592.0	698.0	640.5	680.0	616.6
Dhumad	530.0	620.0	740.0	859.0	891.0	728.0
Jayadhar	210.5	228.3	270.0	193.0	159.3	212.2
Kumta	319.5	450.0	591.0	390.0	294.0	408.9
Mean	383.1	472.6	574.8	520.6	506.1	491.4
For comparing means	SE m±			CD at 5%		
Variety (V)	2.44			6.89		
Salinity (S)	2.73			7.70		
Interaction (V x S)	5.46			15.39		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

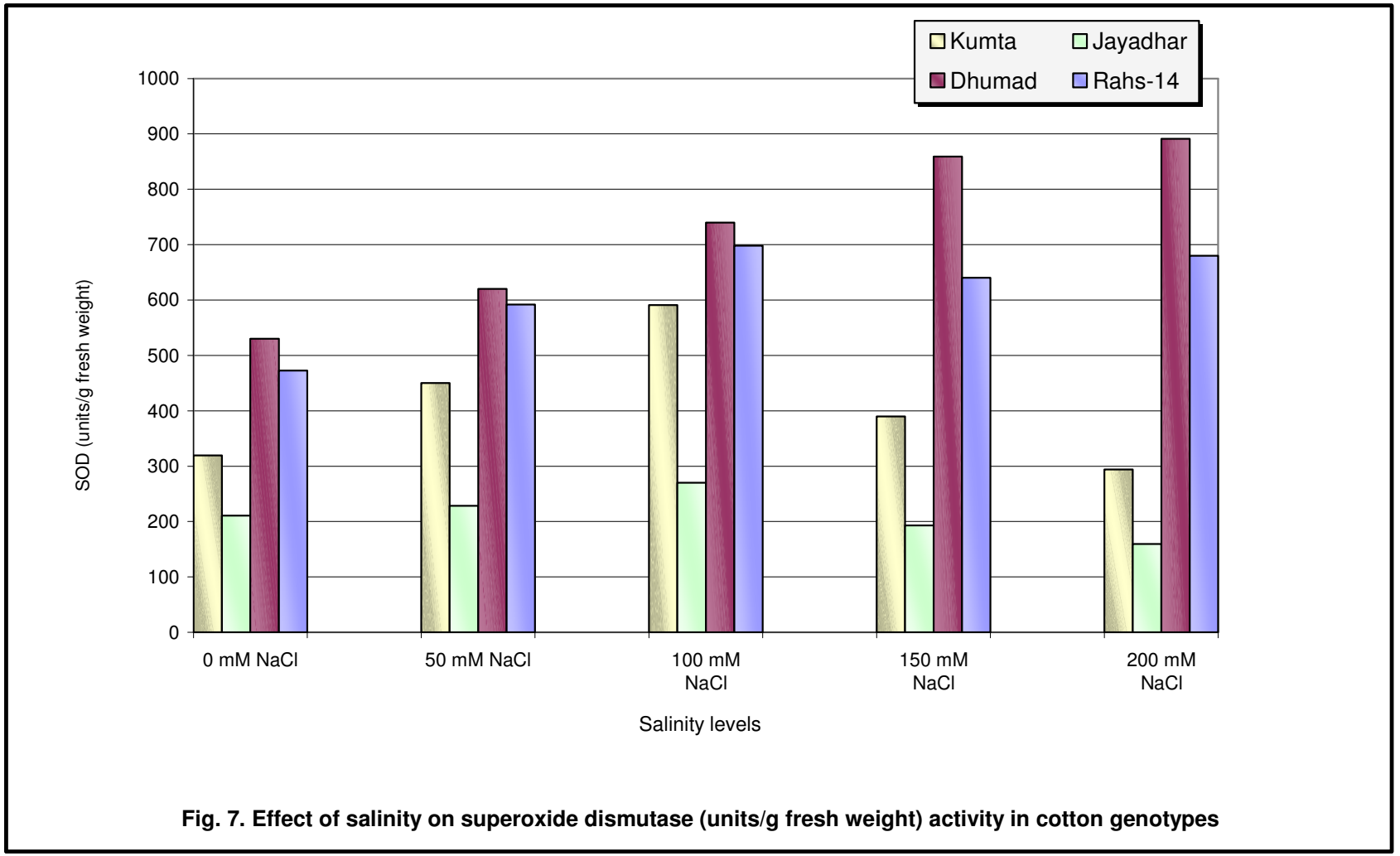

Fig. 7. Effect of salinity on superoxide dismutase (units/g fresh weight) activity in cotton genotypes

Fig. 7. Effect of salinity on superoxide dismutase (units/g fresh weight) activity in cotton genotypes

Table 8: Effect of salinity on catalase (units/g fresh weight) activity in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	275.0	295.5	318.8	325.5	340.0	311.0
Dhumad	345.5	366.8	390.0	418.3	398.0	383.7
Jayadhar	154.0	170.5	134.3	98.0	74.8	126.3
Kumta	215.0	247.0	290.0	280.0	295.0	265.4
Mean	247.4	269.9	283.3	280.4	276.9	271.6
For comparing means	SE m±			CD at 5%		
Variety (V)	2.74			7.71		
Salinity (S)	3.06			8.62		
Interaction (V x S)	6.12			17.25		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

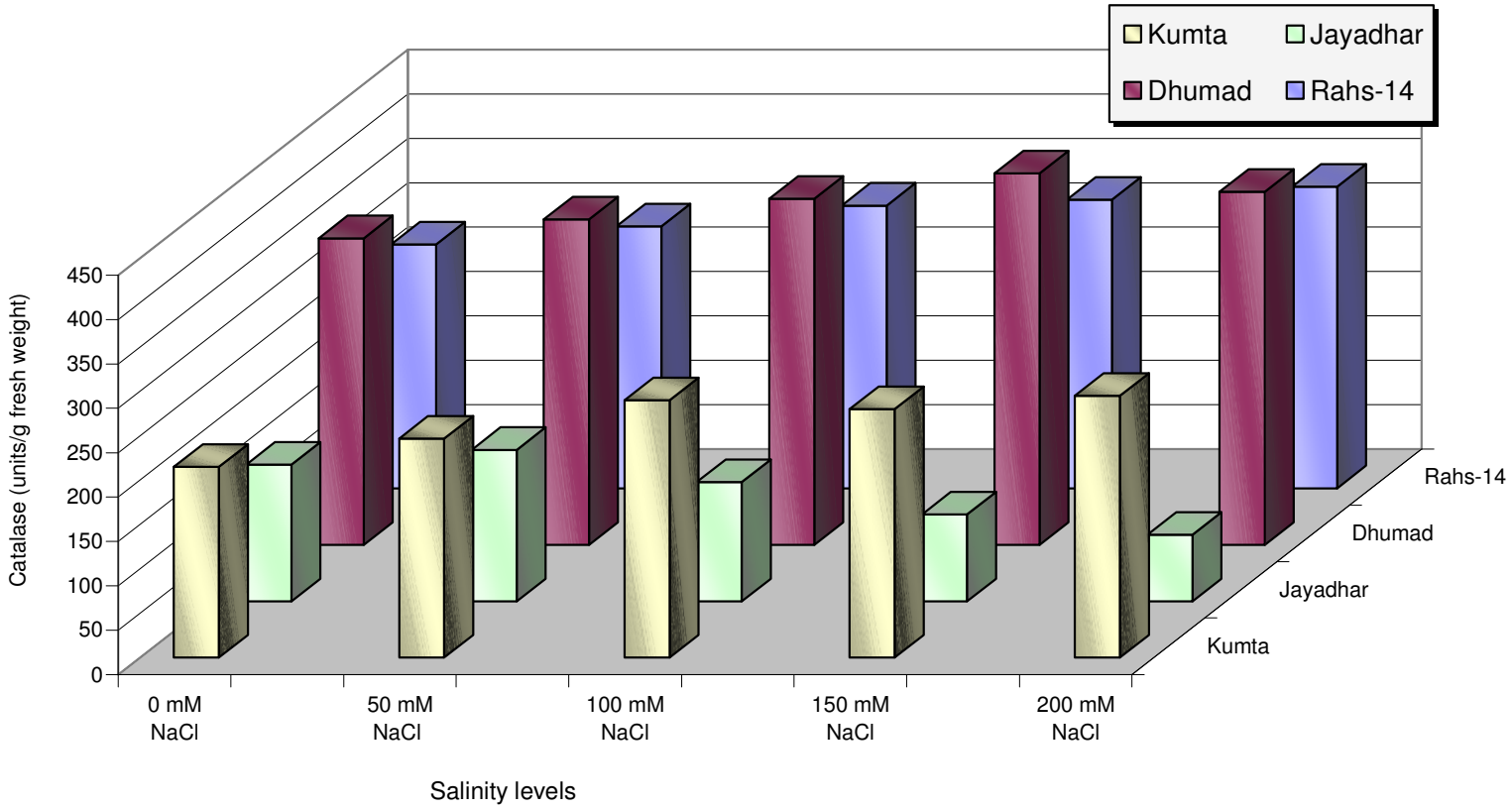

Fig. 8. Effect of salinity on catalase (units/g fresh weight) activity in cotton genotypes

Fig. 8. Effect of salinity on catalase (units/g fresh weight) activity in cotton genotypes

Table 9: Effect of salinity on peroxidase (units/g fresh weight) activity in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	376.0	442.0	485.0	520.0	490.5	462.7
Dhumad	420.0	456.0	540.0	580.0	556.0	510.4
Jayadhar	180.0	290.0	309.0	315.0	285.5	275.9
Kumta	140.0	193.0	181.0	135.0	156.3	161.1
Mean	279.0	345.3	378.8	387.5	372.1	352.5
For comparing means	SE m$\pm$			CD at 5%		
Variety (V)	2.18			6.16		
Salinity (S)	2.44			6.88		
Interaction (V x S)	4.88			13.77		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

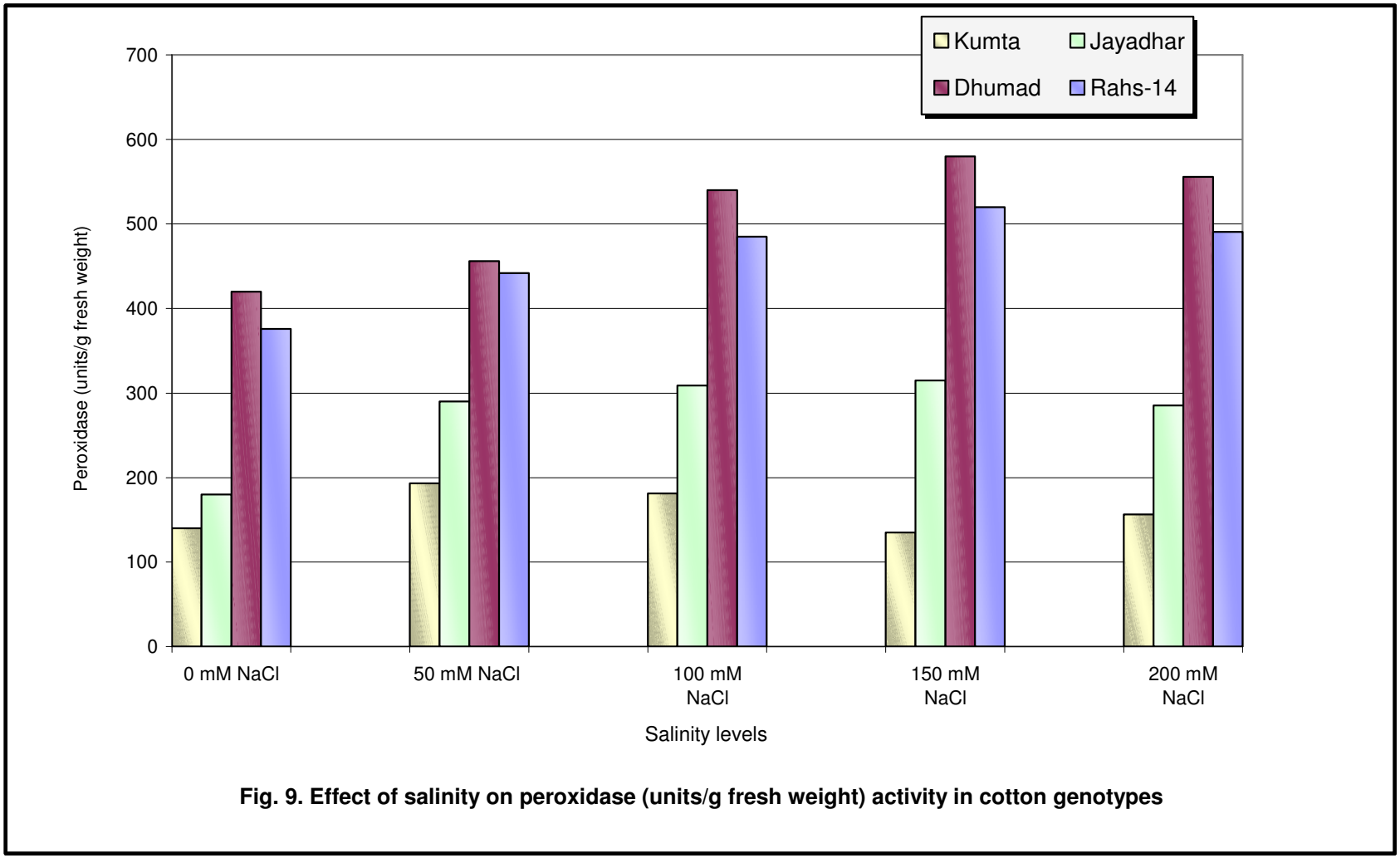

Fig. 9. Effect of salinity on peroxidase (units/g fresh weight) activity in cotton genotypes

Fig. 9. Effect of salinity on peroxidase (units/g fresh weight) activity in cotton genotypes

The activity of catalase did not follow any regular pattern with the levels of salinity. Moderate salinity level S3 (100 mM NaCl) had highest enzyme activity (283.3) followed by S4 (280.4) and S5 (276.9) and these were on par with each other. S1 (control) recorded the least enzyme activity (247.4). There was slight decrease in the activity at S4 and S5 salinity levels.

Interaction effects were found to be significant between the genotypes and salinity level. Among the treatments, enzyme activity did not follow any trend, lot of variation was found among the genotypes with increase in the salinity levels from S1 (control) to S5 (200 mM NaCl) except in case of Dhumad, where catalase activity increased from S1 (345.5) level to S5 (398.0) level. Significantly highest catalase activity was seen at S4 (418.3) level in Dhumad followed by S5 (398.0). Lowest catalase activity was noticed at S5 level in genotype Jayadhar (74.8).

4.2.3 Peroxidase activity (units/g fresh weight)

The results indicated significant difference among cotton genotypes, salinity levels and interaction between the genotypes and salinity levels.

Among the genotypes Dhumad had significantly higher peroxidase activity of 510.4 units per g fresh weight over all other genotypes followed by Rahs-14 (462.7). Least enzyme activity was noticed in the Kumta (161.1) followed by Jayadhar (275.9).

In general, salinity increased the peroxidase activity upto S4 (150 mM NaCl) salinity level and it decreased at S5 (200 mM NaCl) level. S4 level of salinity had significantly higher enzyme activity of 387.5 units per g fresh weight. Whereas, the least peroxidase activity (279.0) was noticed in control (control).

Significant difference was found for interaction between the genotype and salinity level. Genotype Dhumad at S4 (150 mM NaCl) had significantly higher peroxidase activity (580.0) followed by the same genotype at S5 (556.0). Lowest peroxidase activity was found in Kumta at S4 (135.0) and was on par with S1 (140.0) of the genotype. The peroxidase activity also did not follow any regular trend with salinity levels, but all treatments showed higher enzyme activity over control except in case of Kumta at S3 salinity level. The per cent increase was highest in Jayadhar (61.11%) at S2 salinity levels as compared to other genotypes.

4.3 MEMBRANE INTEGRITY AND TOTAL SOLUBLE SUGAR CONTENT

The data on membrane integrity measured as solute leakage and relative leakage ratio are presented in Table 10 and 11.

4.3.1 Solute leakage (absorption value at 260 nm)

Absorption value for solute leakage differed significantly among genotypes, salinity levels and interaction between the genotype and salinity.

The absorption value was significantly higher in genotype Kumta (0.498) and was on par with Jayadhar (0.495). The genotype Dhumad (0.408) had recorded least solute leakage followed by Rahs-14 (0.417) and they were on par with each other.

Table 10: Effect of salinity on solute leakage in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	0.203	0.261	0.465	0.520	0.635	0.417
Dhumad	0.212	0.262	0.452	0.513	0.601	0.408
Jayadhar	0.178	0.305	0.543	0.659	0.789	0.495
Kumta	0.163	0.324	0.554	0.646	0.802	0.498
Mean	0.189	0.288	0.504	0.584	0.706	0.454
For comparing means	SE m$\pm$			CD at 5%		
Variety (V)	0.007			0.020		
Salinity (S)	0.008			0.023		
Interaction (V x S)	0.015			0.045		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

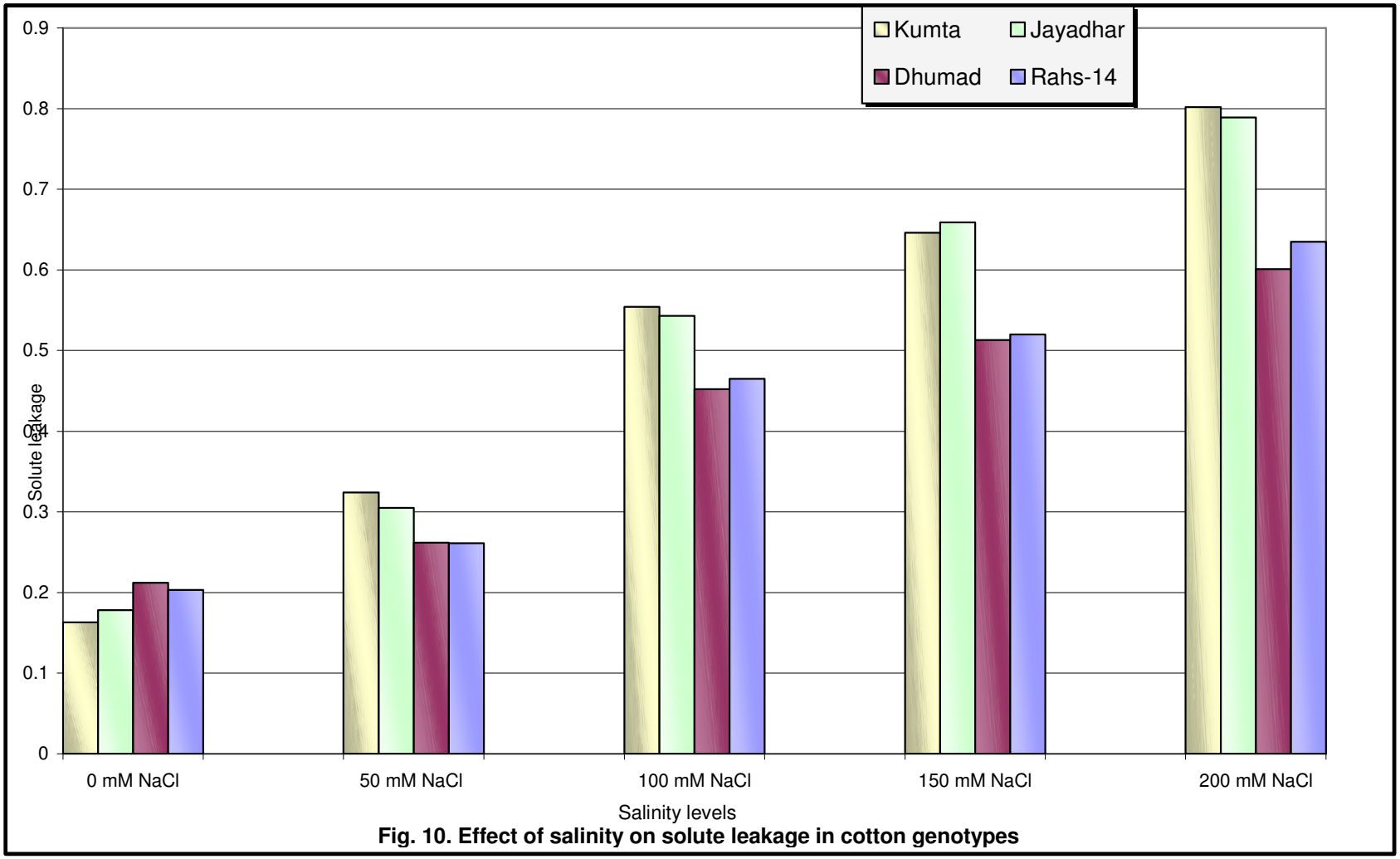

Fig. 10. Effect of salinity on solute leakage in cotton genotypes

Fig. 10. Effect of salinity on solute leakage in cotton genotypes

Salinity increased the solute leakage considerably with increase in the salinity level which reflected in the absorption value and it is increased significantly at all salinity levels. The control (S1) recorded the lowest absorption value (0.189), further with increase in salinity the value increased significantly and maximum value was noticed at S5 (0.706) level.

Interaction between the genotype and salinity was found to be significant with Kumta having the maximum absorption value (0.802) at S5 salinity level followed by Jayadhar (0.789) at same level and were on par with each other. Significantly lower absorption value was noticed in genotype Kumta (0.163) at S1 (control) level and was on par with Jayadhar (0.178) and Rahs-14 (0.203) at the same level of salinity. Salinity increased the absorption values in all the genotypes, but maximum increase in absorption value was found between S2 (50 mM NaCl) and S3 (100 mM) salinity level.

4.3.2 Relative leakage ratio (%)

Genotypes, salinity and interactions were found to be significant for relative leakage ratio (Table 11). The genotype Dhumad had significantly higher relative leakage ratio (52.9%) followed by Jayadhar (51.3%) and Kumta (51.6%). The least relative leakage ratio was observed in Rahs-14 (47.4%).

With increase in salinity level from S1 to S5, relative leakage ratio increased from 36.5 to 63.8 per cent. S1 salinity level recorded least relative leakage ratio of 36.5 per cent, whereas, S5 salinity level had significantly higher relative leakage ratio (63.8%). Maximum increase in relative leakage ratio was found between S2 and S3 salinity level.

Irrespective of genotype, relative leakage ratio increased with increase in the salinity significantly. The least relative leakage ratio was observed in genotype Kumta (28.8%) followed by Jayadhar (31.8%) at S1 salinity level. However, significantly highest relative leakage ratio was found in Jayadhar (74.8%) followed by the Kumta (68.3%) at S5 level indicating the more sensitivity of the membrane to the salt stress. Between S2 (50 mM) and S3 (100 mM NaCl) salinity level the increased relative leakage ratio was found higher.

4.1.7 Sugar content (mg/g dry weight)

Significant differences were observed between genotypes, salinity levels and interaction between the genotype and salinity (Table 12).

On an average of all the salinity levels, the genotype Rahs-14 had significantly higher sugar content (28.50) followed by genotype Dhumad (24.86). Lowest sugar accumulation was noticed in genotype Kumta (20.71).

Salinity had a positive impact on sugar accumulation and it increased from 20.66 mg per g dry weight at S1 to 27.92 mg per g dry weight at S5. All the salinity levels significantly differed with each other.

Genotype $\times$ salinity interaction was significant with Rahs-14 having the highest sugar accumulation at S5 (34.20). Lower sugar accumulation was observed in genotype Dhumad (19.96) and was found to be on par with Kumta (18.46) at control. In general, irrespective of genotype sugar accumulation increased considerably with increase in the salinity level, however the per cent increase varied among the genotypes and the per cent increase was 46.15 per cent in Rahs-14 from S1 to S5 as compared to Dhumad, Jayadhar and Kumta (49.10%, 20.64% and 22.43%, respectively) at the same levels of salinity.

Isozyme profiles

Superoxide dismutase

The genotypes followed certain patterns of SOD, which could be classified into different groups (Plate 1). The genotypes Rahs-14 at S3 and S5 level and Dhumad at S4 and S5 level followed one pattern with mobility value of 0.69, where its intensity was higher when compared to other treatments. In case of genotypes Jayadhar at S3 and Kumta at S3, S4 and S5 followed one pattern with mobility value of 0.62 and in this treatments intensity of zones was very low.

Table 11: Effect of salinity on relative leakage ratio (%) in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	39.5 (38.93)*	45.3 (42.30)	48.9 (44.38)	51.0 (45.57)	52.5 (46.44)	47.4 (43.53)
Dhumad	46.4 (42.93)	51.2 (45.68)	53.5 (47.00)	54.5 (47.58)	58.5 (50.06)	52.9 (46.65)
Jayadhar	31.8 (34.31)	37.8 (37.93)	52.3 (46.31)	59.2 (50.33)	74.8 (59.87)	51.3 (45.75)
Kumta	28.8 (32.47)	43.0 (40.97)	57.1 (49.08)	61.0 (51.35)	68.3 (55.73)	51.6 (45.92)
Mean	36.5 (37.16)	44.30 (41.72)	53.0 (46.69)	56.5 (48.71)	63.8 (53.02)	50.8 (45.46)
For comparing means	SE m±			CD at 5%		
Variety (V)	0.087			0.246		
Salinity (S)	0.097			0.275		
Interaction (V x S)	0.195			0.550		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl
* - Values in parentheses indicate arc sign transformed values

Table 12: Effect of salinity on total soluble sugar (mg/g dry weight) in cotton genotypes

Varieties	Salinity levels					
	S_1	S_2	S_3	S_4	S_5	Mean
Rahs-14	23.40	24.30	28.60	32.03	34.20	28.50
Dhumad	19.96	23.36	24.16	27.03	29.76	24.86
Jayadhar	20.83	21.93	24.23	26.83	25.13	23.79
Kumta	18.46	20.10	20.60	21.76	22.60	20.71
Mean	20.66	22.42	24.40	26.91	27.92	24.46
For comparing means	SE m$\pm$			CD at 5%		
Variety (V)	0.223			0.634		
Salinity (S)	0.250			0.709		
Interaction (V x S)	0.577			1.418		

S_1 – 0 mM NaCl S_2 – 50 mM NaCl S_3 – 100 mM NaCl
S_4 – 150 mM NaCl S_5 – 200 mM NaCl

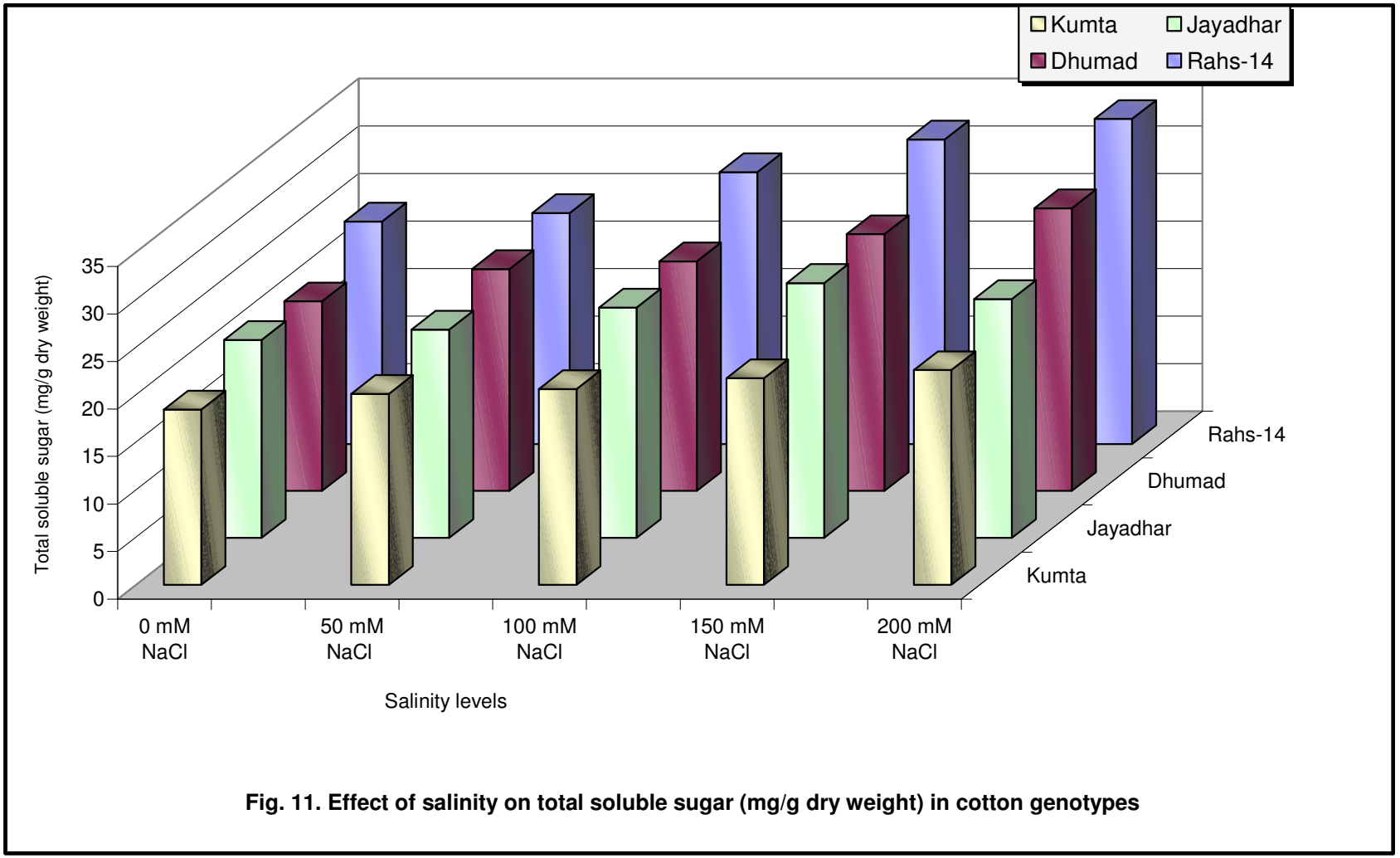

Fig. 11. Effect of salinity on total soluble sugar (mg/g dry weight) in cotton genotypes

Fig. 11. Effect of salinity on total soluble sugar (mg/g dry weight) in cotton genotypes

LEGEND

Genotypes

G_1 : Rahs-14

G_2 : Dhumad

G_3 : Jayadhar

G_4 : Kumta

Salinity levels

1- 0 mM NaCl (Control)

2- 50 mM NaCl

3- 100 mM NaCl

4- 150 mM NaCl

5- 200 mM NaCl

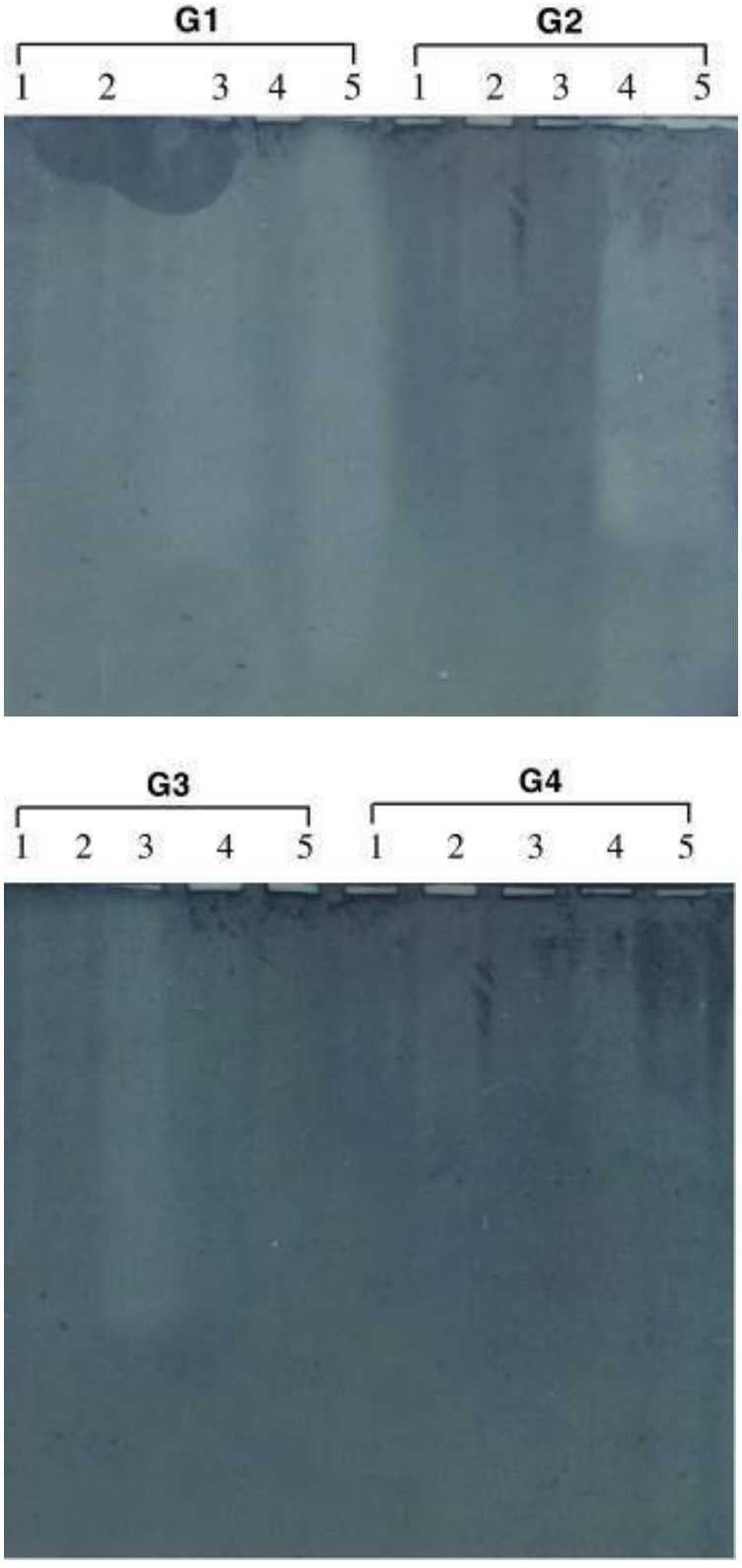

Plate1. Superoxide dismutase activity

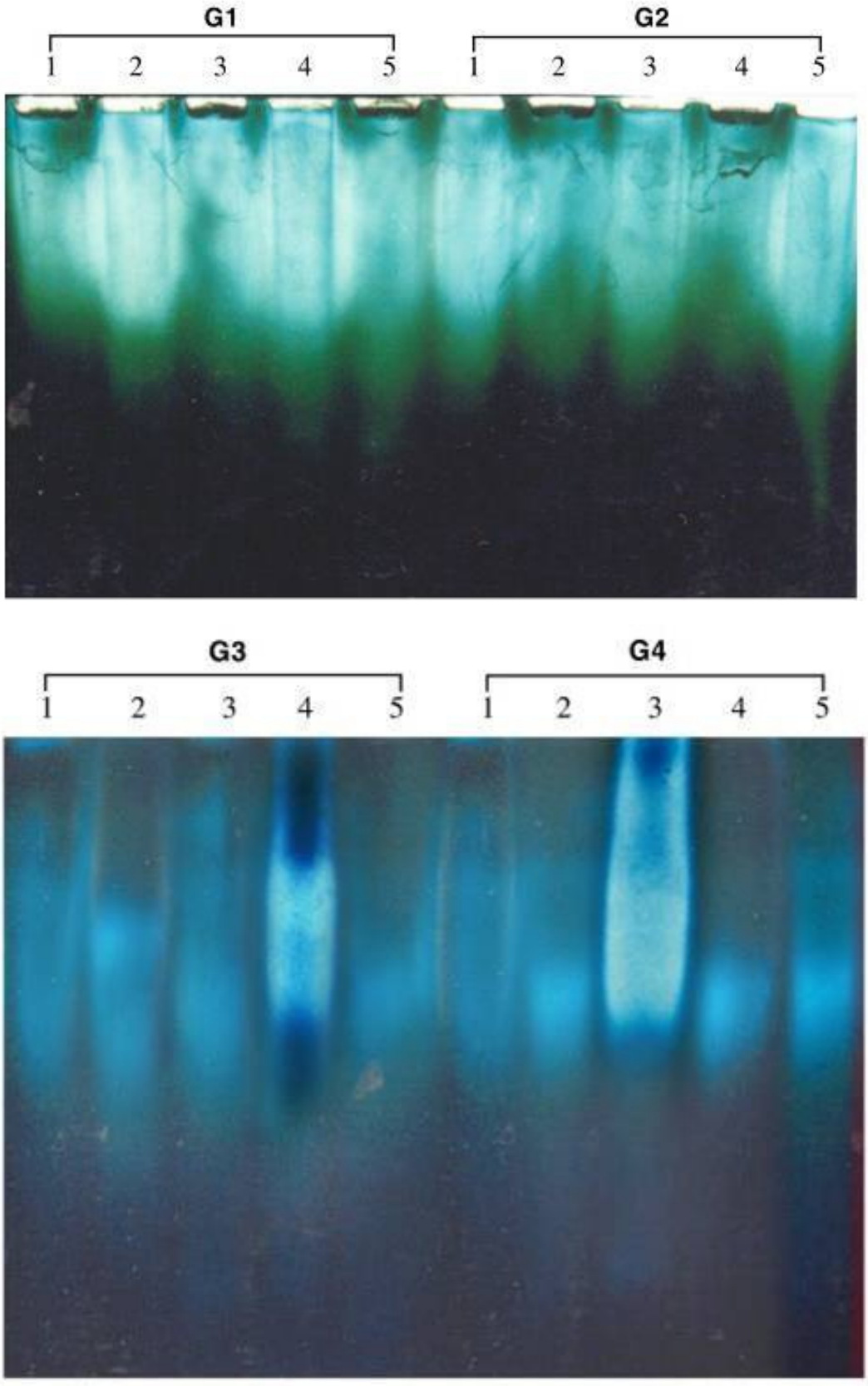

Plate 2. Catalase activity

Catalases

The genotypes varied for isozyme patterns with respect to catalase induction (Plate 2). The genotypes Rahs-14 and Dhumad showed the maximum activity of enzyme at 150 mM and 200 mM salinity levels, respectively with relative mobility value of 0.67. The intensity of band was lower at 150 mM NaCl in case of Dhumad indicating reduction in enzyme activity at higher salinity level. The intensity of bands were similar in Jayadhar and Kumta except for the bands at 150 mM NaCl in case of Jayadhar and 100 mM NaCl in case of Kumta, wherein the intensity was higher with mobility value of 0.70.

Peroxidase

All the genotypes showed no much variation for the peroxidase isozymes (Plate 3) except for the bands of mobility value of 0.75 at 150 and 200 mM. NaCl in genotypes Rahs-14 and Dhumad wherein its intensity was higher when compared to other treatments. In case of Jayadhar and Kumta observed very light bands at all salinity levels indicating similar isozyme pattern of other enzymes.

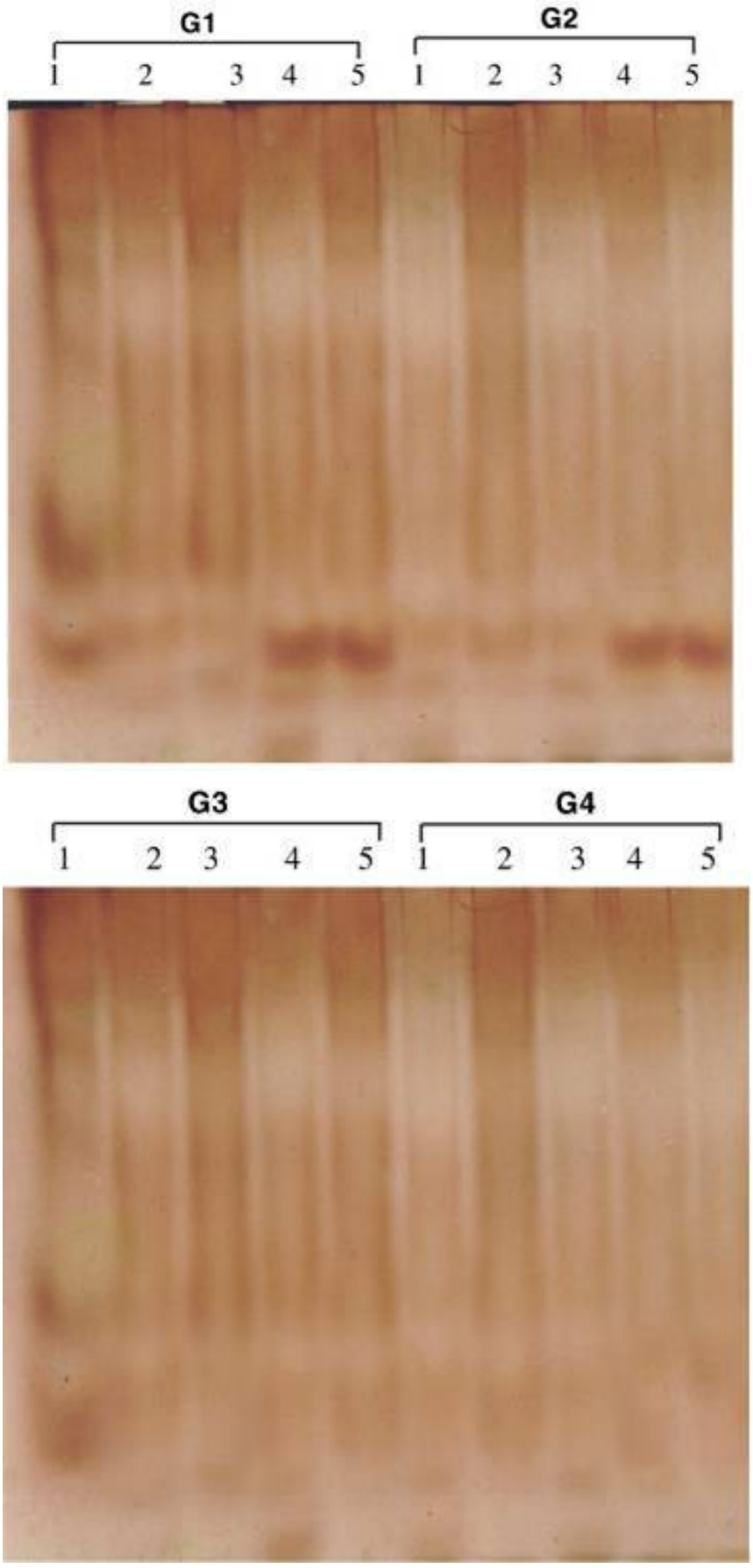

Plate 3: Peroxidase activity

V. DISCUSSION

Soil salinization is a major menace of irrigated agriculture in arid and semi-arid regions. Among the several approaches to solve the problem of saline soils, biological approach to identify and grow salt tolerant plants in such soils and to bring about soil reclamation is promising. Lot of work had already been carried out in this direction in a number of crop species. A few agricultural crops *viz.,* barley, cotton, sorghum, sugar beet *etc.* have moderate tolerance and there can be a wide variation in salt tolerance among varieties or genetic lines. Despite the existence of genetic variation for salt tolerance, only limited number of varieties have been developed with improved tolerance. These new varieties have been evaluated based on agronomic characters such as yield or survival under saline conditions.

The understanding of the physiology and molecular biology of salt tolerance of plants is important for long-term integrated system of farming in areas affected by both soil and water salinity. Several mechanical and chemical methods have been devised by many workers to reclaim salt affected soils, which are expensive and are not readily feasible. Hence, breeding for salt tolerance might be more successful, if selection is based on physiological mechanisms and biochemical characters conferring tolerance. So identification of genotypes with such favourable physiological traits would be of great use, since it can be used directly in breeding programme.

One crop of particular emphasis is cotton. Although, it is fairly a salt tolerant crop; its sensitivity at germination and seedling stage, crop stand and yield is affected. Therefore, there is dire need to focus efforts for developing salt tolerant varieties of cotton. The present investigation reveals the response of four cotton genotypes in relation to physiological, biochemical and molecular parameters at different salinity levels. The results obtained are discussed in this chapter.

5.1 GERMINATION AND SEEDLING GROWTH

The germination of cotton seed and emergence of seedling is generally delayed and reduced by salinity (Qadir and Shams, 1997). However, it differs from one crop to other and even a significant variation has been recorded amongst the different cultivars of same crop (Kumar and Bhardwaj, 1981 and Datta and Dayal, 1988). Similarly, varietal differences in response to salinity have been observed in case of cotton (Khan *et al*., 1998 and Janagoudar *et al*., 2004).

5.1.1 Germination

The effect of different levels of salinity on seed germination in four cotton genotypes revealed that germination per cent decreased with an increase in salinity level from S1 (0 mM NaCl) to S5 (200 mM). Genotypes differed significantly with respect to the extent of reduction in germination under salinity stress. Among the genotypes, RAHS-14 (66%) and Dhumad (62%) had lower reduction in germination per cent at highest salinity level (200 mM NaCl), but Jayadhar and Kumta had higher germination percentage reduction of 98 and 96 per cent, respectively at same salinity level as compared to control. The gradual reduction in germination with linear increase in salinity levels was in line with the results obtained in different crops by several workers (Malik and Makhadum, 1987 and Gupta *et al*., 1995 in cotton; Anuradha and Seetaram Rao, 2002 in rice and Ghoulam *et al*., 2002 in sugar beet). However, lower salinity level (S2) had not affected the germination per cent, but higher salinity level (S5) showed greater reduction and varietal difference in response to salinity have also been observed. The genotypes with least reduction in germination were found tolerant (Khan *et al*., 1998 and Ahmad *et al*., 1991) in cotton. This reduction in germination percentage due to increasing salinity level might be due to the decreased osmotic potential which reduced water imbition in seed (Javid *et al*., 2001) and also due to the accumulation of toxic ions like sodium and chloride in cytoplasm might have damaged the enzymes and organells (Lauchli and Epstein, 1990).

5.1.2 Seedling growth parameters

Different concentrations of NaCl (50, 100, 150 and 200 mM NaCl) significantly reduced seedling growth parameters as compared to the control (0 mM NaCl). The seedling growth parameters *viz.,* root length, shoot length, seedling vigour index and dry weight decreased significantly except root:shoot ratio, which increased with the increase in the salinity levels.

Persual of the data indicated that increase in salinity decreased the root length of seedling significantly. The per cent reduction in the root length of RAHS-14 (46%) and Dhumad (47%) was nearer to the genotypes Jayadhar (41%) and Kumta (45%). However, Rahs-14 and Dhumad recorded comparatively higher root length at all salinity levels. In general, root length decreased with increase in salinity and the results are in conformity with the findings of Rema Devi and GopalKrishnan (1997) in cowpea; Hampson and Simpson (1990a) in wheat and Silberbush and Ben-Asher (1987) in cotton.

Genotypes differed to a large extent in the reduction of shoot length under salinity stress. Irrespective of genotype, the shoot length decreased with increase in salinity. The highest per cent reduction in shoot length was observed in Kumta (54%) and Dhumad (50%), while lower reduction was observed in RAHS-14 (50%), Dhumad (50%) and Jayadhar (51%). Relatively shoots were more sensitive to salinity stress than roots. Similar results were reported by Qadir and Shams (1997) and Brugnoli and Bjorkman (1992) in cotton, Ghoulam *et al.* (2002) in sugar beet. The reduction in seedling length (root + shoot) might be attributed to nutritional disorder or delayed germination due to higher osmotic pressure and toxicity due to ions on metabolism may be another reason for reduction in seedling length (Javid *et al.*, 2001).

Root:shoot ratio varied significantly among genotypes with salinity stress. The least root:shoot ratio value was recorded at 50 mM NaCl salinity level in case of RAHS-14 indicating that low concentration of salts stimulate the growth of the shoot. Similar results were observed by Zidan and Elewa (1995) in caraway and cumin and Pessarakli (1995) in cotton. Further, with increase in the salinity level the root:shoot ratio increased and maximum value was seen in the genotype Kumta (0.782) followed by Jayadhar (0.749). Salinity increased the root:shoot ratio because shoots are more sensitive than roots at higher salinity levels (Brugnoli and Bjorkman, 1992).

In general, seedling vigour index decreased with salinity in all the four genotypes and significant differences were found between treatments. The per cent reduction in the seedling vigour index was found to be maximum in the genotypes Jayadhar (98%) and Kumta (98%) and lower reduction in RAHS-14 (80%). The results indicated that increased salinity had greater influence on seedling vigour index and response varied with genotypes. The results obtained were in line with the findings of Janagoudar *et al.* (2004) and Rajgopal (1999) in cotton. Higher seedling vigour index indicates the genotype has the higher capacity to penetrate through the salt encrusted surface soils.

Seedling dry weight differed significantly among cotton genotypes and salinity levels. The genotypes Dhumad (39%) and RAHS-14 (46%) had lower reduction of seedling dry weight at highest salinity level (200 mM NaCl), whereas, the genotype Jayadhar (56%) had the highest reduction of seedling dry weight over the control. Similarly, salt tolerant cotton genotypes were found to have lower reduction in seedling dry weight (Khan *et al.*, 1995). In general, results showed the significant reduction in accumulation of dry weight in seedling as the salinity levels increased. Similar results were observed by Subbarao *et al.* (1991) in pigeonpea; Remadevi and Gopalkrishnan (1997) in cowpea, Ghoulam *et al.* (2002) in sugarbeet and Neera Garg and Jasleen (2004) in chickpea. The probable cause of this reduction at higher salinity levels could be the toxic effect of added salts or physiological scarcity of water with increasing solute suction of saline growth media (Javid *et al.*, 2001).

5.2 ANTIOXIDANT ENZYMES ACTIVITY

Plants in saline areas are easily exposed to multiple abiotic stresses. Among these stresses, high salinity is the most severe factor limiting plant growth. It is well documented that abiotic stresses exert at least in part of their effects by causing oxidative damage (Smimoff, 1995). Oxidative damages are caused by reactive oxygen species (ROS) and excess amounts of ROS are harmful to many cellular components. However, ROS are

inevitable by products from the essential aerobic metabolisms and they need to be maintained under sublethal levels for normal plant growth. Plants possess a number of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) to protect against the damaging effects of ROS (Asada, 1992) and also salinity induced changes in activities of these enzymes has been reported (Hernandez *et al*., 2000).

5.2.1 Superoxide dismutase activity (SOD)

In this study, SOD activity increased under salt stress in genotypes RAHS-14 and Dhumad and among them Dhumad showed the highest increase in activity (68%). Salt stress also increased SOD activity in both Jayadhar and Kumta upto S3 (100 mM NaCl) salinity levels, further at higher salinity levels, it decreased. This indicates reduction in SOD activity of salt sensitive cotton genotypes under higher NaCl stress. Madanpal *et al.* (2004) have shown reduced activity in salt sensitive rice cultivars with increasing magnitude of salt stress and SOD activity increased considerably in salt tolerant rice cultivars and similar results were obtained by Rajguru (1999) in cotton ovules, Gossett (1996) in NaCl tolerant cell line of cotton cultivar, Hernandez *et al.* (1993) in pea. Several other workers have reported enhanced activity of SOD under increasing salinity [Janagoudar *et al.* (2001) and Ganesan and Jayabalan (2005) in cotton, Ebru *et al.* (2004) in lentil and Sumesh (2003) in transgenic tobacco]. Plants with high levels of SOD, either constitutive or induced have significant role in plants to protect them against the damaging effect of ROS generated during salinity stress (Asada, 1992), SOD catalyses the dismutation of superoxide radical to H_2O_2, which is toxic to cell organell membranes (Madanpal *et al*., 2004).

5.2.2 Catalase activity

Salinity also influenced the activity of catalase of cotton genotypes. In RAHS-14 and Dhumad, the catalase activity increased gradually, whereas in Jayadhar it increased upto S2 (50 mM NaCl) and decreased with further increase in salinity levels. But, in genotype Kumta the activity increased with increase in the salinity except at S4 salinity level. These results are in agreement with Janagoudar *et al.* (2001) and Veerabhadra Rao (2001), who also found higher catalase activity in tolerant cotton and wheat cultivars respectively over the susceptible cultivars. Similarly, Gossett *et al.* (1994) noted the NaCl-tolerant cultivars displayed increased catalase activity under NaCl-stress, when compared to the salt sensitive cultivars. Enhanced induction of catalase activity in cotton cultivars more resistant to NaCl stress was also shown over susceptible cultured ovules obtained from cotton plants (Rajguru *et al*., 1999). Similarly observations were made in case of chickpea (Singh *et al*., 2001) and rice (Shim *et al*., 2003).

5.2.3 Peroxidase activity

In general, peroxidase activity increased with an increase in salinity level upto S4 (150 mM NaCl) and at S5 (200 mM NaCl) little reduction was observed in three genotypes but in case of Kumta, peroxidase activity was maximum at S2 (50 mM NaCl) and further increase in salinity decreased its activity. However, the peroxidase activity was found to be higher in case of RAHS-14 and Dhumad over the genotypes Jayadhar and Kumta indicating the salt sensitive nature of later genotypes. Similarly, Gosset *et al.* (1994) also observed significant increase in peroxidase activity in salt tolerant cotton cultivars as well as significant decrease in salt sensitive cultivars and increased activity of peroxidase was attributed to increased ability of peroxidase coding genes or an increased activation of already existing pre-enzyme. Similar results were reported by several other workers in different crops (Rajguru *et al*., 1999) in cotton ovules and Rajgopal (1999) in cotton.

The increased activities of catalase and peroxidase upon salt stress are often related to the enhanced tolerance to salt stress (Gueta-Dahan *et al*., 1997). This is because of increased role of catalase and peroxidase in hydrogen peroxidase detoxification under salt stress. This detoxification of H_2O, which is cytotoxic under supra optimal concentrations may be one of the reason for maintains higher growth in tolerant plants when compared to susceptible one (Rajguru *et al*., 1999).

5.3 MEMBRANE INTEGRITY AND TOTAL SOLUBLE SUGAR CONTENT

Production of ROS is increased under saline conditions leading to oxidative stress (Hasegawa *et al*., 2000). Oxidative damages are caused by ROS and excess amounts of ROS are harmful to many cellular compartments including membrane lipids. ROS cause peroxidation of polyunsaturated fatty acids in the membranes (Smimoff, 1995) and ROS-mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity and reduction in growth rate of salinity in many field crops. The another cause for membrane damage under salinity is by accumulating higher concentrations of Na^+ and Cl^- in the cells (Ghoulam *et al. et al*., 2002) and altering the cell wall extensibility (Pritchard *et al*., 1991).

5.3.1 Solute leakage

The data indicated an increase in solute leakage under salt stress in all the genotypes. The genotypes Dhumad recorded the lower increase in the solute leakage at all salinity levels over all other genotypes. However, the per cent increase was maximum in genotype Kumta (392%) followed by Jayadhar (342%) when compared to genotypes RAHS-14 (212%) and Dhumad (183%) indicating the salt sensitive nature of the former genotypes. The similar results were obtained by several workers in several crops [Ebru *et al.* (2004) in lentil, Madanpal *et al.* (2004) in rice and Ghoulam *et al. et al.* (2002) in sugar beet]. This increase in the solute leakage may be attributed to increase in the membrane permeability, since calcium is important in regulating membrane integrity and can be displaced from the plasma lemma of cells by higher concentrations of Na^+ ions under salinity (Cramer *et al*., 1987) and also due to oxidative damage by the ROS.

5.3.2 Relative leakage ratio (%)

Relative leakage ratio varied significantly among genotypes with salinity stress. The least relative leakage ratio was recorded at S1 (0 mM NaCl) salinity level in all the genotypes indicating that less or no membrane damage. Further, with increase in the salinity the relative leakage ratio increased and maximum relative leakage ratio was noticed in the genotype Jayadhar (59.9%) followed by Kumta (55.8%) indicating the severe membrane damage and salt sensitive nature over other two genotypes. These results agree with the findings of Ghoulam *et al.* (2002) in sugar beet, Hampson and Simpson (1990b) in wheat and Lutts *et al.* (1996b) in rice.

The lower values of relative leakage ratio in case of salt tolerant genotypes, suggests that a great part of leaf ion content did contribute to the osmotic adjustment of the cells. Thus, the apoplastic ion contents might be more important in salt sensitive genotypes. The contrary could be true for the susceptible genotypes (Ghoulam *et al*., 2002).

5.3.3 Total soluble sugar content

Adaptive features that reduce salinity stress are metabolic changes, particularly of carbohydrates that increase cell osmoticum (Greenway and Munns, 1980). Osmoregulation through accumulation of soluble sugars in roots and leaves are characteristics of salinity stressed plants (Rathert *et al*., 1981).

There was a progressive and gradual accumulation of total soluble sugars with increasing salinity treatment in all the genotypes. Among the genotypes, RAHS-14 and Dhumad had higher soluble sugar content. whereas, in genotype Jayadhar and Kumta though sugar content is increased but the rate of accumulation was less over the former genotypes. These results are in agreement with those obtained in chickpea (Neera Garg and Jasleen, 2004), cumin (Garg *et al*., 2002) and in cotton (Rathert, 1983). In contrast to this, decrease in sugar content with increase in salinity level has been reported by several workers (Rush and Epstein, 1976 and Rathert, 1984). It appears that accumulation of soluble sugars is an adoptive feature to cope with adverse saline conditions (Karadge and Gaikwad, 2003) and served as an index of osmotic adjustment by the plant (Neera Garg and Jasleen, 2004).

Isozyme profiles

The enzymes scavenging free radicals of O_2 such as CAT, POX and SOD showed variations between the genotypes and also in the intensity as the salinity levels are increased. The genotypes varied with having higher activity at higher stress levels and some having

higher activity at moderate stress level and some with similar activity under all the salinity levels.

Superoxide dismutase (SOD)

The selected genotypes Rahs-14, Dhumad, Jayadhar and Kumta showed different patterns of SOD activity as mentioned in the results. The genotypes Rahs-14 and Dhumad maintained higher level of SOD activity under stressed as well as under control than the Jayadhar and Kumta. Similarly, Hernandez *et al.* (1993) noticed that NaCl tolerant plants of pea increased the induction of the SOD isozymes compared to the NaCl sensitive plants. It implies that SOD isoforms function in the molecular mechanisms of plant tolerance to NaCl and also Kim *et al.* (2005) reported significant increase in the activity of antioxidant enzymes like SOD in the NaCl stressed barley roots and it was highly correlated with the increased expression of the constitutive isoforms as well as the induced ones.

Catalases

The genotypes Rahs-14, Jayadhar, Dhumad and Kumta showed variation with induction of catalase activity with increase in the salinity level. In general, the catalase activity was higher in genotypes Rahs-14 and Dhumad than the genotypes Jayadhar and Kumta. Higher catalase activity in salinity tolerant genotypes than the susceptible genotype of wheat was reported by Veerabhadra Rao (2001). Parida *et al.* (2003) reported at high salt concentration loss in catalase activity with four isoforms. However, the extent of decrease was not same for all the isoforms.

Peroxidases

Peroxidase showed no much variation for banding pattern in case of genotypes Jayadhar. However, in case of Rahs-14 and Dhumad at higher salinity levels, the bands of mobility value of 0.75 showed higher intensity indicating the higher enzyme activity in tolerant genotypes. Djanaguiraman *et al.* (2003) also observed the peroxidase activity is inferred to be more in tolerant genotype of rice and produced more isoforms when compared to susceptible genotype. The increased activities of catalase and peroxidase upon salt stress were often related to the enhanced tolerance to salt stress (Gueta-Dahan *et al*., 1997) and also differential increase in some of the isoforms indicated specific upregulation of antioxidative defense system (Parida *et al*., 2003).

Thus, from the above results it could be concluded that per cent germination, seedling growth, vigour index, antioxidant enzyme activities like SOD, CAT and paroxidase, membrane leakage and sugar content could be used as a criteria to screen genotypes for salinity tolerance. From the data, it is clear that plants adopt various mechanisms to cope up salinity stress. Thus, in our results, the genotypes Rahs-14 and Dhumad were found resistant due to adoption of various mechanisms when compared to Jayadhar and Kumta. Hence, these genotypes could be used as a line source for incorporation in crop improvement programme for developing putative genotypes for improving salinity tolerance.

FUTURE LINE OF WORK

1. Screening of large number of cotton genotypes for salt tolerance at early seedling stage and under field conditions.
2. Study on the alleviation of salinity stress at early stages by seed treatments with PGRs and nutrients.
3. Study with DNA markers (RFLP, RAPD) for precise genotypic differentiation.

VI. SUMMARY

Salinity is the most serious growth limiting factor and evolution of crop varieties suitable to salt stress situations, therefore, no longer be ignored. Hence, the present investigation was carried out at the Department of Crop Physiology, College of Agriculture, University of Agricultural Sciences, Dharwad with the main objective of physiological, biochemical and molecular characterization of selected cotton genotypes for salinity tolerance.

The material for the present investigation consisted of four cotton genotypes *viz*., RAHS-14, Dhumad, Jayadhar and Kumta. These genotypes were evaluated in laboratory and pot culture conditions and observations were recorded on different parameters. The isozyme analysis was carried out in these genotypes to see the variations if any.

Data on these traits were subjected to statistical analysis. The salient features of the findings from the investigations are summarized below.

- There was decrease in germination with an increase in the levels of salinity. The genotypes RAHS-14 and Dhumad were found to be tolerant with lower reduction of germination, whereas, Jayadhar and Kumta had comparatively higher reduction showing the susceptible nature of the genotypes.
- The genotypes differed significantly for root length and shoot length. Salinity decreased the root length and shoot length. However, genotype RAHS-14 had higher reduction for root length.
- The shoot growth was more affected than root growth under the stressed situation compared to control. In general, the root:shoot ratio increased under the salinity stress and higher increase in root:shoot ratio was noticed in the Kumta and Jayadhar compared to RAHS-14 and Dhumad. Seedling vigour index also decreased due to salinity stress while the tolerant genotypes Rahs-14 and Dhumad were less affected compared to the susceptible genotypes Jayadhar and Kumta.
- Seedling dry weight was reduced significantly due to salinity. The genotypes Dhumad and RAHS-14 recorded higher dry matter accumulation at all salinity levels. While, susceptible genotypes Kumta and Jayadhar recorded lower seedling dry weight at higher salinity levels.
- The tolerant genotypes RAHS-14 and Dhumad showed higher level of SOD activity at all salinity levels. However, the genotypes Jayadhar and Kumta noticed increased activity under moderate stress conditions but decreased at higher salinity levels showing sensitivity of genotypes at higher salinity levels.
- The catalase activity in tolerant genotypes increased with increase in the salinity levels. Whereas, in susceptible genotypes Kumta also showed enhancement of enzyme activity, but it was relatively less when compared to tolerant genotypes and genotype Jayadhar showed decreased enzyme activity at higher salinity level.
- The genotypes Jayadhar and Kumta showed increase in the enzyme peroxidase activity but genotypes RAHS-14 and Jayadhar had higher enzyme activity at all salinity level, while at higher level the enzyme activity decreased.
- The solute leakage and relative leakage ratio were higher in case of susceptible genotypes when compared to tolerant genotypes, indicating the susceptible genotypes prone to higher membrane damage due to salinity.
- The tolerant genotypes accumulated higher soluble sugars at all salinity levels over susceptible genotypes denoting preferential osmotic adjustment.
- The genotypes representing all the categories of tolerance level showed different pattern of catalase peroxidase and superoxide dismutase activity with higher activity under salinity stress over control. In general activity of these enzymes was higher in tolerant genotypes than the susceptible ones.

It can be concluded that genotypes differed widely in their response to salinity and plants possess different adaptation traits to cope up salinity stress. Based on this study, the genotypes RAHS-14 and Dhumad were found to be physiologically and biochemically efficient in salt stressed environments.

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Appendix I: Preparation of phosphate buffer

Stock solutions
A : 0.2 M solution of monobasic sodium phosphate (27.8 g in 1000 mL)
B : 0.2 M solution of dibasic sodium [phosphate (53.65 g of $Na_2HPO_4.7H_2O$ or 71.7 g of $Na_2HPO_4.12H_2O$ in 1000 ml
x mL of A, y mL of B, diluted to a total of 200 mL

x	y	pH	x	y	pH
93.5	6.5	5.7	45.0	55.0	6.9
92.0	8.0	5.8	39.0	61.0	7.0
90.0	10.0	5.9	33.0	67.0	7.1
87.7	12.3	6.0	28.0	72.0	7.2
85.0	15.0	6.1	23.0	77.0	7.3
81.5	18.5	6.2	19.0	81.0	7.4
77.5	22.5	6.3	16.0	84.0	7.5
73.5	26.5	6.4	13.0	87.0	7.6
68.5	31.5	6.5	10.5	89.5	7.7
62.5	37.5	6.6	8.5	91.5	7.8
56.5	43.5	6.7	7.0	93.0	7.9
51.0	49.0	6.8	5.3	94.7	8.0

PHYSIOLOGICAL AND MOLECULAR STUDIES OF SALINITY TOLERANCE IN COTTON (*Gossypium herbaceum* L.)

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ABSTRACT

Investigation on salt tolerance in cotton was made with four genotypes *viz.,* Rahs-14, Dhumad, Jayadhar and Kumta under five salinity levels in lab and pot experiments. The objective was to study the changes in physiological, biochemical, molecular characters and their relationship under varying salinity levels.

The results showed that germination percentage, root length, shoot length, seedling dry weight decreased relatively lower in genotypes Dhumad and Rahs-14 compared to Jayadhar and Kumta.

In general, the root:shoot ratio increased, while SVI decreased with increase in salinity levels. Higher increase in root:shoot ratio and decrease in the seedling vigour index at higher salinity level was noticed in the Kumta and Jayadhar.

The genotypes Rahs-14 and Dhumad showed higher level of antioxidant enzyme i.e., SOD, catalase and peroxidase activity at all salinity levels. Though, the genotypes Kumta and Jayadhar showed increase in activity with increase in salinity but it was relatively less indicating the susceptible nature of these genotypes.

The genotypes Rahs-14 and Dhumad accumulated higher soluble sugars at all salinity levels denoting the preferential osmotic adjustment.

The solute leakage and relative leakage ratio were higher in genotypes Jayadhar and Kumta indicating higher membrane damage due to salinity in susceptible genotypes.

The genotypes showed different patterns of isozyme profiles for the enzymes SOD, catalase and peroxidase with higher activity under salinity stress over control.

Based on the investigation, the genotypes Rahs-14 and Dhumad were found to be efficient in salt stressed environments because of lesser reduction in germination, root and shoot length, SVI, seedling dry weight, solute leakage, relative leakage ratio and maintenance of higher total soluble sugars and higher SOD catalase and peroxidase activity at higher salinity levels with their varied isozyme profiles which could serve as selection criteria for identification of salinity tolerance at early stages of crop growth in cotton.