# MESO-STRUCTURE OF LEAF IN RELATION TO PHOTOSYNTHESIS AND PRODUCTIVITY IN COTTON (*Gossypium* spp.) UNDER SALT STRESS

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JULY, 2003

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*Thesis submitted to the*
*University of Agricultural Sciences, Dharwad*
*in partial fulfilment of the requirements for the*
*Degree of*

**Master of Science (Agriculture)**

*in*

**CROP PHYSIOLOGY**

*By*

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# DEPARTMENT OF CROP PHYSIOLOGY

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## CERTIFICATE

*This is to certify that the thesis entitled* "**MESO-STRUCTURE OF LEAF IN RELATION TO PHOTOSYNTHESIS AND PRODUCTIVITY IN COTTON (*Gossypium* spp.) UNDER SALT STRESS**" *submitted by* **Mr. CHANDRASHEKHARAGOUDA S. N.**, *for the degree of* **MASTER OF SCIENCE (AGRICULTURE)** *in* **CROP PHYSIOLOGY** *to the University of Agricultural Sciences, Dharwad, is a record of research work carried out by him during the period of his study in this university, under my guidance and supervision, and the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles.*

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JULY, 2003

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# ACKNOWLEDGEMENT

*I take this privilege to express my heartfelt gratitude and thanks to the Almighty who has bestowed his love on me through my beneficiaries in the form of parents, teachers, friends, near and dear ones. It is indeed hard to reasonably evaluate the help, assistance and guidance received but the time has come to look back on the path traversed and to remember the faces and spirits behind the successful completion of this endeavour.*

*It is with immense pleasure that I convey my profound sense of gratitude and indebtedness to* **Dr. B. S. JANAGOUDAR**, *Professor, Department of Crop Physiology, College of Agriculture, Raichur, University of Agricultural Sciences, Dharwad and the esteemed Chairman of my Advisory Committee for his meticulously disciplined guidance, valuable suggestions, critical evaluation and ceaseless help rendered during the course of investigation. I must confess that it has been an honour for me to be associated with him during my M.Sc. degree programme.*

*I am equally grateful to* **Dr. B. C. Patil**, *Cotton Physiologist, Department of Crop Physiology,* **Dr. I. S. Katageri**, *Cotton Breeder, Department of Genetics and Plant Breeding and* **Dr.(Mrs.) A. S. Nalini**, *Professor, Department of Crop Physiology (Botany section), UAS, Dharwad, the members of my Advisory Committee for their supportive inspiration, constant encouragement and constructive criticism during the period of my study and in ameliorating the manuscript.*

*I must offer my special thanks to Dr. M. B. Chetti, Professor and Head, Department of Crop Physiology, UAS, Dharwad and all the staff members for their co-operation and sustained help during my period of study.*

*To express my sincere gratitude to my beloved parents, Shri Shivanagouda & Smt. Basamma, brothers Ravi & Anand, sisters Shakunthala & Poornima and*

guardians Shri Sharanappa & Smt Shanthamma Aramani in the form of words is rather restrictive both in expression and quantum. Yet, at this juncture, it is my esteemed duty to reserve my higher regards to them.

Life is not the work all the time. One necessarily has to have some diversion once in a while, to be able to return back to work with a renewed enthusiasm and vigour and this is where my friends Ramesh, Dayanand Patil, Krishnaraj, Prashant, Satish, Rajashekhar, Shashidhar, Akash, Prabhakar, Dakshayani and Prameela come into picture. I heartily thank all my friends not only for their constant companionship and moral support but also for making my stay in the campus more lively and pleasant.

I appreciate Mr. Santosh and M/s Anup Computers for excellent word processing for the document with skill and patience.

..... any omission in this brief acknowledgement does not mean lack of gratitude. Unlimited thanks to one and all.

DHARWAD
JULY, 2003

( Chandrashekharagouda S. N.)

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# Introduction

# I. INTRODUCTION

Soil salinity is one of the major widespread environmental hazards in the arid and semi arid areas and in the sub–humid and humid climates particularly in the coastal regions where ingress of sea water results in large scale soil and water salinization (Panda, 2001). In many arid and semi arid areas secondary salinization of the soil as a result of the introduction of canal irrigation has resulted in taking productive lands out of cultivation.

It is estimated that 10 per cent of the world's crop lands are affected by salinity. Of the irrigated lands, as much as 20-27 per cent may be salt affected and upto 37 per cent may be saline, sodic or water logged (Ghassemi *et al.*, 1995). Sodium chloride is by far the most prevalent salt abundant in saline soils especially in the root zone, resulting in partial or complete loss of soil productivity. According to the recent estimates, about 12 million hectares of land is affected by salinity in India (Afria and Narnolia, 1999).

In Karnataka, the area under salt affected soils is estimated to be 1,31,631 hectares (Balakrishnan and Patil, 1999). With the introduction of canal water irrigation under Tungabhadra, Malaprabha and Ghataprabha project areas soil salinity problems are mainly found in the districts of Raichur, Dharwad, Belgaum and Bijapur.

Soil salinity is an enormous problem adversely affecting growth and development of crop plants and results into low agricultural production. Salt stress decreases the water potential of soil water and reduced water availability to the plant results in imbalance in plant

metabolic process (Chazen and Neumann, 1994). Due to the toxic effects of various ions like, $Na^{+}$, $Mg^{+2}$, $Cl^{-}$, $SO_4^{-2}$ and $HCO_3^{-}$ there is a salt poisoning in plants and in addition plants also suffer from the hunger of essential plant nutrients resulting in premature senescence (Munns *et al.*, 1995).

Selection of crop varieties tolerant to salt environment will allow the proper use of waste saline areas, because, reclamation of saline land, the other possible way is an expensive proposition. It would therefore be important to identify the morpho-physiological, biochemical, biophysical and anatomical parameters responsible for salinity resistance in crop plants.

The assiduous efforts made during the last few decades to increase the salt tolerance in crops either through selective breeding or genetic manipulation have not yielded any promising result (Sam and Reddy, 2000). Flower and Yeo (1995) suggested pyramiding of complex physiological traits in breeding programme to increase salt tolerance.

Cotton is one of the most important commercial crops of India and is recognised as one of the most salt tolerant plant among the field crops (Richards, 1954). It is cultivated in an area of 9 million hectares with a production of about 170 lakh bales and with a productivity of 320 Kg lint per hectare (Gopal swamy *et al.*, 2001). In Karnataka, cotton occupies 0.67 million hectares of area with the production of 0.85 million bales (Khot *et al.*, 2001). It is widely grown on black cotton soils of command areas with usually high pH, where there is gradual development of salinity and sodicity in black soils causing yield reduction in cotton. Hence, there

is a need to intensify the research work to identify characters inspiring the salt tolerance in cotton.

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

a. To study the effect of salt stress on meso-structure of leaf, biochemical and biophysical parameters in cotton genotypes

b. To establish the relationship if any, between meso-structure of leaf, photosynthesis and productivity in cotton under salt stress conditions

# Review of Literature

# II. REVIEW OF LITERATURE

Salinity stress has been defined as the presence of excessive concentration of soluble salts, which suppresses plant growth (Singh *et al.*, 1994). Salinity affects plant growth right from germination to maturity. Literature on effect of salinity on different aspects of growth, metabolism, structure and yield and yield components of cotton genotypes is reviewed in this chapter.

## 2.1 GERMINATION

The effect of salinity stress on germination in saline soils leads to poor productivity. Salinity stress has been known to affect adversely seed germination in a variety of plants (Hampson and Simpson, 1990). Salinity at lower levels delays the germination and at higher level reduces the germination (Kuhad and Sheoran, 1987). Decrease in germination rate might be due to decrease in water absorption (Dell Quilla, 1992).

Germination percentage for various field crops show that irrespective of variety, tolerance to salinity is in the order of bajra > jowar > rice > maize > wheat > safflower > mustard > cotton (Bhumbla *et al.*, 1968).

Cotton appears to be more sensitive for salinity at germination stage. It can tolerate salinity level of 4 mmhos/cm at 25°c in saturation extracts during germination but can tolerate three times this salt level once the seedlings are well-established (Ayers and Hayward, 1948). Cotton is classified as a tolerant crop, which can withstand salinity of 8 to 12 mmhos/cm (Van den Berg, 1950).

Janardhan *et al.* (1976) observed that at salinity level of 12 mmhos/cm, per cent germination on 30$^{th}$ day was 40 and 25 per cent in cotton cultivars, Varalaxmi and Bhagya and 15 per cent in Hampi.

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 jowar > tobacco > bajra > cotton > bean > tomato > cabbage > pea.

* Akhtar *et al.* (1988) reported that germination percentage of cotton decreased at various salinity sodicity levels and there was no germination at $EC_e$ 30 dS $m^{-1}$ and ESP 44.

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

* Emergence of cotton cultivar H 77 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) reported that cotton seed germination significantly declined with increase in salinity from 3.5 to 21.0 dS $m^{-1}$ and cultivar NIAB-78 was more tolerant at germination stage.

Ye and Liu (1994) reported that low concentration of NaCl (0.1 to 0.2 per cent) and table salt (0.2 to 0.4 per cent) increased the germination percentage and germination energy of seeds of moderately salt tolerant cultivar Zhongmiansuo-16. Moderate concentrations of NaCl and table salt (0.5 to I.O per cent) greatly decreased the germination percentage and

germination energy, while, at high concentrations (1.0 to 2.0 per cent) germination was inhibited. The effect of NaCl was higher than that of table salt.

Khan *et al.* (1995a) reported that germination of cotton seeds decreased with an increase in salinity level. In sand culture, seed germination at 150 and 250 mol NaCl $m^{-3}$ was 68 to 89 per cent and 24 to 40 per cent of the control levels, respectively, where as in soil, germination at 15 and 25 dS $m^{-1}$ was 72 to 89 and 20 to 53 per cent of control levels, respectively, Cv, AU-14, NIAB-78 and AU-59 were the most salt tolerant in germination.

Cotton was germinated in salinities of 0,5,10,15 or 20 dS $m^{-1}$. Increasing salinity decreased germination percentage and rate, root and shoot length and seedling vigour (Varghese *et al.*, 1995).

Tort (1996) reported that, germination percentage of cotton Cv. Nazilli-87 was decreased with an increase in salinity level. Germination was 94 per cent without additional salt, 63 per cent at 175 mM NaCl and only 8.7 per cent at 300 mM. The mean germination time ranged from 5.28 days to 13.50 days at salt concentrations of 0 and 300 mM NaCl respectively.

In a pot experiment, the imposed salinity stress of $EC_e$ levels of 10 and 20 dS $m^{-1}$ on a sandy clay loam soil decreased germination with significant differences among the cotton cultivars, viz., MNH-93, NIAB-78, S-12 and B-557. At 10 dS $m^{-1}$ salinity, seed germination was 47 to 84 per cent, whereas, at 20 dS $m^{-1}$ salinity, it was17 to 54 per cent (Qadir and Shams, 1997).

✳ The mean germination percentage of 20 cotton (*Gossypium* Spp.) varieties decreased with the increase in salinity levels from 1.0 to 15.0 dS $m^{-1}$. Mean germination percentage was 87,77,61 and 31 per cent at the four salinity levels. At the highest salinity level of 15.0 dS $m^{-1}$ varieties differed in their tolerance, with Cv. LD-620, the most salt tolerant having a highest mean germination of 73 per cent and Cv. Laxmi, the most sensitive with 10 per cent mean germination (Gill *et al.*, 1999).

✳ Treatment of wheat seeds with NaCl solution decreased the germination rate of seeds (Dash and Panda, 2000). Phogat *et al.* (2001) reported that, cotton (*Gossypium hirsutum*) genotypes showed a significant reduction in germination percentage, with increasing salinity levels upto $EC_e$ 12 dS $m^{-1}$. H-974 and H-777 genotypes of cotton recorded significantly better germination than the H-1098, H-182 and H-1170.

Ashraf *et al.* (2002) reported that, increasing salinity from 0 to 100 mmol $dm^{-3}$ in the germinating medium resulted in decreasing seed germination of cotton cultivars NIAB-Karishma, NIAB-86 and K-115. K-115 had the highest germination percentage followed by NIAB-Karishma and NIAB-86.

## 2.2 GROWTH AND ITS ATTRIBUTES

The saline soils have the problem of high soluble salts creating a condition of physiological drought which limits plant growth by inhibiting nutrient and water uptake by plants. Morphologically, the most typical symptom of saline injury to a plant is retarded growth, resulting in a stunted growth of the plant and reduced leaf area.

Salinity affects the growth through its effect on osmotic potential and thus, by imposing water stress (Niemann, 1965). It was suggested that the reduction in plant growth on saline media was the result of the combined suppressing action of salinity and transpiration on plant water potential.

The growth of barley, oat, rice, sorghum, maize and cotton was accelerated at low concentration of salt (2000 ppm) applied in three installments and further increase in the level of salt application proved to be detrimental. The concentration of 6000 ppm was found to be critical for growth of most of the crops except for cotton and rice, which showed some stand even at salinity level of 8000 ppm indicating tolerance nature in these crops (Das and Mehrotra, 1971).

In 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 treatments. It was further noticed that the seedling and early vegetative growth stages were found more sensitive, whereas, the initiation of flowering was not as much sensitive as earlier stages (Lashin and Atanasiu, 1972).

The height and dry matter accumulation of 17 cotton cultivars decreased with increasing salinity levels from o to 1500 ppm salts at 18 days after sowing (Abdul-Naas and Omran, 1975).

A salt stress of 200 mmol/l total ion concentration above the threshold level for 14 days reduced the growth of two Egyptian cotton varieties (viz., Dandra and Giza 45) considerably. However, the greater salt tolerance of Giza-45 was demonstrated by small depression in dry matter yield (Rathert, 1983).

Cramer *et al.* (1994) reported that early differences in plant growth would have a large impact on final dry matter accumulation at maturity, eventhough later stages of growth have little or no differences in growth rate under salt stress. Further, they found that NAR was not significantly correlated with differences in salt tolerance between genotypes in maize. Increasing total salinity of irrigation water from 320 to 6400 mg/l resulted in reduced yield and total dry weight of cotton (Abd-Ella and Shalby, 1993). Seedling height and dry weights were negatively correlated with increase in EC of irrigation water in wheat (Khan *et al.*, 1992).

Khan *et al.* (1995b) compared 15 cotton genotypes for salt tolerance in hydroponics nutrient medium containing three salt concentrations (0,150 and 250 mol $m^{-3}$ NaCl) and reported that the fresh weight and dry weights of shoot decreased in all the cultivars with increase in salt concentrations. The Cv. NIAB 78 was more tolerant with smallest reduction in shoot fresh weight and dry weight.

The average growth and dry weights of leaf, stem, structural root, fine root and whole plant were reduced in salinised plants of *Citrus grandis* L. and *Poncirus trifoliata* L. compared to those of control plants. Average shoot dry weight reduction was greatest in *C. grandis* and least in *P. trifoliata* in 40 mM NaCl treatment (Ilhami *et al.*, 2000).

Increased NaCl levels resulted in a significant decrease in root, shoot and leaf growth biomass. Root: shoot ratio increased in response to salt stress. The responses of both cultivars of cotton *(Gossypium hirsutum)* viz., Pora and Guazuncho to NaCl stress were similar (Meloni *et al.*, 2001).

Saline irrigation ($EC_{iw}$ 6 or $EC_{iw}$ 8) significantly decreased germination, crop growth and yield of cotton Cv. H777 compared to non-saline canal water irrigation. Plant height increased in bed sowing method compared to presowing saline irrigation and flat surface sowing method (Chauhan, 2001). The irrigation with saline water reduced cotton growth and lowered plant heights, but did not appear to reduce yield (Moreno *et al.*, 1998)

## 2.3 YIELD AND YIELD ATTRIBUTES

Salinity stress is known to affect all the metabolic processes resulting in reduced crop growth and yield. In general, decrease in the seed cotton yield under saline conditions is attributed to reduction in the total number of fruiting points formed, total number of bolls matured and weight of both the seed and lint per boll in cotton.

Hayward and Bernstein (1958) reported that there is no much reduction of seed cotton yield with salinity below 6.0 mmhos/cm, whereas, electrical conductivity of 16.0 mmhos/cm generally decreased yield by 50 per cent. A decrease in the seed cotton yield by 2.02 tonnes per hectare (76.2 per cent decrease) was observed due to salinity created by accumulation of chloride and sulphate salts (Pultatov, 1970). A critical level of EC at which 50 per cent decrease in the yield was induced for cotton and rice were 12.8 and 8.3 mmhos/cm respectively (Das and Mehrotra, 1971). Whereas El-Saidi (1973) reported that when the salts NaCl and $Na_2SO_4$ were added to the soil at 0.4, 0.7 and 1.0 per cent soil dry weight, cotton yields were reduced by 50 and 64 per cent respectively for 0.7 and 1.0 per cent salinity.

Janardhan *et al.* (1979), who studied the influence of saline water irrigation on kapas yield in cotton cultivars reported that the kapas yield indicated a linear reduction in yield with increased concentration of salts in irrigation water. Based on absolute yields and mean salinity indices, the cultivars Varalaxmi and Bhagya were found to be more tolerant and the reduction in kapas yield was attributed to lesser number of bolls per plant, Latif and Khan (1976) by subjecting cotton cultivar AC-134 to different salinity levels of 6, 12, 18 and 24 mmhos/cm at different growth stages found that the effect of salinity was most severe at germination. A salinity level of 6 mmhos/cm tended to increase yield of seed cotton, whereas, in plants subjected to 24 mmhos/cm salinity, there was reduction in fruiting branches, number of bolls and seed cotton yield.

The increasing levels of salinity from 3.9 to 21.4 dS $m^{-1}$ markedly suppressed the plant population and growth characteristics such as plant height, number of sympodia, boll number, boll weight, seed cotton yield and stalk dry weight of cotton. It was estimated that 50 per cent reduction in seed cotton yield of different cultivars was between 8.2 and 10.1 dS/m (Ahmad *et al.*, 1991). Similar results were reported by Sharma *et al.*, (1991) and Abd-Ella and Shalby (1993) in cotton.

Munk and Roberts (1995) evaluated eight Acala (*Gossypium hirsutum*) and four Pima (*Gossypium barbadense*) cotton cultivars for salt tolerance and reported that, plant height, total number of nodes and fruiting node number were all reduced with increase in soil salinity, while vegetative node number increased. Thus, plant maturity was delayed with a rise in soil solution salts. Average Acala cotton yields decreased by 100 lb lint/acre, while Pima yields were reduced by about 400 lb lint/acre.

Subbaiah *et al.* (1995) reported that with an increase in the soil salinity level from 0.94 to 24 dS/m there was a decrease in seed cotton yield. Uma and Patil (1996) reported that growth and yield parameters were reduced in cotton Cv. Sarvottam (*Gossypium herbaceum*), Laxmi (*G.hirsutum*) and G.Cot.15 (*G.arboreum*) with an increase in the salinity levels. The percentage reduction in total number of squares initiated, number of squares shed, number of bolls matured, growth and yield parameters at the highest salinity level were lower in *Gossypium herbaceum* than the other two species. A reduction in number of matured bolls/plant and a lower weight of seed cotton/boll were the two main yield components for yield reduction in all species at high salinity.

Vulkan *et al.* (1998) studied the effect of the amount of water and its salinity level on the yield of Pima cotton cultivar and reported that an increase in water salinity caused a reduction in the seed cotton yield and the salinity threshold increased with an increasing amount of water. The maximum yield of seed cotton of about 5000 Kg $ha^{-1}$ was obtained with a water application of 50cm and water salinity between 4 to 5 dS $m^{-1}$. Phogat *et al.* (2001) reported that, cotton (*Gossypium hirsutum*) genotypes viz., H-974, H-777, H-1098, H-182 and H-1170 showed a significant reduction in plant height and seed cotton yield with increasing salinity levels upto $EC_e$ 12 dS $m^{-1}$.

## 2.4 BIOPHYSICAL AND PHYSIOLOGICAL PARAMETERS

### 2.4.1 Stomatal density and size

In salt stressed kenaf, abaxial stomatal density increased with salinity but that was further offset by the greater decrease in leaf area,

which led to the decrease in total number of stomata. Adaxial stomata exhibited similar trend. The number of stomata in abaxial was 60 per cent greater than the number of adaxial stomata regardless of salt treatment (Curtis and Lauchli, 1987). Whereas, Jafri *et al.* (1995) reported that stomatal density decreased in cotton under salt stress and was compensated by an increase in stomatal size.

Buttery *et al.* (1992) observed increase in stomatal density as a result of water stress in soybean. They suggested that this be presumably brought about by decrease in leaf expansion but with little or no increase in stomatal number per leaf.

In non-halophytes, stomatal function was damaged by sodium ions under salt stress and disruption of normal regulation of transpiration may be a possible contributor to their inability to survive in salt laden soils (Robinson *et al.,* 1997).

### 2.4.2 Leaf area

Salinity known to affect leaf expansion. Leaf area indicates the size of assimilatory surface of plant. The rate of leaf expansion has greater influence on dry matter production.

The reduction in crop growth and yield by salinity has been well-documented (Maas and Hoffman, 1977), although different physiological processes have been put forward to account for this reduction in different species. Leaf elongation and decrease in photosynthetic capacity were attributed for the reduction under salt stress (Munns *et al.,* 1982 and Downtown, 1977).

Treatment of bean seedlings with low level of salinity (50 or 100 mM NaCl) decreased the rate of leaf cell elongation in primary leaves may be because of cell wall extensibility and cell turgor (Neumann *et al.*, 1988).

Curtis and Lauchli (1986) reported that Kenaf (*Hibiscus cannabinus* L.) plants grown at 37 mM NaCl differed significantly from control plants with respect to leaf area and showed 26 per cent reduction in leaf area. Similarly kenaf plants grown at 75 mM NaCl were more severely affected showing a 42 per cent reduction in leaf area at harvest.

Lahiri *et al.* (1987) reported that leaf area and dry weights of shoots were significantly reduced at salinity level beyond 4 dS $m^{-1}$ in cluster bean. Similarly Chetti (1980) reported that with the increase in salinity level, leaf area decreased in six cotton cultivars.

Brugnoli and Lauteri (1991) found that the plant growth, leaf area development and stomatal conductance were reduced due to salinity (Nabil and Coudret, 1995).

Brugnoli and Bjorkman (1992) reported that plants which were grown in flowing culture solutions containing 0, 26 and 55 per cent sea water, the ratio of leaf area to plant dry weight fell by 32 per cent in 26 per cent sea water and by 50 per cent in 55 per cent sea water in cotton Cv. Acala SJC-1.

Reduced growth in maize under salinity was attributed more to reduced leaf area than reduction in photosynthetic rate, further, the decrease in leaf area was attributed to reduction in osmotic potential of leaf cells (Patil *et al.*, 1996). Tolerant cotton Cv. Z-407 showed higher leaf,

stem, root growth, leaf area and number of leaves than sensitive genotype P-792 (Leidi and Saiz, 1997) under salinity. Leaf area and dry weights of shoots and roots of three broadbean *(Vicia faba* L.) lines, 900-3, 67 and 13 were significantly reduced with the increase in salinity (Radi *et al.*, 2001).

### 2.4.3 Photosynthesis, stomatal conductance and transpiration rate

Physiological parameters like photosynthesis, stomatal conductance and transpiration rate are greatly influenced by the external factors such as light, temperature and nutritional conditions. Gale and Poljakoff Mayber (1970) found that the reduced growth in saline media (NaCl or $Na_2SO_4$) was due to increase in stomatal resistance to $CO_2$ and chloroplast function did not appear to be the limiting factor.



Gale *et al.* (1967) demonstrated through *in vivo* studies that high salinity had an adverse effect on the light reduction of photosynthesis in cotton. Sankha and Huber (1974) reported that, sodium chloride inhibited the rate of 14 $CO_2$ fixation and the activities of PEP-carboxylase and RuBP-carboxylase, but increased the activities of both NAD- and NADP–specific malate dehydrogenase. A shift from $C_3$ to CAM in response to salinity has also been recorded by Winter and Luttge (1976) in *Mesembryanthemum Crystallinum.*

Reddy and Das (1978) observed that the rates of Hill reaction activity, photophosphorylation and NADP reducing activity of chloroplast ferredoxin in peanut were depressed under salt stress. Stress created by salinity caused plasmolysis, swelling of thylakoids and disappearance of chloroplastic ribosomes in *Gossypium hirsutum* (Vieira da silva, 1976).

Robinson *et al.* (1983) reported that for spinach, salt stress does not result in any major decrease in photosynthetic potential of leaf. Actual photosynthesis may be reduced by other factors such as decreased stomatal conductance and reduced leaf area.

A reduction in photosynthetic capacity resulting from salinity stress has been suggested to be a consequence of end product inhibition because of other salt inhibited reactions (Rawson and Munns, 1984). Non–stomatal inhibition of photosynthesis caused by direct effect of NaCl on photosynthetic apparatus independent of stomatal closure has also been reported (Seeman and Sharkey, 1986). This inhibition of photosynthesis has been attributed to a reduced efficiency of RuBP-carboxylase. Further, sensitivity of PS-II to NaCl salinity has also been reported to be a factor involved in reduction in photosynthetic capacity (Ball and Anderson, 1986). The quantum yield of leaves from *Phaseolus vulgaris* grown at 100 mM NaCl was approximately 25 per cent below that of control plants (Seeman and Critchley, 1985), while Brugnoli and Lauteri (1991) reported that the photochemical efficiency of cotton and bean plant was unaffected by salinity, since, apparent photon yield of $CO_2$ evolution was insensitive to salinity.

Garg *et al.* (1986) found that increasing salinity of irrigation water displayed a progressive decline in plant water potential and an increase in leaf diffusive resistance in cluster bean.

Leidi *et al.* (1993) observed a decrease in net photosynthesis, transpiration rate and stomatal conductance in cotton genotypes as soil water availability diminished. Similarly Patil *et al.* (1996) observed gradual

and significant reduction in the rate of photosynthesis with increase in salinity beyond 6 dS $m^{-1}$ in maize. Katergi *et al.* (1996) reported that stomatal conductance of salt sensitive sunflower was higher and was more severely affected by salinity than salt tolerant maize.

Dong *et al.* (1996) reported that, compared with the drought sensitive cotton cultivar Xuzhou-1818, the tolerant variety Wuganda-3 showed higher photosynthetic rate (11.8%) and lower stomatal conductance (58.1%) and transpiration rate (20.6%).

The net $CO_2$ assimilation rate decreased by 64 per cent in *Atriplex lentiformis* plants grown at 50 mM NaCl and decreased sharply above this salinity level (Jhu and Frederick, 1999).

Ashraf and O'Leary (1996) reported that stomatal conductance was reduced due to salt stress in all cultivars of wheat, however, salt tolerant cultivar S-24 and S-36 showed relatively higher stomatal conductance than other susceptible cultivars. Similarly, transpiration rate reduced consistently with increase in salinity, but S-36 and S-24 had higher transpiration rate than other lines.

## 2.5 BIOCHEMICAL CHARACTERS

### 2.5.1 Chlorophyll content

Garg and Garg (1982) observed genotypic differences in growth and metabolic performances of greengram under the influence of $Na_2CO_3$ and $NaHCO_3$. The concentration of chlorophyll and RNA decreased with increase in concentration of $Na_2CO_3$ and $NaHCO_3$. Both the salts ($Na_2CO_3$ and $NaHCO_3$) decreased chlorophyll content and net photosynthesis and

decrease was more in greengram compared with pea (Garg and Grag, 1985).

- NaCl markedly decreased linear growth, chlorophyll content and Indole Acetic Acid (IAA) content of leaves at 12 dS $m^{-1}$ in rice Cv. GR-3 (Prakash and Prathapasenan, 1989).

- Munjal and Goswami (1995) reported that NaCl (0,3 and 9 dS $m^{-1}$ $EC_e$) treatments decreased the total chlorophyll content in cotyledonary leaves of cotton. The reduction in chlorophyll under salinity was attributed to destruction of chlorophyll and instability of pigments and proteins (Somani, 1991). A reduction in chlorophyll content under NaCl salinity has been reported in mungbean by several investigations (Saha and Gupta, 1993 and Singh *et al.*, 1994).

The chlorophyll 'a' and chlorophyll 'b' contents gradually decreased with increasing salt intensity in mulberry and a relatively higher rate of depletion was found with chlorophyll 'a' than chlorophyll 'b' (Ramanujalu *et al.*, 1993). Similar reports were made by Sudhakar *et al.* (1991) and this was attributed to the increased chlorophyllase activity and also partly due to the interference of salt ions with *de novo* synthesis of proteins, the structural component of chloroplast. The chlorophyll 'a' and 'b' and total chlorophyll contents were reduced significantly at higher concentrations of salts in mungbean cultivars and variation between cultivars was evident (Ashraf and Rasul, 1988). It has been suggested that the specific enzyme, which is responsible for synthesis of chlorophyll, be suppressed by higher concentration of salt.

Among the two varieties of rice *(Oriza sativa)* viz., Nonabokra (salt tolerant) and IR-5931-110-1 (Salt sensitive), total chlorophyll and chlorophyll 'a' were higher in both the varieties and chlorophyll 'b' was higher only in Nonabokra grown in saline medium (EC= 5.2 dS $m^{-1}$) compared to control. Chlorophyll a/b ratio was lower in Nanabokra and higher in IR-5931-110-1 in saline medium compared to control (Boniface *et. al.*, 1992). Similarly chickpea genotypes, viz., SG-11 and DHG-84-11 (tolerant) showed minimum reduction in total chlorophyll., chlorophyll 'a' and chlorophyll 'b', compared to susceptible genotypes Pusa-256 and Phule G-5 (Singh and Singh, 1999).

The chlorophyll content of both *Vigna radiata* (Cv.Sujata) and *Brassica juncea* (Cv. Pusa Bold) plants decreased with 1 per cent NaCl, but there was an increase at 0.5 per cent in the more salinity resistant *Brassica juncea* (Sahu *et al.*, 1998).

### 2.5.2 Sugar content

Solute accumulation was considered as a suitable screening parameter for salinity tolerance (Ashraf *et al.*, 1991). Sugar is known to accumulate in plants under salinity.

Rathert (1983) while working on carbohydrate content of salt sensitive (Dandara) and salt tolerant (Giza 45) cultivars of cotton in relation to salinity reported that salt stress caused an increase in carbohydrate content in both varieties, sucrose consistently increased in both cultivars, but especially in Dandara. The NaCl treatments increased the concentration of soluble carbohydrate in elongating tissues of growing leaf of barley Cv. Beheer. This shows that photosynthesis was not limiting, since starch content did not change (Munns *et al.*, 1982).

Dubey (1987) concluded that sugar content decreased with increase in salt stress in rice seeds. The reduction in sugar may be attributed to suppressed amylase activity (Prasad, 1990) which cause reduction in the hydrolysis of polysaccharides.

Ding *et al.* (1995) reported that with increasing salinity protein content in cotton seedlings decreased while soluble sugar content increased. Whereas, Salam and Awadalla (1989) reported that soluble sugar content of cotton decreased with increase in salinity.

In rice, sucrose concentration was higher and starch and protein concentrations were lower in plants exposed to NaCl. The total carbohydrate content in resistant rice cultivars was higher than susceptible (Prakash and Prathapasenan, 1989).

Leidi and Saiz (1997) reported that, sugar and amino acid contents were higher in salt sensitive cotton cultivar P-792 than salt tolerant cultivar Z-407 under salinity stress.

### 2.5.3 Proline accumulation

Osmoprotectants such as proline and sugar are usually accumulated during exposure to salinity stress. These osmoprotectants help the plants to overcome stress condition (McNeil *et al.*, 1999). The proline is known to accumulate rapidly in a variety of crop plants in response to salt stress (Gill and Sharma, 1999). Accumulation of free proline under stress conditions has been taken as a criteria to screen genotypes for drought and salinity tolerance.

Free proline level in leaves increased rapidly in all the genotypes of plant species in response to salinity stress (Stewart and Lee, 1974) and in response to water deficit (Singh *et al.*, 1972).

Stewart and Lee (1974) observed the accumulation of proline in halophytes and level of proline increased with increase in salinity suggesting that capacity to accumulate proline was correlated with salt tolerance and also served as a source of solute for intercellular osmotic adjustment under saline conditions. Patil *et al.* (1996) found the differential accumulation of proline in 27 species. Some species found to accumulate more proline than others.

Chu *et al.* (1976) suggested that the accumulation of proline during both water and salinity stress followed as a consequence of reduction in cell osmotic potential. NaCl promoted less proline accumulation than did PEG at comparable water potential in intact barley roots.

Plunneke and Johan (1972) observed that in cotton a marginal increase in proline content was noticed under salt stress. The total free amino acid concentration in NaCl treated cotton leaves was considerably higher than the plants grown under normal conditions. The data suggested that sodium might have a role in the synthesis of amino acids in cotton plants.

Salinity decreased proline concentration more in the salt tolerant Cv. 9871-1 than in the more susceptible 149-F (Kuznetsov *et al.*, 1992). The induced proline synthesis and its accumulation observed in *Crotalaria strita* plants appeared to help the plants in tolerating the stress by acting as compatible solute (Chandrashekhar and Sandhyarani, 1996).

Proline accumulation is frequently reported in salt stressed plants. Although the precise role of this accumulation is still debated, proline is often considered to act as compatible solute involved in osmotic adjustment at plant cell level, because of high solubility it accumulates in cytoplasm without having any detrimental effects on cytosolic enzyme activities (Stewart and Lee, 1974). On the contrary, Manetas (1990) clearly demonstrated that proline decreased phosphoenol pyruvate carboxylase affinity towards substrate, As a result the role of proline in drought and salt stressed plants is still vigorously debated.

The highest salinity levels increased proline accumulation in the leaf and petiole – derived calli of cotton Cv. Acala SJ-2. The correlation between relative growth rate and proline concentration was -0.98 (Leaf callus) and -0.97 (Petiole callus). It is concluded that salinity reduced callus growth and proline acted as a compatible solute. (Lal-Hussain *et al.,* 1999).

Free proline content of six cotton cultivars increased with increasing water stress upto 11 days after the onset of water stress, then decreased, under greenhouse pot trial (Ronde *et al.*, 2000).

Salinity significantly enhanced the rate of proline accumulation in the dark induced senescing leaves of both salt sensitive (Ratna) and salt tolerant (Getu) rice cultivars, the trend being more pronounced in the salt tolerant than in the salt sensitive cultivar (Sahoo *et al.*, 2001).

## 2.6 ANATOMICAL CHARACTERS

*Gossypium arboreum* and *Gossypium herbaceum* were anatomically similar with well-developed adaxial pallisade parenchyma but less

developed abaxial pallisade. They were also characterized by low tissue ratio, thicker lamina and two layers of pallisade parenchyma which indicate their suitability for rainfed conditions (Singh, 1992).

David *et al.* (1979) observed that, increasing salinity led to substantially higher ratios of mesophyll surface area to leaf area ($A^{mes}/A$) for *Phaseolus vulgaris* and *Gossypium hirsutum* and a smaller increase for *Atriplex patula*, a salt-tolerant species.

The increase in thickness of leaves of the salt treated *Phaseolus vulgaris* plants was brought about by the increase in thickness of the spongy parenchyma layer. The pallisade parenchyma layer was thinner than the control leaves (Wignarajah *et al.*, 1975).

Jarfri *et al.* (1995) reported that, adoption to saline environments was adjusted by increasing mesophyll surface area to ensure normal exchange of gases and photosynthetic activities in cotton cultivars. Singh and Mohan (1999) attributed higher thickness of pallisade cells to resistance to abiotic stress in cotton.

# Material and Methods

# III. MATERIAL AND METHODS

A study was made with six genotypes of cotton including two cultivars of *Gossypium hirsutum viz.*, NHH-44 and LRA–5166, three cultivars of *Gossypium herbaceum viz.*, RAHS-14, Dhumad and Jayadhar and one cultivar of *Gossypium arboreum viz.*, AK-235, under four levels of salinity *viz.*, 0.8, 4.5, 8.0 and 14.8 dS $m^{-1}$ of EC. The present investigation was carried out in pot experiment during 2001-2002 at Agricultural Research Station, Dharwad.

## 3.1 EXPERIMENTAL SITE

Pot experiment was conducted at Agricultural Research Station, Dharwad.

## 3.2 SOIL PREPARATION AND MAINTENANCE OF SALINITY

The experiment was carried out in pots of size 2x1.5x1.5 feet. These pots were filled with finely ground soil of natural salinity levels brought from ARS, Gangavathi. Accordingly various salinity levels were fixed for the experiment and later on pots were irrigated with water containing NaCl, $CaCl_2$, $NaHCO_3$ and $MgSO_4$ in the ratio of 4:1.7:1 (Na:Mg:Ca) to maintain the required Electrical Conductivity (EC). At regular intervals, the EC of the soil was monitored and required EC be maintained till the experiment was completed.

## 3.3 CLIMATE

The Agricultural Research Station, Dharwad Farm, which is situated at an altitude of 678m above mean sea level at latitude 15° 26'

**Table 1. Monthly meteorological data for the year 2001- 02 and the average of past 28 years (1974 to 2001) recorded at Agricultural Research Station, Dharwad**

| Months | 2001- 02 | | 1974-2001(28 years) | | Relative Humidity (%) | Temperature(°C) | |
|---|---|---|---|---|---|---|---|
| | Rainfall (mm) | No. of rainy days | Rainfall (mm) | No. of rainy days | | Maximum | Minimum |
| April, 2001 | 53.8 | 5 | 45.2 | 03 | 69.5 | 35.7 | 22.0 |
| May, 2001 | 5.7 | 1 | 86.2 | 08 | 80.3 | 34.8 | 21.5 |
| June, 2001 | 65.4 | 10 | 106.2 | 13 | 91.7 | 30.3 | 21.3 |
| July, 2001 | 52.6 | 9 | 140.9 | 23 | 93.7 | 26.8 | 21.1 |
| Aug, 2001 | 89.8 | 12 | 91.9 | 21 | 99.4 | 27.2 | 20.9 |
| Sept, 2001 | 119.6 | 8 | 106.5 | 12 | 86.2 | 30.1 | 20.2 |
| Oct, 2001 | 17.4 | 5 | 126.0 | 11 | 84.3 | 30.1 | 19.9 |
| Nov, 2001 | 15.8 | 1 | 35.7 | 03 | 64.4 | 31.0 | 17.9 |
| Dec, 2001 | 2 | 1 | 7.5 | 0.8 | 65.4 | 29.6 | 18.7 |
| Jan, 2002 | 0 | 0 | 2.06 | 0.02 | 67.3 | 32.8 | 17.7 |
| Feb, 2002 | 0 | 0 | 0.90 | 0.01 | 64.0 | 34.25 | 20.14 |
| Mar, 2002 | 0 | 0 | 6.17 | 0.05 | 63.0 | 36.5 | 23.3 |
| **Total** | **422.1** | **52** | **755.53** | **94.88** | - | - | - |

North and at longitude 76° 7′ East and has well drained fertile medium black soil best suited for cotton cultivation. It receives an average annual rainfall of 780 mm.

The meteorological data (Table-1) revealed that seasonal conditions prevailing during the crop growth deviated from the average of past 25 years. Average monthly maximum and minimum temperatures recorded were 36.5° and 17.7°c respectively. The total rainfall received during the crop growth period was 743.6 mm which was less than the average of 25 years, but the number of rainy days was less over the average of 25 years.

### 3.4 EXPERIMENTAL DETAILS

There were 24 treatment combinations comprising of six genotypes of cotton and four salinity levels.

#### 3.4.1 Genotypes

Totally six cotton genotypes were taken for the study belonging to different species of cotton.

| *Gossypium hirsutum* | *Gossypium herbaceum* | *Gossypium arboreum* |
|---|---|---|
| NHH–44 | RAHS–14 | AK-235 |
| LRA–5166 | Dhumad | |
| | Jayadhar | |

#### 3.4.2 Salinity levels (dS $m^{-1}$)

| Salinity levels | $EC_e$ |
|---|---|
| $S_1$ | 0.8 dS $m^{-1}$ (control) |
| $S_2$ | 4.5 dS $m^{-1}$ |
| $S_3$ | 8.0 dS $m^{-1}$ |
| $S_4$ | 14.8 dS $m^{-1}$ |

### 3.4.3 Design and plan of layout

The treatment combinations were allotted to pots in a Split plot design with three replications (Figure 1).

## 3.5 CULTURAL PRACTICE

The soil samples having natural salinity gradient, brought from ARS, Gangavathi, were finely ground and filled in the pots according to various EC levels as mentioned above. The soil in the pots was loosened followed by addition of water containing salts to maintain required natural level of salinity.

### 3.5.1 Fertilizer application

Each pot was supplied with required quantities of fertilizer @ 150:75:75 N, P and K per hectare. Entire dose of fertilizer was applied on the day of sowing in all the treatments.

### 3.5.2 Sowing

Twenty seeds of each of the genotype were sown in each pot during June, 2001 and germination count was recorded till 30$^{th}$ day. Seedlings were thinned at 30 DAS (days after sowing) to maintain only five seedlings per pot for studying the morpho-physiological, biochemical and yield parameters.

### 3.5.3 After care

At 30 days after sowing rogor was sprayed to control jassids and thrips. In order to control leaf minor and bollworms, monocrotophos was sprayed twice.

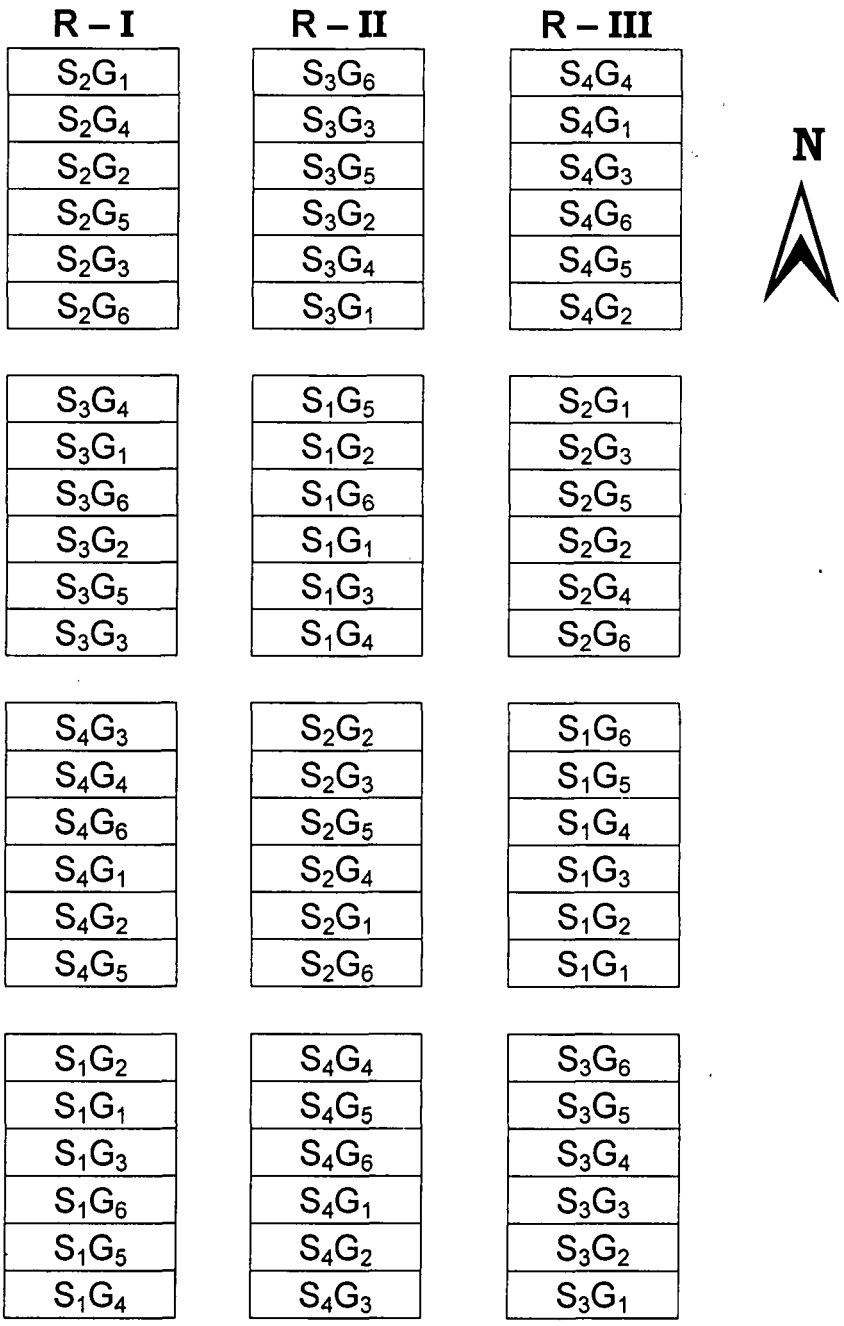


**Figure 1. Plan of layout of pot experiment**

**Genotypes**

$G_1$ – NHH-44
$G_2$ – LRA-5166
$G_3$ – RAHS-14
$G_4$ – Dhumad
$G_5$ – Jayadhar
$G_6$ – AK-235

**Salinity levels**

$S_1$- 0.8 dS $m^{-1}$ (control)
$S_2$- 4.5 dS $m^{-1}$
$S_3$- 8.0 dS $m^{-1}$
$S_4$- 14.8 dS $m^{-1}$

## 3.6 OBSERVATIONS



### 3.6.1 Germination percentage

Germination percentage was calculated at 30 DAS by counting the number of germinated seeds in each pot and expressed as percentage of the total number of seeds sown in each pot.

$$\text{Germination percentage} = \frac{100 \times \text{Number of seeds germinated}}{\text{Total number of seeds sown}}$$

### 3.6.2 Shoot length, root length and root to shoot ratio

Shoot length and root length was measured at 30 DAS by uprooting three plants samples in each replication and root to shoot ratio was calculated.

### 3.6.2 Shoot vigour index and root vigour index

Shoot and root vigour indices were calculated at 30 DAS as described by Abdul – Baki and Anderson (1973),

Shoot Vigour Index = Shoot length (cm) x Germination percentage

Root Vigour Index = Root length (cm) x Germination percentage

### 3.6.3 Dry matter production at 30 and 60 DAS

Total dry matter production, leaf dry weight and stem dry weight at 30 and 60 DAS were determined by uprooting three plant samples in each replication and oven dried at 72°c for 48 hours. Dry weights were recorded as gram per plant.

### 3.6.5 Leaf area

Leaf area was measured by disc method as suggested by Vivekanandan *et al.* (1972). Twenty leaf discs of known size were taken using the cork borer from two plants. Both discs and remaining leaf blades were oven dried at 75-80°c and the leaf area was calculated by using formula at different growth stages of crop growth.

$$LA = \frac{W_a \times A}{W_d}$$

Where,

LA = Leaf area ($dm^2$/plant)

$W_a$ = Weight of all leaves (Inclusive of 20 discs weight)

$W_d$ = Weight of discs (g)

A = Area of the disc

### 3.6.6 Determination of stomatal density and size

The study of stomatal frequency in epidermal cells was made by following the xylene- thermocole method.

To sufficient xylene, a piece of thermocole was added to until a thick paste was formed. The paste was smeared on both the surfaces of the leaf. After 5 minutes, the thermocole layer was peeled off carefully and mounted on a slide with a cover slip and observed under microscope. Number of stomata was recorded under microscope at 40x magnification and expressed in terms of number of stomata per square millimeter leaf area. At the same time, length and breadth of stomata were recorded using ocular and the stage micrometers.

### 3.6.7 Measurement of rate of photosynthesis (μmol $CO_2$ /$m^2$/sec), transpiration rate (mmol/$m^2$/sec) and stomatal conductance (μmol/$m^2$/sec)

Measurements of rate of photosynthesis, transpiration rate and stomatal conductance were made on the top fully expanded leaf at 30 and 60 DAS using portable photosynthesis system (LI – COR 6400). These measurements were made between 10.00 AM to 12.00 noon at all the sampling dates. Photosynthesis was expressed as μmol $CO_2$ $m^{-2}$ $sec^{-1}$, where as, transpiration rate was expressed as mmol $m^{-2}$ $sec^{-1}$ and stomatal conductance as μmol $m^{-2}$ $sec^{-1}$.

### 3.6.8 Biochemical parameters

#### 3.6.8.1 Estimation of chlorophyll (mg/g fresh weight)

Chlorophyll content in the leaves of different cotton genotypes subjected to different salinity levels was determined colorimetrically as per the DMSO (Dimethyl sulfoxide) method.

Fresh leaf tissue of 100 mg was cut into small pieces and incubated in 7 ml of DMSO at 65°c for 30 minutes. At the end of the incubation period, supernatant was decanted and leaf tissue was discarded. Volume was made to 10 ml with DMSO. Absorbance of the extract was read at 645, 652 and 663 nm using DMSO as blank. The chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents were calculated using the formulae,

Chlorophyll 'a' =12.7 ($A_{663}$) – 2.69 ($A_{645}$) x $\frac{V}{1000xWxa}$ (mg/g fresh weight)

Chlorophyll 'b' = 22.9 ($A_{645}$) – 4.68 ($A_{663}$) x $\frac{V}{1000xWxa}$ (mg/g fresh weight )

Total chlorophyll = 20.2 ($A_{645}$) + 8.02 ($A_{663}$) x $\frac{V}{1000xWxA}$ (mg/g fresh weight)

OR

= 27.8 ($A_{652}$) x $\frac{V}{1000xWxa}$ (mg/g fresh weight )

Where,

A = Absorbance at specific wave lengths (645, 652 and 663 nm)

V = Final volume of the chlorophyll extract (ml)

W = Fresh weight of the sample (g)

a = Path length of light (1 cm)

### 3.6.8.2 Measurement of total soluble sugars

The amount of total soluble sugars present in the leaf extract was estimated by anthrone method (Dobois *et al.*, 1951 and Yoshida, 1972.)

**Sample extraction**

Known quantities of dry leaf samples (0.2 to 0.3 g) were transferred to test tubes containing 80 per cent ethanol and were mixed well by stirring the test tubes for 3-5 minutes. The test tubes were then kept on hot water bath and allowed to boil for 10 minutes, cooled and filtered. The extraction process was repeated three to four times and the extracts were pooled. This was used for the estimation of total sugars by using anthrone reagent.

**Estimation of sugar (mg/g fresh weight)**

Test tubes containing alcohol extract were kept on hot water bath until most of the alcohol was removed and volume was made upto 25 ml by adding distilled water. From this extract, 2.3 ml was pipetted out to other test tubes. The test tubes were kept on ice bath for about 15-20 minutes and to which, 5 ml of 2 per cent anthrone reagent (0.2 g of anthrone was dissolved in 10 ml concentrated $H_2SO_4$) was allowed to rundown from the side of the test tube. The test tubes containing sample with anthrone reagent were boiled on hot water bath for exactly 7.5 minutes. Test tubes were removed from hot water bath immediately and cooled in ice bath and absorbance was measured at 630 nm in spectrophotometer. Total sugar content was calculated by using glucose standard curve and expressed in mg/g dry weight of leaf.

**3.6.8.3 Determination of proline content (µg / g dry weight)**

Free proline content in the leaves of different cotton genotypes subjected to different salinity levels was determined colorimetrically as per the method of Bates *et al.* (1973).

A known weight (0.5g) of fresh leaf sample was ground well in a morter using fine sand and extracted with 10 ml of 3 per cent sulphosalicylic acid. The extract was filtered and 2 ml of the filtrate was used for proline estimation. For this 2 ml of filtrate, 2 ml of acid ninhydrin reagent (2.5 g of ninhydrin was dissolved in 40 ml of 6 M orthophosphoric acid and 60 ml of glacial acetic acid) and 2 ml of glacial acetic acid were added and placed in a boiling water bath for one hour. Following this the test tubes containing the samples were transferred to

ice water bath for cooling. The content of each test tube was transferred to a separating funnel and 6 ml of toluene was added, shaken thoroughly and allowed for few minutes for separation of two layers. The lower layer was discarded and the upper toluene layer containing the colour complex was taken into a test tube. The optical density of the colour complex was calculated using the formula,

$$\text{Proline content } (\mu g\ g^{-1} \text{ dry weight}) = \frac{36.2311 \times OD \times V \times d}{2 \times f}$$

Where,

OD = Optical density at 520 nm.

V = Total volume of the extract in ml

d = Fresh weight / dry weight ratio.

f = Milligrams of fresh sample taken for proline estimation.

2 = Volume of the extract taken for proline estimation.

### 3.6.9 Anatomical characters

The study of meso-structure of leaf was made by following the Histological Paraffin technique followed by staining.

Slides of leaf sample were prepared by paraffin technique involving following steps.

**1. Tissue preparation and fixation**

Cotton leaf tissues of different genotypes at different salinity levels were collected separately at 90 DAS. Tissues were freed from dirt, sand and other materials and fixed using fixative.

| | |
|---|---|
| Fixative used was, Formalin-Acetic acid -Alcohol | - (FAA) |
| Ethyl Alcohol : 50% or 70% | - 90 ml |
| Glacial acetic acid | - 5 ml |
| Commercial formalin :40% formaldehyde | - 5 ml |

The tissue was fixed in this for 24 hours and stored in it indefinitely.

**2. Dehydration**

Dehydration is the process of removing water from the fixed and hardened tissues, which was done by transferring tissues to solvent paraffin.

a) Washing : Tissues were washed using 70% alcohol.

b) Gradual replacement of water : Water gradually replaced with alcohol.

70% - 70 cc absolute alcohol, 30 cc water

90% - 90 cc absolute alcohol, 10 cc water

100% - absolute alcohol

Tissue material was kept in each concentration at least for 2 hours.

c) Dehydration by butyl alcohol series (Paraffin solvent) :

To remove alcohol and replace paraffin solvent, tissues were kept in each of these mixtures for 2 hours each,

1) 3 parts alcohol + 1 part butanol
2) 1 part alcohol + 1 part butanol
3) 1 part alcohol + 3 parts butanol
4) Pure butanol

**3. Infiltration**

It is the process of gradual replacement of paraffin solvent with pure paraffin wax.

1. Tissue was placed in a small veil with enough butanol just to cover it. Equal quantity of molten paraffin was added to it and veil was placed in an oven at a temperature 5 C, higher than the melting point of paraffin used.

2. Approximately 4 hours after the paraffin has melted, paraffin was replaced with fresh molten paraffin taking care not to loose the tissue.

3. Above procedure was repeated again till the last trace of butanol was lost.

**4. Embedding**

1. Paper boat method was followed ; paper boats were prepared and coated inside with glycerin to peel off from the paraffin block easily after it was solidified.

2. Tissue with paraffin was taken and poured into the paper boat and enough molten paraffin was added to cover the tissue adequately.

3. Tissues were arranged and oriented using warm needle such that individual piece can be cut easily from finished block. Labels were also placed inside the block, which can be read when the block was solid.

4. After solidification of paraffin block, paper boat was peeled away from the block and block was stored in a cool location.

**5. Microtoming**

Paraffin infiltrated and embedded tissue was sectioned on a microtome, which is a delicate and an expensive instrument used to take thin and uniform sections at rapid rate. Sections were obtained in the form of ribbon.

A) Sectioning of paraffin material :

1. Paraffin block containing material were cut to convenient size and fixed to the holder by melting the block and pressed to the holder immediately and allowed it to solidify by placing in cold water.

2. Trimming :

Trimming was done before microtoming to remove excess paraffin from around tissue and to make the faces of the block that are going to strike the knife parallel to one another.

3. Sectioning :

After trimming, the block with holder was placed in the microtome. When the block was in place and correctly oriented, sections of 0.9 mm thickness were taken.

B) Affixing sections to slide :

1. Shorter unit of paraffin ribbon (approximately two thirds the length of the cover slip to be used) was placed on clean slide which was covered with a thin coating of gelatin adhesive (1 gm of gelatin + 100 cc warm

distilled water + 5 g of potassium dichromate → dissolved well and filtered) and segments were arranged neatly and compactly on the slide.

2. Slide then transferred to a slide warmer several degrees lower the melting point of paraffin. Formalin (4%) was added to tissue section to stretch as it warms. When the tissue was fully extended, slide was removed from the slide warmer and allowed it to cool.

**6. Staining**

Staining is the process of increasing the visibility and contrast of cell and tissue parts by their differential reactions to dyes.

1. Slides were deparaffinised by passing them in xylene, xylene : absolute alcohol (1 :1), 100% alcohol, 70% and 50% alcohol (5 minutes each)

2. Slides were stained in safranin (1% solution in 50% alcohol) for 2 hours.

3. Then slides were passed in 50%, 70% and 90% alcohol (3 minutes each).

4. Then slides were stained in fast green (0.5% solution in 95% alcohol) just for 3 to 5 minutes.

5. Sections were then dehydrated by using 50%, 70%, 90% and 100% alcohol (3minutes each).

6. Finally slides were cleared in xylene and mounted in DPx mount and observed under microscope at 10x X 10x magnification.

Size of the mesophyll cells, Pallisade cells and Spongy parenchyma cells was recorded using ocular and stage micrometer.

### 3.6.10 Yield and yield components

#### 3.6.10.1 Yield of seed cotton per plant

Cotton was picked separately from tagged plants. The number of bolls picked during each picking were counted and weighed. From this, mean yield of seed cotton per plant from over all pickings was worked out.

#### 3.6.10.2 Harvest Index

Harvest index was calculated by using the formula of Donald (1962) and expressed in percentage.

$$\text{Harvest Index} = \frac{\text{Economic yield}}{\text{Biological yield}} \times 100$$

#### 3.6.10.3 Number of bolls harvested per plant

At each picking the total number of bolls picked from tagged plants were counted and from this number of bolls harvested per plant was worked out.

#### 3.6.10.4 Mean boll weight

It was worked out by dividing the seed cotton yield per plant by the number of bolls harvested per plant.

### 3.6.11 Statistical analysis

Fisher method of analysis of variance was applied for the analysis of the data and interpretation of results as suggested by Panse and Sukhatme (1969). The level of significance used in 'F' and 't' test was, P=0.05. Critical Difference (CD) values were calculated at 5 per cent probability level, wherever 'F' tests was significant.

Correlation for various morpho-physiological, biochemical, anatomical and yield and yield components were worked out by the method as suggested by Steel and Torrie (1960).

# Experimental Results

# IV. EXPERIMENTAL RESULTS

The effect of different levels of salinity on six cotton genotypes belonging to different species *viz.*, NHH-44, LRA-5166, RAHS-14, Dhumad, Jayadhar and AK-235 was studied in pots during the year 2001-2002. The genotypes were evaluated for germination, growth and yield parameters and changes in physiological, biochemical and anatomical characters. The data recorded on these parameters in pot experiment was statistically analyzed. The results of the pot experiment are presented in this chapter.

## 4.1 GERMINATION PERCENTAGE, SHOOT LENGTH (cm) AND ROOT LENGTH (cm)

The data on germination percentage, shoot length (cm) and root lengths (cm) of six cotton genotypes in response to salinity levels are presented in Table 2.

### 4.1.1 Germination percentage

The results indicated significant difference among cotton genotypes and salinity levels. The interaction effect between cotton genotypes and salinity levels was non-significant.

Among cotton genotypes, NHH-44 had significantly higher germination percentage (81.60) and was on par with LRA-5166 (78.73). Significantly lowest germination percentage was recorded in Dhumad (71.91).

Significant difference in the germination percentage was observed due to salinity levels. Salinity level $S_1$ (0.8 dS $m^{-1}$) had higher germination

**Table 2. Effect of salinity on germination (%), shoot length (cm) and root length (cm) in cotton**

| Genotypes | Germination percentage | | | | | Shoot length | | | | | Root length | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 93.12 | 89.15 | 78.92 | 65.22 | 81.60 | 16.2 | 13.8 | 10.9 | 08.5 | 12.4 | 8.7 | 6.9 | 6.5 | 6.1 | 7.1 |
| LRA-5166 | 90.35 | 86.87 | 74.34 | 63.36 | 78.73 | 14.7 | 11.6 | 09.8 | 07.6 | 10.9 | 8.5 | 6.4 | 6.3 | 5.8 | 6.8 |
| RAHS-14 | 89.97 | 81.24 | 73.52 | 61.54 | 76.57 | 12.1 | 10.1 | 07.8 | 06.7 | 09.2 | 7.3 | 6.1 | 4.9 | 4.8 | 5.8 |
| DHUMAD | 86.13 | 79.17 | 66.93 | 55.41 | 71.91 | 12.4 | 09.5 | 06.6 | 05.3 | 08.5 | 7.4 | 6.7 | 5.8 | 5.1 | 6.3 |
| JAYADHAR | 89.15 | 80.49 | 70.95 | 60.14 | 75.18 | 11.1 | 09.3 | 07.6 | 06.2 | 08.6 | 6.7 | 6.1 | 5.3 | 5.0 | 5.8 |
| AK-235 | 87.67 | 82.73 | 74.17 | 63.33 | 76.98 | 10.6 | 09.8 | 08.2 | 06.4 | 08.8 | 6.6 | 6.0 | 5.2 | 5.1 | 5.7 |
| Mean | 89.40 | 83.28 | 73.14 | 61.50 | 76.83 | 12.9 | 10.7 | 08.5 | 06.8 | 09.7 | 7.5 | 6.4 | 5.7 | 5.3 | 6.2 |
| For comparing | SEm± | | CD at 5% | | | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 1.81 | | 5.02 | | | 0.30 | | 0.83 | | | 0.21 | | 0.58 | | |
| Salinity (S) | 1.37 | | 4.74 | | | 0.19 | | 0.66 | | | 0.14 | | 0.48 | | |
| Interaction (GxS) | 3.62 | | NS | | | 0.60 | | 1.66 | | | 0.42 | | 1.16 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

percentage (89.40), where as, $S_4$ (14.8 dS $m^{-1}$) showed significantly lowest germination per cent (61.50).

Interaction effect was found to be non-significant. However, genotype NHH-44 showed higher germination percentage in $S_1$ salinity level (93.12) and least was recorded by Dhumad (55.41) in $S_4$ salinity level.

### 4.1.2 Shoot length (cm)

Shoot length differed significantly at 30 DAS among cotton genotypes, salinity levels and interaction between genotypes and salinity levels.

NHH-44 had significantly higher shoot length (12.4). Under all salinity levels it had higher shoot length. Significantly lower shoot length was observed in Dhumad (8.5) and it was on par with Jayadhar (8.6) and AK-235 (8.8).

Salinity levels had significant effect on shoot length. Shoot length decreased significantly from $S_1$ (12.9) to $S_4$ (6.8).

The interaction effect of genotypes and salinity levels indicated that NHH-44 recorded higher shoot length under salinity level $S_1$ (16.2). The genotype Dhumad at $S_4$ (14.8 dS $m^{-1}$) salinity level recorded the lowest shoot length (5.3).

### 4.1.3 Root length (cm)

Significant difference was observed in root length among cotton genotypes, salinity levels and in genotypes x salinity interaction.

The root length was maximum in NHH-44 (7.1) followed by LRA-5166 (6.8) and these differed significantly with other genotypes. Lowest root length was observed in the genotype AK-235 (5.7).

Significant reduction in the root length was observed as the salinity levels increased from 0.8 to 14.8 dS $m^{-1}$. The root length was maximum in $S_1$ (7.5). Higher salinity level of $S_4$ showed significantly lower root length (5.3).

Interaction effect of genotypes and salinity levels showed that significantly higher root length was noticed in NHH-44 (8.7) at $S_1$ followed by LRA-5166 at $S_1$ (8.5) and these differed significantly with other genotypes. Significantly lower root length was recorded in Jayadhar (5.0) at $S_4$.

## 4.2 ROOT TO SHOOT RATIO, SHOOT VIGOUR INDEX AND ROOT VIGOUR INDEX

The data on the effect of genotypes and salinity levels on root to shoot ratio, shoot vigour index and root vigour index is presented in Table 3.

### 4.2.1 Root to shoot ratio

Genotypes, salinity levels and interaction effect were found to differ significantly for root to shoot ratio at 30 DAS.

Dhumad recorded significantly higher root to shoot ratio (0.786) over all other genotypes. Similarly NHH-44 had significantly lower root to shoot ratio (0.588). Next lowest root to shoot ratio was observed in LRA-5166 (0.634) and it was on par with RAHS-14 (0.636).

**Table 3. Effect of salinity on root to shoot ratio, shoot vigour index and root vigour index in cotton**

| Genotypes | Root to shoot ratio | | | | | Shoot vigour index | | | | | Root vigour index | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 0.537 | 0.500 | 0.596 | 0.718 | 0.588 | 1509 | 1230 | 853 | 554 | 1037 | 810 | 615 | 513 | 398 | 584 |
| LRA-5166 | 0.578 | 0.552 | 0.643 | 0.763 | 0.634 | 1328 | 1009 | 729 | 482 | 0887 | 768 | 556 | 468 | 368 | 540 |
| RAHS-14 | 0.603 | 0.604 | 0.628 | 0.709 | 0.636 | 1089 | 0821 | 573 | 412 | 0724 | 657 | 496 | 360 | 295 | 452 |
| DHUMAD | 0.597 | 0.705 | 0.879 | 0.962 | 0.786 | 1068 | 0752 | 442 | 294 | 0639 | 637 | 530 | 388 | 282 | 459 |
| JAYADHAR | 0.604 | 0.656 | 0.697 | 0.807 | 0.691 | 0990 | 0749 | 539 | 373 | 0663 | 597 | 491 | 376 | 301 | 441 |
| AK-235 | 0.623 | 0.612 | 0.634 | 0.797 | 0.667 | 0929 | 0811 | 608 | 405 | 0688 | 579 | 496 | 386 | 323 | 446 |
| Mean | 0.590 | 0.605 | 0.678 | 0.793 | 0.667 | 1152 | 0895 | 624 | 420 | 0773 | 675 | 531 | 415 | 328 | 487 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.02 | | 0.06 | | | 24.99 | | 69.27 | | | 15.12 | | 41.91 | | |
| Salinity (S) | 0.01 | | 0.04 | | | 12.76 | | 44.16 | | | 09.33 | | 32.29 | | |
| Interaction (GxS) | 0.04 | | 0.11 | | | 49.99 | | 138.57 | | | 30.23 | | 83.79 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

Root to shoot ratio differed significantly due to salinity levels from $S_1$ to $S_4$. Root to shoot ratio increased significantly from $S_1$ (0.590) to $S_4$ (0.793).

The interaction effect of genotypes and salinity levels indicated that Dhumad recorded higher root to shoot ratio under salinity level $S_4$ (0.962). The genotype NHH-44 at $S_2$ (4.5 dS $m^{-1)}$ salinity level recorded the lowest root to shoot ratio (0.500).

### 4.2.2 Shoot vigour index

Significant difference was noticed in shoot vigour index among cotton genotypes, salinity levels and genotypes x salinity interaction.

Irrespective of salinity levels, the genotype NHH-44 recorded significantly higher shoot vigour index (1037) over all other genotypes. Significantly lower shoot vigour index of 639 was observed in Dhumad as compared to rest of the genotypes.

Salinity levels had significant effect on shoot vigour index. Shoot vigour index decreased significantly from $S_1$ (1152) to $S_4$ (420).

The interaction effect of genotypes and salinity levels indicated that NHH-44 recorded higher shoot vigour index under salinity level $S_1$ (1509). The genotype Dhumad at $S_4$ (14.8 dS $m^{-1}$) salinity level recorded the lowest shoot vigour index (294).

### 4.2.3 Root vigour index

Significant differences were observed among cotton genotypes, salinity levels and interaction for root vigour index.

NHH-44 had significantly higher root vigour index (584). Under all salinity levels it had higher root vigour index. Significantly lower root vigour index was observed in Jayadhar (441) and it was on par with AK-235 (446).

Root vigour index differed significantly due to salinity levels from $S_1$ to $S_4$. $S_1$ recorded significantly higher root vigour index (675). Highest salinity level of $S_4$ recorded the least root vigour index (328) and differed significantly with all the remaining salinity levels.

Significant interaction effect was observed with NHH-44, which had significantly higher root vigour index at $S_1$ level (810). This was followed by LRA-5166 at $S_1$ level (768). However, significantly lower root vigour index (282) was observed in Dhumad at $S_4$ (14.8 dS $m^{-1}$)

## 4.3 TOTAL DRY MATTER (g/plant)

The results of total dry matter (g/plant) at 30 and 60 DAS are presented in Table 4 and Figure 2.

### 4.3.1 Total dry matter (g/plant) at 30 DAS

Total dry matter differed significantly at 30 DAS among cotton genotypes, salinity levels and interaction between genotypes and salinity levels.

The dry matter accumulation was maximum in NHH-44 (0.54) followed by LRA-5166 (0.48) and these differed significantly with other genotypes. Lowest dry matter accumulation was observed in the genotype Dhumad (0.28).

**Table 4. Effect of salinity on total dry matter (g/plant) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 0.68 | 0.59 | 0.50 | 0.39 | 0.54 | 5.84 | 4.71 | 3.92 | 3.21 | 4.42 |
| LRA-5166 | 0.63 | 0.51 | 0.43 | 0.34 | 0.48 | 5.35 | 4.28 | 3.27 | 2.66 | 3.89 |
| RAHS-14 | 0.49 | 0.43 | 0.36 | 0.29 | 0.39 | 4.43 | 3.72 | 3.36 | 2.43 | 3.49 |
| DHUMAD | 0.39 | 0.31 | 0.24 | 0.18 | 0.28 | 3.50 | 2.58 | 1.72 | 0.84 | 2.16 |
| JAYADHAR | 0.47 | 0.36 | 0.30 | 0.25 | 0.35 | 3.72 | 3.39 | 2.54 | 1.51 | 2.79 |
| AK-235 | 0.44 | 0.39 | 0.33 | 0.27 | 0.36 | 4.35 | 3.62 | 3.11 | 2.65 | 3.43 |
| Mean | 0.52 | 0.43 | 0.36 | 0.29 | 0.40 | 4.53 | 3.72 | 2.99 | 2.22 | 3.36 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.02 | | 0.06 | | | 0.14 | | 0.39 | | |
| Salinity (S) | 0.01 | | 0.04 | | | 0.08 | | 0.28 | | |
| Interaction (GxS) | 0.04 | | 0.11 | | | 0.27 | | 0.75 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

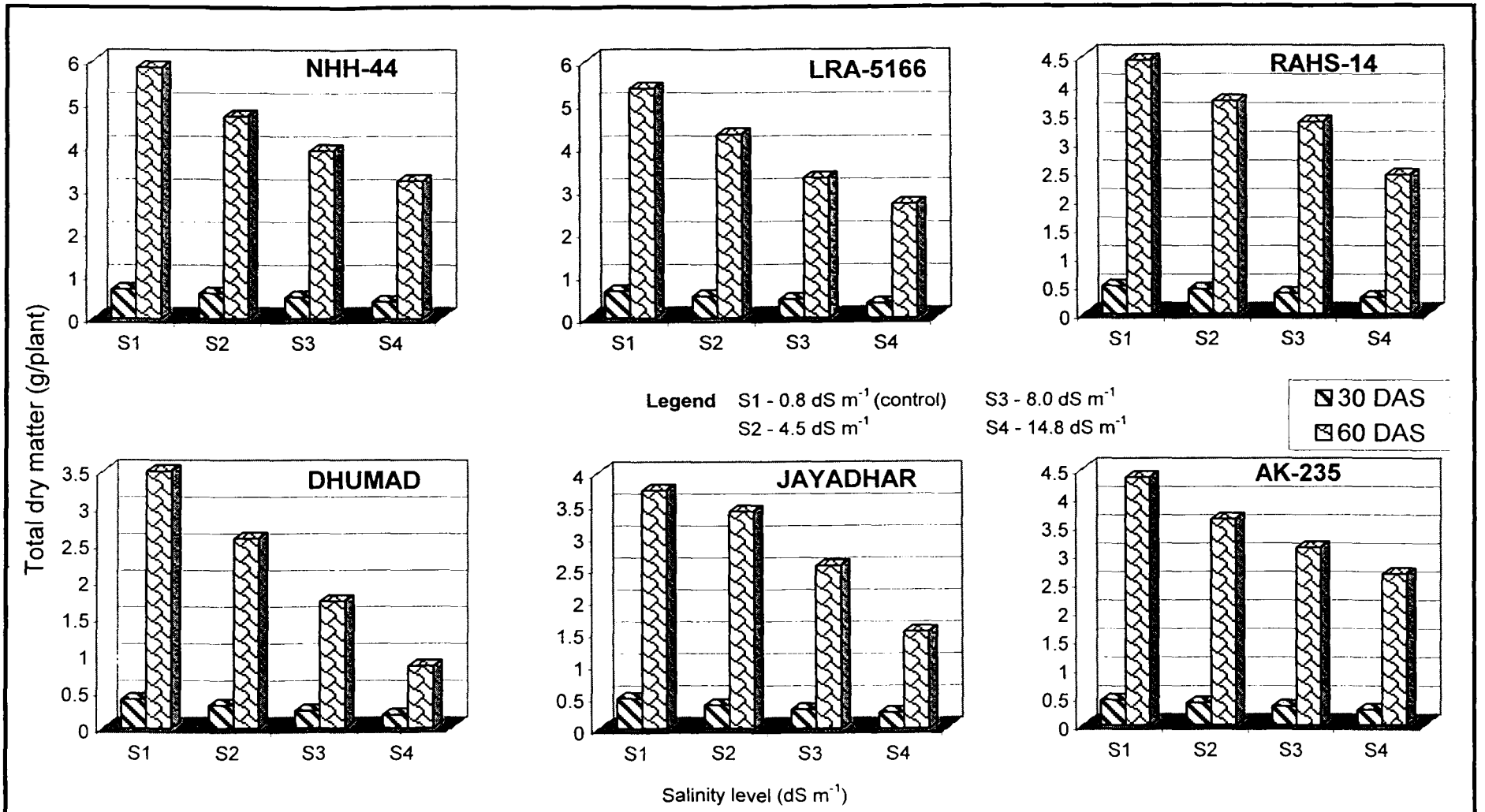


**Figure 2. Effect of salinity on total dry matter (g/plant) at different growth stages in cotton genotypes**

Significant reduction in the total dry mater was observed as the salinity levels increased from 0.8 to 14.8 dS $m^{-1}$. The total dry matter was maximum in $S_1$ (0.52). Higher salinity level of $S_4$ showed significantly lower dry matter (0.29).

Interaction effect of genotypes and salinity levels showed that significantly higher dry matter was noticed in NHH-44 at $S_1$ (0.68) followed by LRA-5166 at $S_1$ (0.63) and these differed significantly with other genotypes. Significantly lower dry matter was observed in Dhumad (0.18) at $S_4$ salinity level

### 4.3.2 Total dry matter (g/plant) at 60 DAS

Genotypes, salinity and their interaction differed significantly in the total dry matter production at 60 DAS.

Total dry matter was significantly higher with genotype NHH-44 (4.42) as compared to other genotypes. It accumulated 51 per cent more dry matter compared to the lowest performing genotype Dhumad (2.16). The genotype LRA-5166 showed greater dry matter accumulation (3.89) after NHH-44 followed by RAHS-14 (3.49) and AK-235 (3.43).

Lower salinity level of $S_1$ plants had significantly higher total dry matter (4.53) compared to plants in higher salinity $S_4$ (2.22). The deleterious effect of salinity was more at 60 DAS after sowing compared to 30 DAS after sowing.

Interaction effect indicated that the genotype NHH-44 accumulated significantly more dry matter at $S_1$ (5.84) followed by LRA-5166 at $S_1$ (5.35). The genotype Dhumad showed the least total dry matter (0.84) at $S_4$ level of salinity followed by Jayadhar (1.51) at $S_4$ (14.8 dS $m^{-1}$) level

## 4.4 LEAF DRY WEIGHT (g/plant)

The results of leaf dry weight (g/plant) at 30 and 60 DAS are presented in Table 5.

### 4.4.1 Leaf dry weight (g/plant) at 30 DAS

Leaf dry weight differed significantly at 30 DAS among cotton genotypes, salinity levels and interaction between genotypes and salinity levels.

The maximum leaf dry weight was observed in NHH-44 (0.34) followed by LRA-5166 (0.29) and these differed significantly with other genotypes. The least (0.16) was observed in Dhumad.

Significant reduction in the leaf dry matter was observed as the salinity levels increased from $S_1$ (0.8 dS $m^{-1}$) to $S_4$ (14.8 dS $m^{-1}$). The leaf dry weight was maximum in $S_1$ (0.30). Significantly lower leaf dry weight (0.16) was observed in higher salinity level $S_4$ (14.8 dS $m^{-1}$).

Interaction effect of genotypes and salinity levels, showed that significantly higher leaf dry weight was noticed in NHH-44 at $S_1$ (0.43) followed by LRA-5166 at $S_1$ (0.39) and NHH-44 at $S_2$ (0.38) and these differed significantly with other genotypes. Significantly lower leaf dry weight was observed in Dhumad at $S_4$ (0.10).

### 4.4.2 Leaf dry weight (g/plant) at 60 DAS

Genotypes, salinity and their interaction differed significantly in the leaf dry weight at 60 DAS.

**Table 5. Effect of salinity on leaf dry weight (g/plant) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 0.43 | 0.38 | 0.31 | 0.22 | 0.34 | 3.21 | 2.58 | 2.21 | 1.81 | 2.45 |
| LRA-5166 | 0.39 | 0.30 | 0.26 | 0.19 | 0.29 | 2.95 | 2.38 | 1.76 | 1.27 | 2.09 |
| RAHS-14 | 0.28 | 0.24 | 0.21 | 0.16 | 0.22 | 2.48 | 2.07 | 1.77 | 1.28 | 1.90 |
| DHUMAD | 0.23 | 0.18 | 0.14 | 0.10 | 0.16 | 2.01 | 1.37 | 0.91 | 0.38 | 1.17 |
| JAYADHAR | 0.25 | 0.20 | 0.17 | 0.15 | 0.19 | 2.18 | 1.88 | 1.36 | 0.86 | 1.57 |
| AK-235 | 0.24 | 0.21 | 0.18 | 0.15 | 0.20 | 2.43 | 1.95 | 1.49 | 1.29 | 1.79 |
| Mean | 0.30 | 0.25 | 0.21 | 0.16 | 0.23 | 2.54 | 2.04 | 1.58 | 1.15 | 1.83 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.008 | | 0.022 | | | 0.06 | | 0.17 | | |
| Salinity (S) | 0.005 | | 0.017 | | | 0.04 | | 0.14 | | |
| Interaction (GxS) | 0.015 | | 0.042 | | | 0.12 | | 0.33 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

Leaf dry weight was significantly higher with genotype NHH-44 (2.45) as compared to other genotypes. Low leaf dry weight was observed in the genotype Dhumad (1.17).

Significant reduction in the leaf dry weight was observed as the salinity levels increased from 0.8 to 14.8 dS $m^{-1}$. The leaf dry weight was maximum in $S_1$ (2.43). Higher salinity level of $S_4$ showed significantly lower leaf dry weight (1.29).

Interaction effect of genotypes and salinity levels showed that significantly higher leaf dry weight was noticed in NHH-44 at $S_1$ (3.21) followed by LRA-5166 (2.95) at $S_2$ level of salinity. Significantly lower leaf dry weight was noticed in Dhumad (0.38) at $S_4$ salinity level.

**4.5 STEM DRY WEIGHT (g/plant)**

The results of stem dry weight (g/plant) at 30 and 60 DAS are presented in Table 6.

**4.5.1 Stem dry weight (g/plant) at 30 DAS**

Stem dry weight differed significantly at 30 DAS among cotton genotypes, salinity levels and interaction between genotypes and salinity levels.

The maximum stem dry weight was noticed in NHH-44 (0.21) and this differed significantly with other genotypes. The least was recorded in Dhumad (0.12).

Significant reduction in the stem dry weight was observed as the salinity levels increased from $S_1$ to $S_4$. The stem dry weight was maximum

**Table 6. Effect of salinity on stem dry weight (g/plant) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| **NHH-44** | 0.25 | 0.22 | 0.19 | 0.17 | 0.21 | 2.63 | 2.13 | 1.71 | 1.40 | 1.97 |
| **LRA-5166** | 0.24 | 0.21 | 0.17 | 0.15 | 0.19 | 2.40 | 1.90 | 1.51 | 1.39 | 1.80 |
| **RAHS-14** | 0.21 | 0.19 | 0.15 | 0.13 | 0.17 | 1.95 | 1.65 | 1.59 | 1.15 | 1.59 |
| **DHUMAD** | 0.16 | 0.13 | 0.10 | 0.08 | 0.12 | 1.49 | 1.21 | 0.81 | 0.46 | 0.99 |
| **JAYADHAR** | 0.22 | 0.16 | 0.13 | 0.10 | 0.15 | 1.54 | 1.51 | 1.18 | 0.65 | 1.22 |
| **AK-235** | 0.20 | 0.18 | 0.15 | 0.12 | 0.16 | 1.92 | 1.67 | 1.63 | 1.36 | 1.65 |
| **Mean** | 0.21 | 0.18 | 0.15 | 0.13 | 0.17 | 1.99 | 1.68 | 1.41 | 1.07 | 1.54 |
| **For comparing** | **S.Em±** | | **CD at 5%** | | | **S.Em±** | | **CD at 5%** | | |
| **Genotypes (G)** | 0.010 | | 0.028 | | | 0.05 | | 0.14 | | |
| **Salinity (S)** | 0.007 | | 0.024 | | | 0.03 | | 0.10 | | |
| **Interaction (GxS)** | 0.020 | | 0.055 | | | 0.10 | | 0.28 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

in $S_1$ (0.20). Significantly lower stem dry weight (0.12) was observed in higher salinity level $S_4$.

Interaction effect of genotypes and salinity levels indicated that NHH-44 recorded higher stem dry weight under salinity level $S_1$ (0.25) followed by LRA-5166 at $S_1$ (0.24). The genotype Dhumad at $S_4$ (14.8 dS $m^{-1}$) salinity level recorded the lowest stem dry weight of 0.08.

### 4.5.2 Stem dry weight (g/plant) at 60 DAS

Stem dry weight was significantly influenced by genotypes, salinity levels and interaction of genotypes x salinity at 60 DAS.

Irrespective of salinity levels, significantly higher stem dry weight was observed in NHH-44 (1.97) and was on par with LRA-5166 (1.80). The genotype Dhumad had least stem dry weight of 0.99 g/plant.

Increase in the salinity from $S_1$ (0.8 dS $m^{-1}$) to $S_4$ (14.8 dS $m^{-1}$) decreased the stem dry weight significantly. Stem dry weight was maximum in $S_1$ (1.92) and the least (1.36) was observed in $S_4$.

Interaction effect showed higher stem dry weight in NHH-44 with $S_1$ level of salinity (2.63) followed by LRA-5166 (2.40) at $S_1$. The genotype Dhumad had significantly lower stem dry weight of 0.46 g/plant at $S_4$ (14.8 dS $m^{-1}$) salinity level.

## 4.6 LEAF AREA ($dm^2$/ plant)

Leaf area of cotton genotypes as influenced by salinity levels at 30 and 60 DAS is presented in Table 7.

**Table 7. Effect of salinity on leaf area ($dm^2$/plant) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| **NHH-44** | 4.53 | 4.18 | 3.03 | 2.42 | 3.54 | 25.61 | 23.19 | 20.92 | 17.63 | 21.84 |
| **LRA-5166** | 4.42 | 3.76 | 2.82 | 2.31 | 3.33 | 24.31 | 22.36 | 18.16 | 15.41 | 20.06 |
| **RAHS-14** | 3.45 | 3.11 | 2.57 | 1.68 | 2.70 | 24.56 | 21.29 | 17.61 | 14.39 | 19.46 |
| **DHUMAD** | 2.56 | 2.19 | 1.95 | 1.43 | 2.03 | 22.58 | 19.46 | 14.87 | 10.68 | 16.90 |
| **JAYADHAR** | 3.40 | 2.42 | 2.06 | 1.59 | 2.37 | 23.87 | 20.78 | 16.72 | 13.57 | 18.74 |
| **AK-235** | 3.21 | 2.56 | 2.16 | 1.86 | 2.45 | 25.12 | 20.18 | 16.59 | 15.68 | 19.89 |
| **Mean** | 3.60 | 3.04 | 2.43 | 1.88 | 2.74 | 24.34 | 21.54 | 17.48 | 14.56 | 19.48 |
| **For comparing** | **S.Em±** | | **CD at 5%** | | | **S.Em±** | | **CD at 5%** | | |
| **Genotypes (G)** | 0.06 | | 0.17 | | | 0.49 | | 1.36 | | |
| **Salinity (S)** | 0.04 | | 0.14 | | | 0.31 | | 1.07 | | |
| **Interaction (GxS)** | 0.20 | | 0.55 | | | 1.17 | | 3.24 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

### 4.6.1 Leaf area ($dm^2$/plant) at 30 DAS

Genotypes, salinity levels and interaction effect were found to differ significantly for leaf area at 30 DAS.

NHH-44 recorded significantly higher leaf area (3.54) and was on par with LRA-5166 (3.33). Similarly, Dhumad had lowest leaf area (2.03). Next lowest leaf area was observed in Jayadhar (2.37), which was on par with AK-235 (2.45).

Increase in the salinity from $S_1$ to $S_4$ decreased the leaf area significantly. Leaf area in $S_1$ was 3.60 $dm^2$ $plant^{-1}$ and the least (1.88) was observed in $S_4$.

Interaction effect was significant with NHH-44 having highest leaf area at $S_1$ (4.53) followed by LRA-5166 at $S_1$ (4.42). The genotype Dhumad had significantly the lower leaf area of 1.43 $dm^2$ $plant^{-1}$ at $S_4$ (14.8 dS $m^{-1}$) level.

### 4.6.2 Leaf area ($dm^2$/plant) at 60 DAS

Leaf area was significantly influenced by genotypes, salinity levels and interaction of genotypes x salinity at 60 DAS.

Irrespective of salinity levels, significantly higher leaf area was observed in NHH-44 (21.84) over all other genotypes. The genotype Dhumad had the least leaf area of 16.90 $dm^2$ $plant^{-1}$. Next lowest leaf area was observed in Jayadhar (18.74) followed by AK-235 (19.89) and RAHS-14 (19.46).

Salinity levels had the similar effect on leaf area at 60 DAS as it was observed at 30 DAS. Lower salinity level $S_1$ had maximum leaf area (24.34). Significantly lower leaf area was observed in $S_4$ (14.56).

Interaction effect showed higher leaf area in NHH-44 with $S_1$ level of salinity (25.61) followed by AK-235 (25.12) at $S_1$. The genotype Dhumad had significantly lower leaf area of 10.68 $dm^2$ $plant^{-1}$ at $S_4$ (14.8 dS $m^{-1}$) salinity level.

## 4.7 STOMATAL DENSITY (no. $mm^{-2}$), LENGTH (μm) AND BREADTH (μm)

The data on the effect of genotypes and salinity levels on stomatal density, length and breadth are presented in Tables 8, 9 and 10.

### 4.7.1 Stomatal density (no. $mm^{-2}$) on abaxial and adaxial leaf surfaces

Stomatal density on both upper and lower surfaces was found to be significant for genotypes, salinity and genotype x salinity interaction (Table 8).

In all the genotypes, stomatal density was more on lower surface in comparison to the upper surface. Significantly higher stomatal density on both abaxial and adaxial leaf surfaces was observed in LRA-5166 (244.6 and 140.5 respectively) followed by Dhumad on both surfaces (243.0 and 138.5 respectively) as compared to other genotypes. However, RAHS-14 possessed the lowest density (233.7 and 131.6 respectively) on both abaxial and adaxial surfaces.

Genotypes AK-235 on lower leaf surface and NHH-44 on upper leaf surface showed lower per cent increase in stomatal density, where as, genotypes Dhumad and Jayadhar showed maximum increase in stomatal density on both surfaces.

**Table 8. Effect of salinity on stomatal density (no. $mm^{-2}$) on abaxial and adaxial leaf surfaces at 50 DAS in cotton**

| Genotypes | Abaxial | | | | | Adaxial | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 227.6 | 232.8 | 234.5 | 251.3 | 236.6 | 121.5 | 127.4 | 136.3 | 143.2 | 132.1 |
| LRA-5166 | 232.3 | 240.8 | 248.4 | 256.7 | 244.6 | 129.6 | 137.0 | 143.1 | 152.4 | 140.5 |
| RAHS-14 | 221.8 | 229.3 | 236.6 | 247.1 | 233.7 | 120.9 | 125.5 | 133.3 | 146.6 | 131.6 |
| DHUMAD | 227.7 | 237.6 | 247.2 | 259.6 | 243.0 | 121.3 | 132.6 | 144.2 | 153.7 | 138.5 |
| JAYADHAR | 224.6 | 233.4 | 243.3 | 254.3 | 238.2 | 123.5 | 131.5 | 142.6 | 151.2 | 137.2 |
| AK-235 | 226.5 | 231.8 | 242.6 | 248.8 | 237.4 | 121.5 | 129.7 | 137.6 | 145.9 | 133.7 |
| Mean | 226.8 | 234.3 | 242.1 | 253.0 | 239.1 | 123.1 | 130.6 | 139.5 | 148.8 | 135.5 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 6.93 | | 19.21 | | | 3.93 | | 10.89 | | |
| Salinity (S) | 4.06 | | 14.05 | | | 2.67 | | 9.24 | | |
| Interaction (GxS) | 13.85 | | 38.39 | | | 7.85 | | 21.76 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

Increase in the salinity levels increased the stomatal density on both the surfaces. Significantly more number of stomata were observed on lower surface. Lower surface at $S_1$ level had the stomatal density of 226.8 $mm^{-2}$, while $S_4$ had 253.0 stomata $mm^{-2}$. Similarly for upper surface $S_1$ had 123.1 and $S_4$ had 148.8 stomata $mm^{-2}$.

Significantly higher stomatal density on lower surface (259.6) and upper surface (153.7) was observed in Dhumad at $S_4$ level of salinity. RAHS-14 at $S_1$ had the lowest stomatal density on both lower (221.8) and upper (120.9) surfaces.

### 4.7.2 Stomatal length (μm) and breadth (μm) of lower leaf surface (abaxial)

Stomatal length and stomatal breadth varied significantly among genotypes and salinity levels but interaction effect was non significant (Table 9).

The genotype NHH-44 had significantly higher breadth (18.31) and length (26.65) over other genotypes. Dhumad recorded significantly lower breadth (13.58) and length (21.37).

Increase in salinity had a negative effect on the stomatal breadth and length and they decreased from 16.77 ($S_1$) to 14.77 ($S_4$) and from 25.11 ($S_1$) to 22.54 ($S_4$) for stomatal breadth and length respectively.

Interaction effect was found non-significant between genotypes and salinity for stomatal breadth and length. However, maximum stomatal breadth and length was noticed in NHH-44 (19.27 and 27.38 respectively) at $S_1$ level.

**Table 9. Effect of salinity on stomatal breadth (μm) and stomatal length (μm) of abaxial leaf surface at 50 DAS in cotton**

| Genotypes | Stomatal breadth | | | | | Stomatal length | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 19.27 | 18.68 | 17.83 | 17.45 | 18.31 | 27.38 | 26.34 | 26.83 | 26.03 | 26.65 |
| LRA-5166 | 18.25 | 17.83 | 17.15 | 16.11 | 17.34 | 25.89 | 24.68 | 23.74 | 23.55 | 24.47 |
| RAHS-14 | 17.32 | 16.74 | 15.79 | 15.12 | 16.24 | 24.49 | 24.12 | 23.78 | 22.69 | 23.77 |
| DHUMAD | 14.13 | 13.84 | 13.52 | 12.82 | 13.58 | 23.54 | 21.50 | 20.64 | 19.81 | 21.37 |
| JAYADHAR | 15.49 | 14.65 | 13.74 | 13.32 | 14.30 | 24.65 | 23.52 | 22.68 | 21.34 | 23.05 |
| AK-235 | 16.14 | 15.62 | 14.15 | 13.78 | 14.92 | 24.71 | 23.94 | 22.76 | 21.82 | 23.31 |
| Mean | 16.77 | 16.23 | 15.36 | 14.77 | 15.78 | 25.11 | 24.02 | 23.41 | 22.54 | 23.77 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.46 | | 1.28 | | | 0.69 | | 1.91 | | |
| Salinity (S) | 0.29 | | 1.00 | | | 0.48 | | 1.66 | | |
| Interaction (GxS) | 0.92 | | NS | | | 1.38 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

### 4.7.3 Stomatal length (μm) and breadth (μm) of upper leaf surface (adaxial)

Significant difference was observed for genotypes and salinity with no interaction effect between genotypes and salinity levels (Table 10).

The genotype NHH-44 had significantly higher stomatal breadth (17.30) and was on par with LRA-5166 (16.46). Dhumad recorded significantly lower stomatal breadth and was on par with Jayadhar (14.75) and RAHS-14 (14.98). Similarly NHH-44 had significantly higher stomatal length (27.42). Genotype Dhumad had significantly lower stomatal length (21.75) and was on par with Jayadhar (22.81).

Increase in the salinity level had a decreasing effect on breadth and length of stomata and they decreased from 16.12 ($S_1$) to 14.72 ($S_4$) and from 25.98 ($S_1$) to 22.28 ($S_4$) for stomatal breadth and length respectively.

Interaction effect was found non-significant for stomatal breadth and length. However, NHH-44 had higher stomatal breadth (18.20) and length (29.73) at $S_1$ level, whereas, Dhumad recorded least stomatal breadth (13.54) and length (20.45) at $S_4$ level.

## 4.8 PHOTOSYNTHETIC RATE (μmol $CO_2/m^2/sec$)

The photosynthetic rate of different cotton genotypes grown at four salinity levels and studied at 30 and 60 DAS, indicated significant differences between genotypes and salinity levels (Table 11 and Figure 3). From the average of all the salinity levels and genotypes, it is evident that photosynthetic rate was higher at 60 DAS compared to 30 DAS.

**Table 10. Effect of salinity on stomatal breadth (μm) and stomatal length (μm) of adaxial leaf surface at 50 DAS in cotton**

| Genotypes | Stomatal breadth | | | | | Stomatal length | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| **NHH-44** | 18.20 | 17.58 | 16.95 | 16.45 | 17.30 | 29.73 | 27.95 | 26.36 | 25.65 | 27.42 |
| **LRA-5166** | 17.38 | 16.76 | 16.06 | 15.63 | 16.46 | 26.89 | 24.32 | 23.56 | 22.15 | 24.23 |
| **RAHS-14** | 16.89 | 15.75 | 14.94 | 14.34 | 14.98 | 26.78 | 23.56 | 22.65 | 21.74 | 23.68 |
| **DHUMAD** | 14.48 | 14.24 | 14.15 | 13.54 | 14.10 | 23.29 | 21.89 | 21.35 | 20.45 | 21.75 |
| **JAYADHAR** | 15.70 | 15.04 | 14.30 | 13.94 | 14.75 | 24.34 | 23.00 | 22.27 | 21.63 | 22.81 |
| **AK-235** | 16.06 | 15.64 | 14.73 | 14.42 | 15.21 | 24.85 | 23.35 | 22.83 | 22.04 | 23.27 |
| **Mean** | 16.12 | 15.84 | 15.19 | 14.72 | 15.47 | 25.98 | 24.01 | 23.17 | 22.28 | 23.86 |
| **For comparing** | | **S.Em±** | **CD at 5%** | | | | **S.Em±** | **CD at 5%** | | |
| **Genotypes (G)** | | 0.45 | 1.25 | | | | 0.69 | 1.91 | | |
| **Salinity (S)** | | 0.32 | 1.11 | | | | 0.51 | 1.77 | | |
| **Interaction (GxS)** | | 0.90 | NS | | | | 1.39 | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

**Table 11. Effect of salinity on photosynthetic rate (µmol $CO_2/m^2/sec$) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 32.19 | 30.35 | 27.86 | 25.70 | 29.03 | 37.54 | 34.15 | 29.52 | 25.36 | 31.64 |
| LRA-5166 | 31.52 | 28.35 | 25.78 | 22.81 | 27.12 | 34.79 | 31.81 | 27.65 | 24.53 | 29.70 |
| RAHS-14 | 26.85 | 23.63 | 20.67 | 19.86 | 22.75 | 30.65 | 28.89 | 24.71 | 20.65 | 26.23 |
| DHUMAD | 23.98 | 22.65 | 19.96 | 18.80 | 21.35 | 29.58 | 25.69 | 23.56 | 19.54 | 24.59 |
| JAYADHAR | 25.68 | 21.30 | 18.92 | 17.37 | 20.82 | 30.16 | 26.21 | 22.12 | 18.35 | 24.21 |
| AK-235 | 25.35 | 23.87 | 21.76 | 19.47 | 22.61 | 29.81 | 27.83 | 23.72 | 21.69 | 25.76 |
| Mean | 27.60 | 25.03 | 22.49 | 20.67 | 23.95 | 32.09 | 29.10 | 25.21 | 21.69 | 27.02 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.70 | | 1.94 | | | 0.80 | | 2.22 | | |
| Salinity (S) | 0.49 | | 1.70 | | | 0.56 | | 1.94 | | |
| Interaction (GxS) | 1.41 | | NS | | | 1.60 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

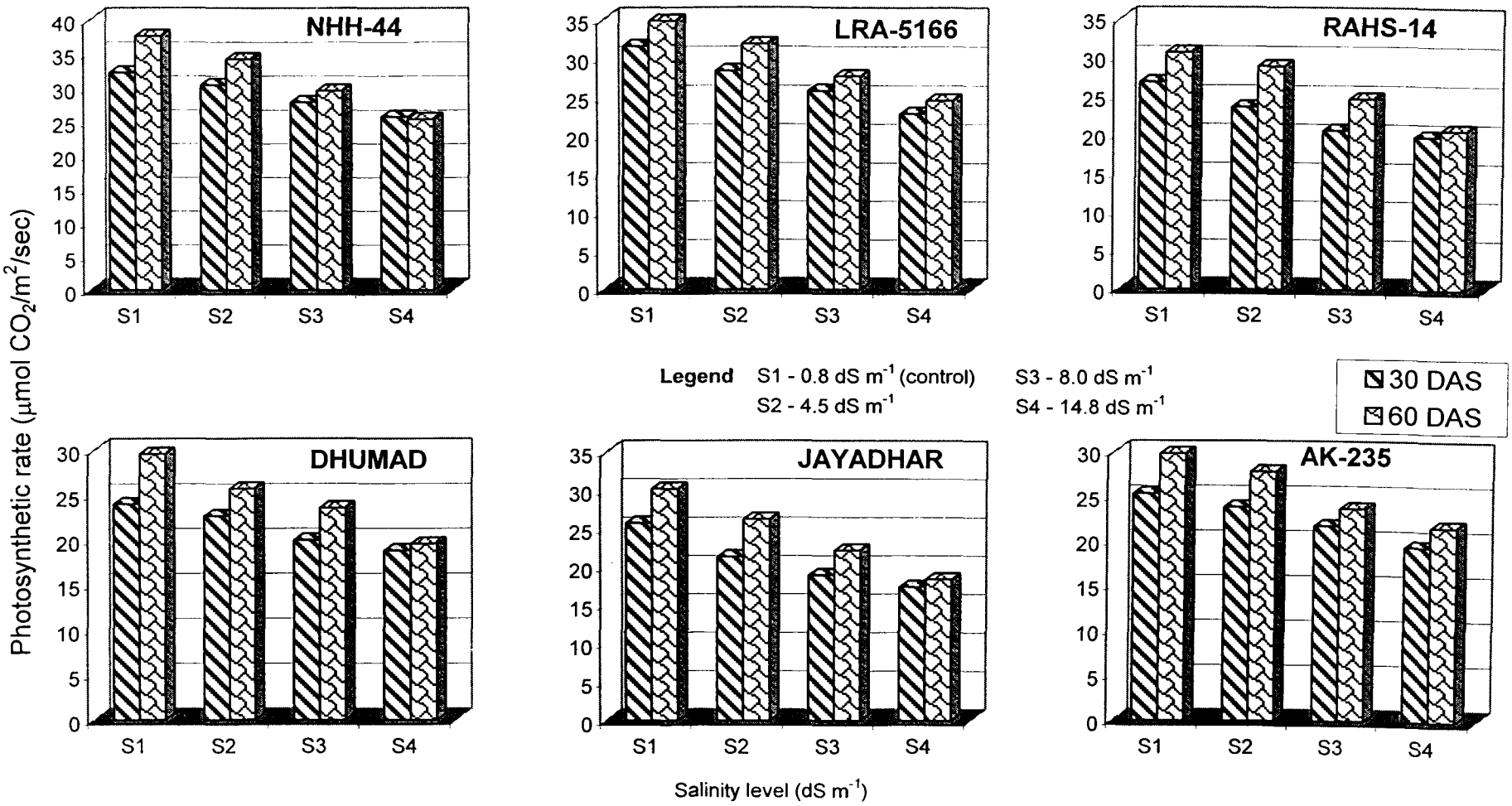


**Figure 3. Effect of salinity on photosynthetic rate (µmol $CO_2/m^2$/sec) at different growth stages in cotton genotypes**

At 30 DAS maximum photosynthetic rate was observed in NHH-44 (29.03) and least was noticed in Jayadhar (20.82). The genotypes NHH-44 and LRA-5166 recorded significantly higher photosynthetic rate at both the stages. At 60 DAS also NHH-44 (31.64) recorded higher photosynthetic rate and was on par with LRA-5166 (29.70), whereas, Jayadhar (24.21) recorded least rate of photosynthesis and was on par with Dhumad (24.59).

The photosynthetic rate decreased with increase in the salinity at both the stages. At 30 DAS it was reduced from 27.60 in $S_1$ level to 20.67 in $S_4$ level. A similar trend of reduction in photosynthetic rate with increase in salinity level was noticed at 60 DAS from 32.09 in $S_1$ level to 21.69 in $S_4$ level.

Interaction of genotypes and salinity levels was not significant. However, at 30 DAS higher photosynthetic rate was observed in NHH-44 (32.19) followed by LRA-5166 (31.52) at $S_1$ level of salinity. Similar interaction effect was noticed at 60 DAS also. Lowest photosynthetic rate was observed in Jayadhar at $S_4$ level at both 30 and 60 DAS (17.37 and 18.35 respectively).

**4.9 STOMATAL CONDUCTANCE ($\mu mol/m^2/sec$)**

The genotypes, salinity levels and their interaction effect showed significant differences with respect to stomatal conductance both at 30 and 60 DAS (Table 12) and stomatal conductance increased from 30 to 60 DAS as that of photosynthetic rate.

At 30 DAS, the genotype NHH-44 (0.328) recorded significantly higher stomatal conductance as compared to rest of the genotypes. The

**Table 12. Effect of salinity on stomatal conductance (μ mol/m$^2$/sec) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 0.412 | 0.375 | 0.298 | 0.227 | 0.328 | 0.446 | 0.389 | 0.336 | 0.223 | 0.349 |
| LRA-5166 | 0.384 | 0.320 | 0.247 | 0.194 | 0.286 | 0.414 | 0.326 | 0.284 | 0.174 | 0.300 |
| RAHS-14 | 0.280 | 0.228 | 0.201 | 0.195 | 0.226 | 0.325 | 0.264 | 0.208 | 0.178 | 0.244 |
| DHUMAD | 0.245 | 0.182 | 0.163 | 0.140 | 0.183 | 0.301 | 0.231 | 0.201 | 0.170 | 0.226 |
| JAYADHAR | 0.265 | 0.210 | 0.174 | 0.125 | 0.194 | 0.295 | 0.234 | 0.195 | 0.163 | 0.222 |
| AK-235 | 0.234 | 0.205 | 0.169 | 0.147 | 0.189 | 0.312 | 0.258 | 0.189 | 0.181 | 0.235 |
| Mean | 0.303 | 0.253 | 0.209 | 0.171 | 0.234 | 0.349 | 0.284 | 0.236 | 0.182 | 0.263 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.007 | | 0.019 | | | 0.008 | | 0.022 | | |
| Salinity (S) | 0.004 | | 0.014 | | | 0.005 | | 0.017 | | |
| Interaction (GxS) | 0.014 | | 0.039 | | | 0.016 | | 0.044 | | |

$S_1$ : 0.8 dS m$^{-1}$, $S_2$ : 4.5 dS m$^{-1}$, $S_3$ : 8.0 dS m$^{-1}$, $S_4$ : 14.8 dS m$^{-1}$

genotypes Dhumad (0.183) and AK-235 (0.189) had significantly lower stomatal conductance. At 60 DAS also NHH-44 (0.349) recorded maximum stomatal conductance followed by LRA-5166 (0.300) and least was observed in Jayadhar (0.222) followed by Dhumad (0.226).

There were significant differences among salinity levels with respect to stomatal conductance. The salinity level $S_1$ recorded significantly higher stomatal conductance at both stages. In general, there was more than 40 per cent reduction in stomatal conductance in highest level of salinity compared to lowest salinity level.

The genotypes NHH-44 (0.412) followed by LRA-5166 (0.384) recorded significantly higher stomatal conductance under $S_1$ (0.8 dS $m^{-1}$) level and least was observed in Jayadhar at $S_1$ level (0.125) at 30 DAS. Whereas at 60 DAS, NHH-44 (0.446) recorded significantly higher stomatal conductance followed by LRA-5166 (0.414) at $S_1$ and least was observed in Jayadhar at $S_4$ level (0.163) followed by Dhumad (0.170).

### 4.10 TRANSPIRATION RATE (mmol $H_2O$ / $m^2$ /sec)

The data on transpiration rate indicated significant differences due to salinity levels and genotypes at both stages of plant growth, whereas, interaction effect was found non-significant. In general, similar to photosynthetic rate and stomatal conductance, transpiration rate increased from 30 to 60 DAS (Table 13 and Figure 4).

Transpiration rate was maximum in NHH-44 (10.11) followed by LRA-5166 (9.13), which had significantly higher transpiration rate than other genotypes at 30 DAS and least was noticed in Dhumad (5.96). At 60

**Table 13. Effect of salinity on transpiration rate (mmol /$m^2$/sec) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 12.75 | 11.25 | 9.19 | 7.26 | 10.11 | 12.92 | 11.75 | 9.83 | 8.31 | 10.71 |
| LRA-5166 | 11.56 | 9.72 | 8.31 | 6.93 | 9.13 | 12.24 | 11.23 | 9.21 | 7.36 | 10.01 |
| RAHS-14 | 7.96 | 6.91 | 6.10 | 5.16 | 6.53 | 9.25 | 7.94 | 7.27 | 6.14 | 7.65 |
| DHUMAD | 7.51 | 6.34 | 5.22 | 4.76 | 5.96 | 8.34 | 7.31 | 6.58 | 5.67 | 6.98 |
| JAYADHAR | 8.73 | 6.74 | 5.12 | 4.55 | 6.29 | 8.96 | 7.46 | 6.79 | 5.88 | 7.27 |
| AK-235 | 7.84 | 6.79 | 5.87 | 5.18 | 6.42 | 9.26 | 7.68 | 7.26 | 5.91 | 7.53 |
| Mean | 9.36 | 7.96 | 6.62 | 5.64 | 7.40 | 10.16 | 8.90 | 7.82 | 6.55 | 8.36 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.22 | | 0.61 | | | 0.25 | | 0.69 | | |
| Salinity (S) | 0.14 | | 0.49 | | | 0.12 | | 0.42 | | |
| Interaction (GxS) | 0.45 | | NS | | | 0.50 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

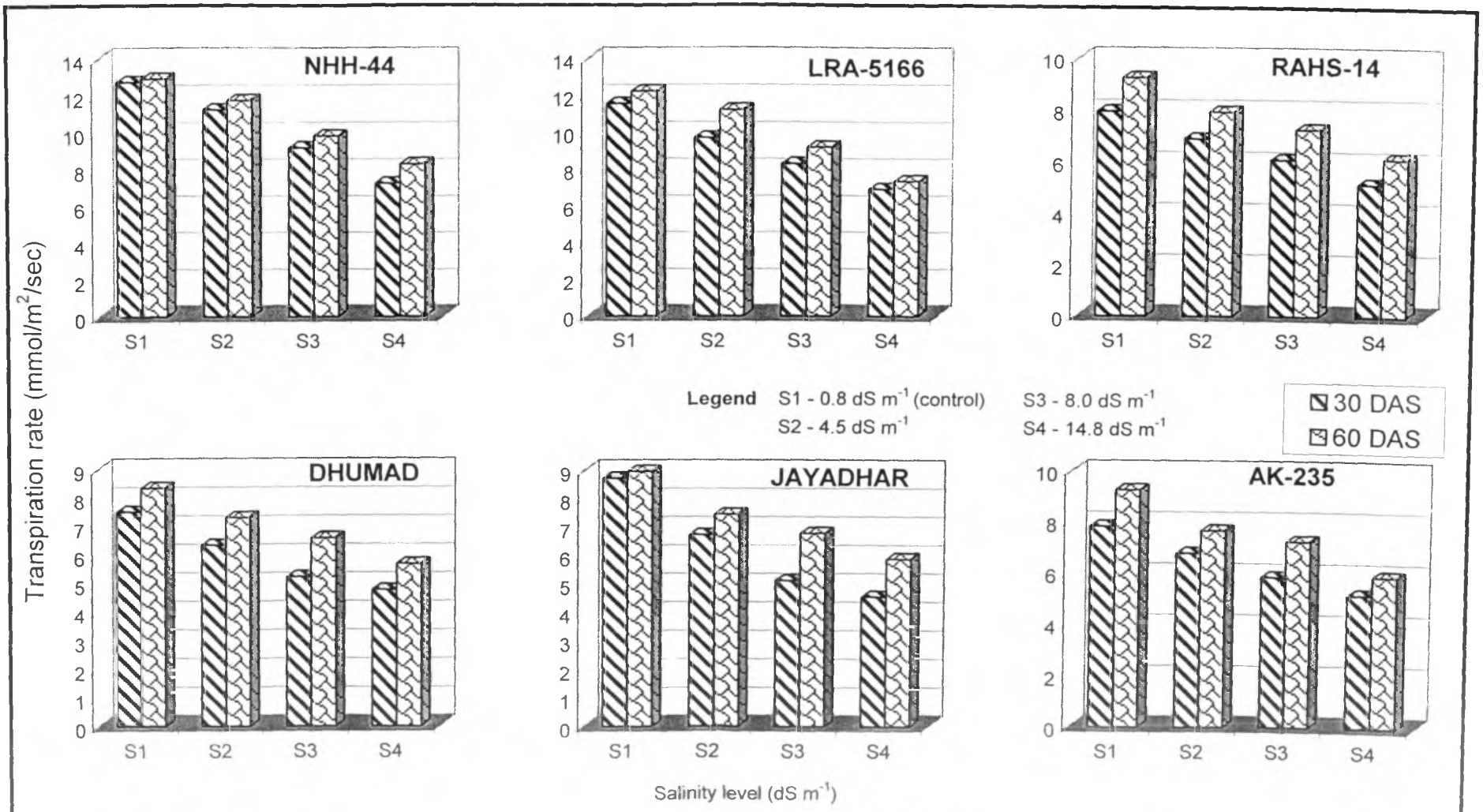


**Figure 4. Effect of salinity on transpiration rate (mmol/m$^2$/sec) at different growth stages in cotton genotypes**

DAS also NHH-44 (10.71) and LRA-5166 (10.01) had maximum transpiration rate and Dhumad recorded least transpiration rate (6.98) followed by Jayadhar (7.27).

Salinity reduced the transpiration rate from 9.36 in $S_1$ to 5.64 in $S_4$ at 30 DAS and from 10.16 in $S_1$ to 6.55 in $S_4$ at 60 DAS. At 30 DAS, $S_1$ (9.36) recorded significantly higher transpiration rate, while $S_4$ (5.64) registered significantly lower transpiration rate. A similar trend of reduction in transpiration rate with increase in salinity level was noticed at 60 DAS.

Genotype x Salinity interaction was not significant at both stages. However, highest transpiration rate was noticed in NHH-44 at both 30 and 60 DAS at $S_1$ salinity level.

## 4.11 BIOCHEMICAL PARAMETERS

The data on biochemical parameters, viz., chlorophyll content, proline content and sugar content of six cotton genotypes in response to salinity levels are presented in Tables 14, 15, 16, 17 and 18.

### 4.11.1 Chlorophyll content (mg/g fresh weight)

The data on chlorophyll 'a', 'b' and total chlorophyll contents at 30 and 60 DAS are presented in Tables 14,15 and 16.

#### 4.11.1.1 Chlorophyll 'a' content (mg/g fresh weight)

Significant difference in the chlorophyll 'a' content was observed among genotypes and salinity levels both at 30 and 60 DAS. However, there was no interaction effect between the genotypes and salinity levels at both the stages (Table 14).

**Table 14. Effect of salinity on chlorophyll 'a' content (mg/g fresh weight) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 0.60 | 0.55 | 0.49 | 0.44 | 0.52 | 0.82 | 0.76 | 0.67 | 0.61 | 0.72 |
| LRA-5166 | 0.52 | 0.49 | 0.46 | 0.40 | 0.47 | 0.74 | 0.69 | 0.60 | 0.54 | 0.64 |
| RAHS-14 | 0.54 | 0.51 | 0.47 | 0.42 | 0.49 | 0.75 | 0.68 | 0.63 | 0.58 | 0.66 |
| DHUMAD | 0.52 | 0.48 | 0.43 | 0.37 | 0.45 | 0.68 | 0.63 | 0.59 | 0.50 | 0.60 |
| JAYADHAR | 0.53 | 0.48 | 0.45 | 0.39 | 0.46 | 0.72 | 0.66 | 0.60 | 0.55 | 0.63 |
| AK-235 | 0.55 | 0.50 | 0.47 | 0.40 | 0.48 | 0.71 | 0.65 | 0.62 | 0.58 | 0.64 |
| Mean | 0.54 | 0.50 | 0.46 | 0.40 | 0.48 | 0.74 | 0.68 | 0.62 | 0.56 | 0.65 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.014 | | 0.039 | | | 0.029 | | 0.080 | | |
| Salinity (S) | 0.010 | | 0.035 | | | 0.018 | | 0.062 | | |
| Interaction (GxS) | 0.031 | | NS | | | 0.037 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

The genotype NHH-44 had significantly higher chlorophyll 'a' content of 0.52 and 0.72 mg/g fresh weight at 30 and 60 DAS respectively. RAHS-14 (0.49) and AK-235 (0.48) were on par with NHH-44 at 30 DAS, while at 60 DAS, it was only RAHS-14 (0.66), which was on par with NHH-44. Lowest chlorophyll 'a' content was found in Dhumad both at 30 and 60 DAS (0.45 and 0.60 respectively). In general, in all the genotypes the chlorophyll 'a' content was 35 per cent higher at 60 DAS in comparison to 30 DAS.

Salinity decreased the chlorophyll 'a' content significantly from a salinity level of 0.8 dS $m^{-1}$ ($S_1$) to 14.8 dS $m^{-1}$ ($S_4$). Chlorophyll 'a' content decreased by 26 and 24 per cent at highest salinity level compared to control (0.8 dS $m^{-1}$) at 30 and 60 DAS respectively.

**4.11.1.2 Chlorophyll 'b' content (mg/g fresh weight)**

Significant differences for chlorophyll 'b' content were observed among genotypes and salinity levels at 30 and 60 DAS, whereas, interaction effect was found non significant at both the stages (Table 15).

The genotype NHH-44 had significantly higher chlorophyll 'b' content at both 30 (0.39) and 60 DAS (0.52). Genotypes RAHS-14 and AK-235 each having 0.37 mg/g fresh weight of chlorophyll 'b' content were on par with NHH-44 at 30 DAS. Average genotypic content of chlorophyll 'b' was higher by 22 per cent at 60 DAS compared to 30 DAS. All the genotypes had lesser chlorophyll 'b' in comparison to chlorophyll 'a' at both stages.

Salinity decreased the chlorophyll 'b' content from 0.41 to 0.31 mg/g fresh weight and 0.51 to 0.39 mg/g fresh weight at 30 and 60 DAS

**Table 15. Effect of salinity on chlorophyll 'b' content (mg/g fresh weight) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 0.44 | 0.41 | 0.37 | 0.35 | 0.39 | 0.59 | 0.54 | 0.48 | 0.46 | 0.52 |
| LRA-5166 | 0.41 | 0.37 | 0.35 | 0.30 | 0.36 | 0.52 | 0.48 | 0.43 | 0.41 | 0.46 |
| RAHS-14 | 0.42 | 0.39 | 0.36 | 0.32 | 0.37 | 0.50 | 0.46 | 0.38 | 0.37 | 0.43 |
| DHUMAD | 0.38 | 0.35 | 0.31 | 0.27 | 0.33 | 0.47 | 0.43 | 0.39 | 0.31 | 0.40 |
| JAYADHAR | 0.40 | 0.37 | 0.35 | 0.29 | 0.35 | 0.47 | 0.41 | 0.40 | 0.39 | 0.42 |
| AK-235 | 0.43 | 0.38 | 0.36 | 0.31 | 0.37 | 0.49 | 0.46 | 0.40 | 0.38 | 0.43 |
| Mean | 0.41 | 0.38 | 0.35 | 0.31 | 0.36 | 0.51 | 0.46 | 0.41 | 0.39 | 0.44 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.014 | | 0.039 | | | 0.021 | | 0.058 | | |
| Salinity (S) | 0.010 | | 0.035 | | | 0.015 | | 0.052 | | |
| Interaction (GxS) | 0.022 | | NS | | | 0.030 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

respectively with increase in salinity levels from 0.8 to 14.8 dS $m^{-1}$. Genotype NHH-44 had lower reduction in chlorophyll 'b' content, whereas, AK-235 had maximum reduction at 30 DAS, while at 60 DAS, Jayadhar had lower reduction and Dhumad recorded maximum reduction in chlorophyll 'b' content.

#### 4.11.1.3 Total chlorophyll content (mg/g fresh weight)

Significant differences between genotypes and salinity levels were observed for total chlorophyll content at 30 and 60 DAS (Table 16 and Figure 5).

NHH-44 had significantly higher total chlorophyll content of 0.91 and 1.24 mg/g fresh weight at 30 and 60 DAS respectively. The genotype RAHS-14 (0.86) and AK-235 (0.85) were on par with NHH-44 at 30 DAS. Total chlorophyll content was more at 60 DAS compared to 30 DAS.

Salinity decreased the total chlorophyll content significantly from 0.96 to 0.71 mg/g fresh weight and from 1.24 to 0.95 mg/g fresh weight at 30 and 60 DAS respectively with increase in salinity level from $S_1$ (0.8 dS $m^{-1}$) to $S_4$ (14.8 dS $m^{-1}$) Lower reduction in total chlorophyll content with increase in salinity was observed in RAHS-14 at 30 DAS and AK-235 at 60 DAS, whereas, Dhumad had maximum reduction at highest salinity level compared to control at both stages.

Interaction effect was found to be non-significant at both stages.

### 4.11.2 Proline content (µg/g dry weight)

Genotypes, salinity and interaction were found to be significant for proline both at 30 and 60 DAS (Table 17 and Figure 6).

**Table 16. Effect of salinity on total chlorophyll content (mg/g fresh weight) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 1.04 | 0.96 | 0.86 | 0.79 | 0.91 | 1.41 | 1.30 | 1.15 | 1.07 | 1.23 |
| LRA-5166 | 0.93 | 0.86 | 0.81 | 0.70 | 0.83 | 1.26 | 1.17 | 1.03 | 0.95 | 1.10 |
| RAHS-14 | 0.96 | 0.90 | 0.83 | 0.74 | 0.86 | 1.25 | 1.14 | 1.01 | 0.95 | 1.09 |
| DHUMAD | 0.90 | 0.83 | 0.74 | 0.64 | 0.78 | 1.15 | 1.06 | 0.98 | 0.81 | 1.00 |
| JAYADHAR | 0.93 | 0.85 | 0.80 | 0.68 | 0.82 | 1.19 | 1.07 | 1.00 | 0.94 | 1.05 |
| AK-235 | 0.98 | 0.88 | 0.83 | 0.71 | 0.85 | 1.20 | 1.11 | 1.02 | 0.96 | 1.07 |
| Mean | 0.96 | 0.88 | 0.81 | 0.71 | 0.84 | 1.24 | 1.14 | 1.03 | 0.95 | 1.09 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.030 | | 0.083 | | | 0.032 | | 0.089 | | |
| Salinity (S) | 0.021 | | 0.073 | | | 0.024 | | 0.083 | | |
| Interaction (GxS) | 0.050 | | NS | | | 0.073 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

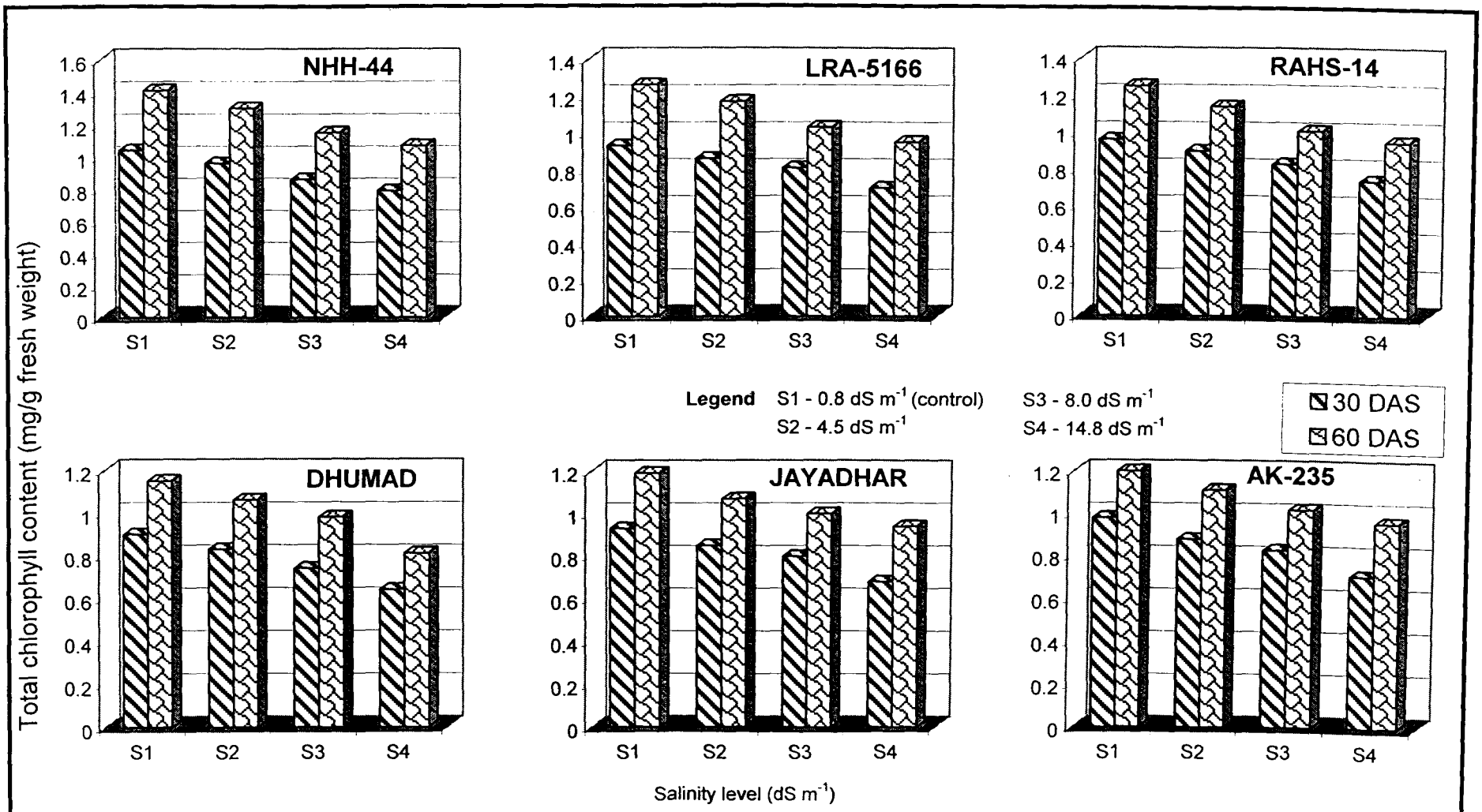


**Figure 5. Effect of salinity on total chlorophyll content (mg/g fresh weight) at different growth stages in cotton genotypes**

**Table 17. Effect of salinity on proline content in leaf (μg/g dry weight) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 37.6 | 47.2 | 55.3 | 61.2 | 50.3 | 59.6 | 74.8 | 85.4 | 99.7 | 79.9 |
| LRA-5166 | 39.7 | 49.2 | 59.4 | 66.1 | 53.6 | 54.6 | 68.3 | 81.2 | 96.6 | 75.2 |
| RAHS-14 | 36.2 | 46.1 | 54.3 | 62.4 | 49.8 | 53.1 | 66.3 | 82.4 | 95.3 | 74.3 |
| DHUMAD | 33.4 | 38.6 | 49.7 | 56.6 | 44.6 | 47.5 | 54.2 | 69.3 | 79.4 | 62.6 |
| JAYADHAR | 30.5 | 37.4 | 46.2 | 53.7 | 42.0 | 49.7 | 55.9 | 72.5 | 90.3 | 67.1 |
| AK-235 | 35.4 | 41.8 | 52.1 | 57.2 | 46.6 | 51.4 | 63.6 | 79.7 | 94.4 | 72.3 |
| Mean | 35.5 | 43.4 | 52.8 | 59.5 | 47.8 | 52.7 | 63.9 | 78.4 | 92.6 | 72.0 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 1.41 | | 3.91 | | | 2.14 | | 5.93 | | |
| Salinity (S) | 1.03 | | 3.56 | | | 1.83 | | 6.33 | | |
| Interaction (GxS) | 2.83 | | 7.84 | | | 4.27 | | 11.84 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

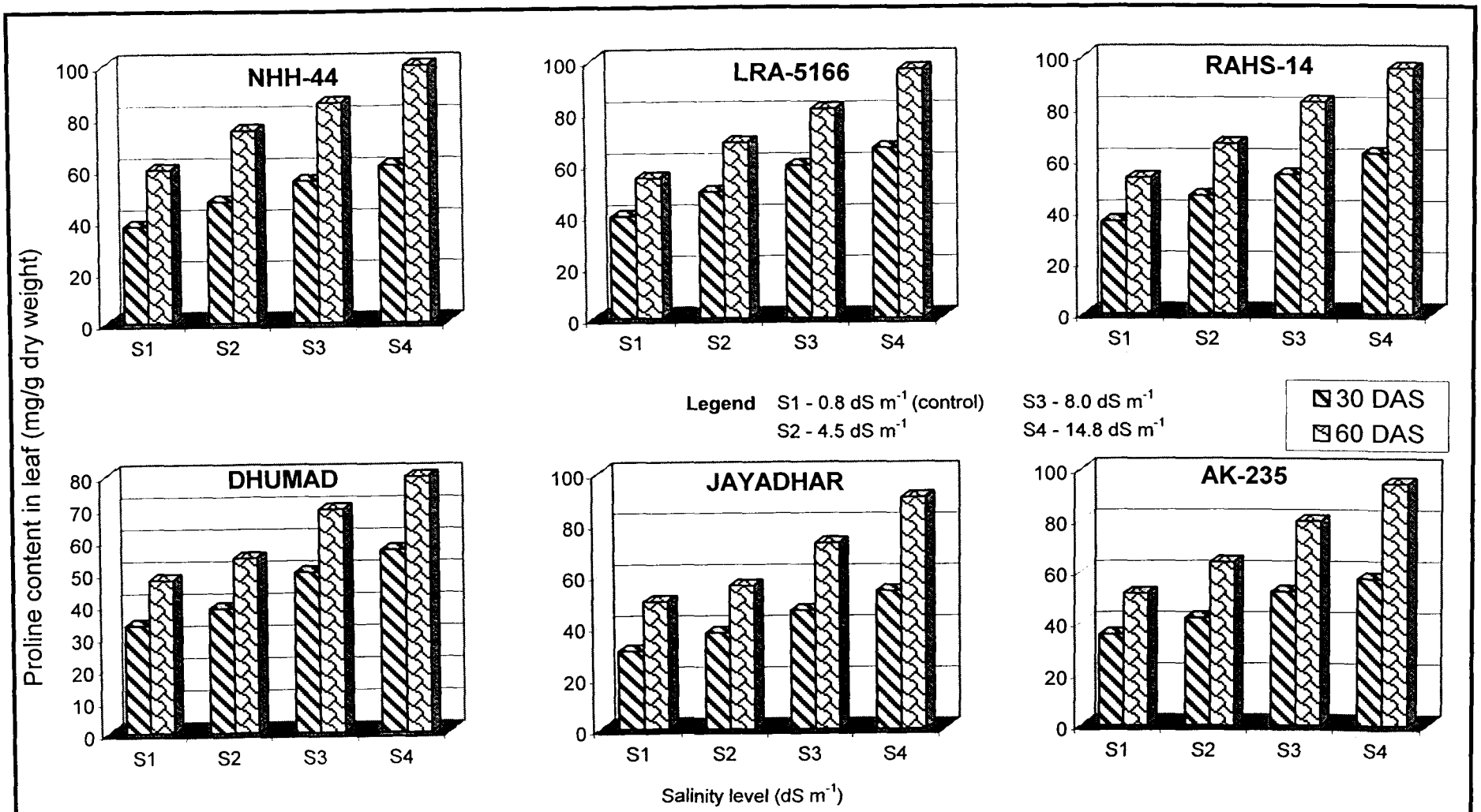


**Figure 6. Effect of salinity on proline content in leaf (mg/g dry weight) at different growth stages in cotton genotypes**

The genotype LRA-5166 had significantly higher proline content at 30 DAS (53.6), while NHH-44 recorded significantly higher proline content at 60 DAS (79.9). Least proline was observed in Jayadhar at 30 DAS (42.0) and in Dhumad at 60 DAS (62.6).

Salinity had a positive impact on proline accumulation and it increased from 35.5 μg/g dry weight at $S_1$ to 59.5 μg/g dry weight at $S_4$ at 30 DAS and from 52.7 ($S_1$) to 92.6 μ g/g dry weight ($S_4$) at 60 DAS. All the salinity levels were significantly differing with each other. However, per cent increase in proline content was higher at 60 DAS (76%) over 30 DAS (68%) from $S_1$ to $S_4$.

The genotype LRA-5166 with $S_4$ salinity had significantly higher proline content (66.1) at 30 DAS, while, NHH-44 had maximum proline content (99.7) at 60 DAS. The genotypes Jayadhar with $S_1$ (30.5) at 30 DAS and Dhumad with $S_1$ (47.5) at 60 DAS recorded significantly lowest proline content.

### 4.11.3 Total sugar content (mg/g dry weight)

Significant differences were observed between genotypes and salinity levels for total sugar content at both 30 and 60 DAS. Interaction effect was non-significant (Table 18 and Figure 7).

The genotype RAHS-14 (29.0) had significantly higher total sugar content at 30 DAS and was on par with Jayadhar (28.5). Lowest sugar accumulation was noticed in AK-235 (20.9) at 30 DAS. At 60 DAS also RAHS-14 recorded highest sugar content (30.2) followed by LRA-5166 (28.5) and least was observed in AK-235 (21.8).

**Table 18. Effect of salinity on total sugar content (mg/g dry weight) at different growth stages in cotton**

| Genotypes | 30 DAS | | | | | 60 DAS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 22.8 | 23.9 | 25.3 | 26.7 | 24.7 | 22.5 | 24.2 | 26.7 | 27.2 | 25.2 |
| LRA-5166 | 24.3 | 25.4 | 27.6 | 28.3 | 26.4 | 26.6 | 27.9 | 30.2 | 29.4 | 28.5 |
| RAHS-14 | 25.7 | 27.6 | 30.4 | 32.2 | 29.0 | 26.9 | 29.3 | 32.6 | 32.1 | 30.2 |
| DHUMAD | 19.8 | 22.6 | 25.5 | 26.8 | 23.7 | 22.5 | 24.2 | 26.7 | 27.2 | 25.2 |
| JAYADHAR | 26.3 | 27.9 | 29.2 | 30.5 | 28.5 | 21.6 | 22.1 | 25.4 | 24.6 | 23.4 |
| AK-235 | 18.2 | 19.8 | 22.3 | 23.1 | 20.9 | 17.6 | 21.4 | 24.2 | 23.9 | 21.8 |
| Mean | 22.9 | 24.5 | 26.7 | 27.9 | 25.5 | 23.0 | 24.9 | 27.6 | 27.4 | 25.7 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.76 | | 2.11 | | | 0.81 | | 2.25 | | |
| Salinity (S) | 0.51 | | 1.77 | | | 0.53 | | 1.83 | | |
| Interaction (GxS) | 1.52 | | NS | | | 1.48 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

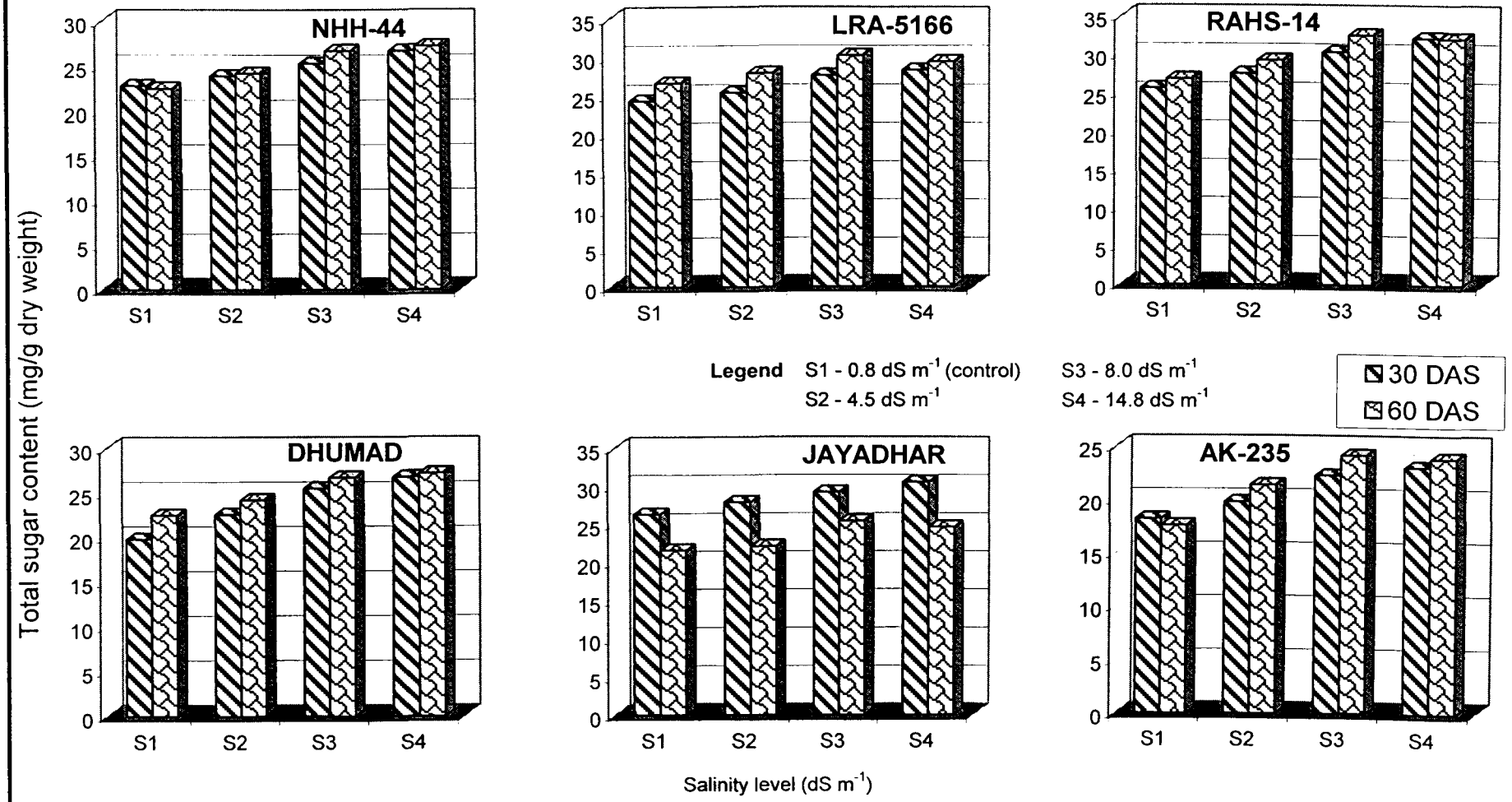


**Figure 7. Effect of salinity on total sugar content (mg/g dry weight) at different growth stages in cotton genotypes**

All genotypes had more sugar content at 60 DAS except Jayadhar compared to 30 DAS.

Salinity increased the accumulation of sugar considerably. It increased from 22.9 ($S_1$) to 27.9 mg/g dry weight ($S_4$) at 30 DAS and from 23.0 ($S_1$) to 27.4 mg/g dry weight ($S_4$) at 60 DAS.

Interactions between genotypes and salinity levels were found non-significant at both stages.

## 4.12 ANATOMICAL CHARACTERS

The data on the effect of genotypes and salinity levels on meso-structure of leaf are presented in tables 19 and 20.

### 4.12.1 Mesophyll thickness (μm), pallisade layer thickness (μm) and spongy parenchyma layer thickness (μm)

Mesophyll thickness, pallisade layer thickness and spongy layer thickness differed significantly among genotypes and salinity levels but interaction effect was found non-significant (Table 19).

The genotype NHH-44 had significantly higher mesophyll thickness and spongy layer thickness (206 and 125 respectively) over other genotypes, while genotype Dhumad had significantly higher pallisade layer thickness (97) and Jayadhar recorded significantly lower mesophyll thickness (180).

Increase in salinity level had an increasing effect on mesophyll thickness, palliside layer and spongy layer thickness. Mesophyll layer thickness increased from 183 ($S_1$) to 200 ($S_4$), pallisade thickness

Table 19. Effect of salinity on mesophyll thickness (μm), palisade layer thickness (μm) and spongy parenchyma layer thickness (μm) at 90 in cotton

| Genotypes | Mesophyll thickness | | | | | Palisade layer thickness | | | | | Spongy parenchyma layer thickness | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 201 | 203 | 208 | 212 | 206 | 80 | 80 | 82 | 83 | 81 | 121 | 123 | 126 | 129 | 125 |
| LRA-5166 | 194 | 197 | 204 | 210 | 202 | 84 | 85 | 88 | 90 | 87 | 110 | 112 | 116 | 120 | 115 |
| RAHS-14 | 178 | 183 | 189 | 193 | 186 | 87 | 90 | 92 | 93 | 91 | 91 | 93 | 97 | 100 | 95 |
| DHUMAD | 175 | 180 | 191 | 205 | 188 | 89 | 92 | 98 | 107 | 97 | 86 | 88 | 93 | 98 | 91 |
| JAYADHAR | 172 | 175 | 183 | 189 | 180 | 88 | 90 | 96 | 99 | 93 | 84 | 85 | 87 | 90 | 87 |
| AK-235 | 176 | 183 | 189 | 192 | 185 | 86 | 87 | 89 | 90 | 88 | 90 | 96 | 100 | 102 | 97 |
| Mean | 183 | 187 | 194 | 201 | 192 | 86 | 87 | 91 | 94 | 90 | 97 | 100 | 103 | 107 | 102 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 3.50 | | 9.70 | | | 1.75 | | 4.85 | | | 2.13 | | 5.90 | | |
| Salinity (S) | 2.29 | | 7.93 | | | 1.07 | | 3.70 | | | 1.18 | | 4.08 | | |
| Interaction (GxS) | 6.45 | | NS | | | 4.50 | | NS | | | 5.10 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

increased from 86 ($S_1$) to 94 ($S_4$) and spongy layer thickness increased from 97 ($S_1$) to 107($S_4$).

Interaction effect was found non-significant for mesophyll thickness, pallisade thickness and spongy layer thickness. However, NHH-44 had higher mesophyll thickness (201) at $S_1$ level.

### 4.12.2 Size of pallisade cell (μm) and spongy cell (μm)

Size (width) of pallisade cell and spongy cell differed significantly among genotypes and salinity levels but interaction effect was found non significant (Table 20).

Among genotypes, NHH-44 followed by LRA-5166 had maximum pallisade cell width (29 and 28 respectively), as well as spongy cell width (35 and 34 respectively) and these genotypes differed significantly over other genotypes. Genotype Dhumad recorded minimum pallisade cell width (23), while RAHS-14 and Jayadhar had minimum spongy cell width (27 each).

Increase in salinity level had a positive effect on size (width) of pallisade and spongy cell. Pallisade cell width increased from 24 ($S_1$) to 28 μm ($S_4$), while spongy cell width increased from 29 ($S_1$) to 32 μm ($S_4$) with the increase in salinity level from $S_1$ to $S_4$.

Interaction effect was non-significant. However, NHH-44 at $S_4$ (14.8 dS $m^{-1}$) salinity level (31) and Dhumad at $S_1$ (0.8 dS $m^{-1}$) salinity level had maximum and minimum pallisade cell width respectively. Similar results were observed in case of spongy cell width.

**Table 20. Effect of salinity on size (width) of pallisade cell (μm) and spongy cell (μm) at 90 DAS in cotton**

| Genotypes | Pallisade cell width | | | | | Spongy cell width | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 28 | 27 | 29 | 31 | 29 | 34 | 34 | 35 | 36 | 35 |
| LRA-5166 | 26 | 27 | 29 | 30 | 28 | 32 | 33 | 34 | 37 | 34 |
| RAHS-14 | 23 | 22 | 25 | 26 | 24 | 27 | 26 | 27 | 28 | 27 |
| DHUMAD | 22 | 21 | 24 | 26 | 23 | 25 | 27 | 30 | 30 | 28 |
| JAYADHAR | 24 | 23 | 25 | 27 | 25 | 26 | 26 | 28 | 29 | 27 |
| AK-235 | 23 | 22 | 24 | 25 | 24 | 29 | 28 | 29 | 31 | 29 |
| Mean | 24 | 24 | 26 | 28 | 26 | 29 | 29 | 31 | 32 | 30 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.65 | | 1.80 | | | 0.75 | | 2.08 | | |
| Salinity (S) | 0.42 | | 1.45 | | | 0.51 | | 1.77 | | |
| Interaction (GxS) | 1.56 | | NS | | | 1.64 | | NS | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

## 4.13 YIELD AND YIELD COMPONENTS

The data on yield and yield components are presented in Tables 21 and 22.

### 4.13.1 Seed cotton yield (g/plant)

In general, the seed cotton yield (Table 21 and Figure 8) decreased significantly from 41.06 to 22.43 with progressive increase in salinity from $S_1$ to $S_4$ level. Among varieties, NHH-44 recorded higher yield of 34.95 g plant$^{-1}$ and RAHS–14 (34.27) stood second and were on par with AK-235 (34.09). The genotype Dhumad produced the lowest yield of 25.86 g plant$^{-1}$, NHH-44 produced 35 per cent more yield over the lowest yielding genotype Dhumad.

$S_1$ level of salinity had highest yield of 41.06 g plant$^{-1}$. Lowest yield (22.43) was observed at $S_4$ salinity level.

Genotype x salinity interaction effect showed higher yield in RAHS-14 with $S_1$ level of salinity (45.29). The genotype Dhumad had significantly lower yield of 11.06 g plant$^{-1}$ at $S_4$ (14.8 dS m$^{-1}$) salinity level.

### 4.13.2 Total dry matter at harvest (g/plant)

Salinity, genotypes and their interaction differed significantly in total dry matter production at harvest (Table 21).

The genotype NHH-44 (107.6) followed by AK-235 (101.3) produced significantly higher dry matter than other genotypes, whereas, Dhumad (82.3) produced significantly lower total dry matter at harvest.

**Table 21. Effect of salinity on seed cotton yield (g/plant), total dry matter (g/plant) and harvest index (%) in cotton genotypes**

| Genotypes | Seed cotton yield | | | | | Total dry matter | | | | | Harvest index | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| NHH-44 | 39.19 | 35.18 | 33.49 | 31.93 | 34.95 | 129.9 | 118.7 | 101.4 | 80.3 | 107.6 | 0.302 | 0.296 | 0.330 | 0.398 | 0.332 |
| LRA-5166 | 36.69 | 32.89 | 32.27 | 28.09 | 32.29 | 115.0 | 99.3 | 88.6 | 68.6 | 92.9 | 0.319 | 0.331 | 0.364 | 0.410 | 0.356 |
| RAHS-14 | 45.29 | 38.06 | 31.03 | 22.68 | 34.27 | 121.6 | 104.2 | 94.2 | 69.6 | 97.4 | 0.373 | 0.365 | 0.329 | 0.326 | 0.348 |
| DHUMAD | 38.92 | 30.40 | 23.04 | 11.06 | 25.86 | 110.2 | 89.4 | 74.4 | 55.3 | 82.3 | 0.353 | 0.340 | 0.310 | 0.200 | 0.301 |
| JAYADHAR | 44.36 | 36.54 | 30.32 | 16.96 | 32.05 | 117.4 | 98.7 | 81.3 | 65.3 | 90.7 | 0.378 | 0.370 | 0.373 | 0.260 | 0.345 |
| AK-235 | 41.88 | 37.03 | 33.57 | 23.87 | 34.09 | 127.7 | 110.6 | 94.9 | 71.8 | 101.3 | 0.328 | 0.335 | 0.354 | 0.332 | 0.337 |
| Mean | 41.06 | 35.02 | 30.62 | 22.43 | 32.28 | 120.3 | 103.5 | 89.1 | 68.5 | 95.4 | 0.342 | 0.340 | 0.343 | 0.321 | 0.337 |
| For comparing | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | | S.Em± | | CD at 5% | | |
| Genotypes (G) | 0.783 | | 2.17 | | | 1.76 | | 4.88 | | | 0.23 | | 0.64 | | |
| Salinity (S) | 0.548 | | 1.90 | | | 1.29 | | 4.46 | | | 0.12 | | 0.42 | | |
| Interaction (GxS) | 1.566 | | 4.34 | | | 1.37 | | 3.80 | | | 0.88 | | 2.44 | | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

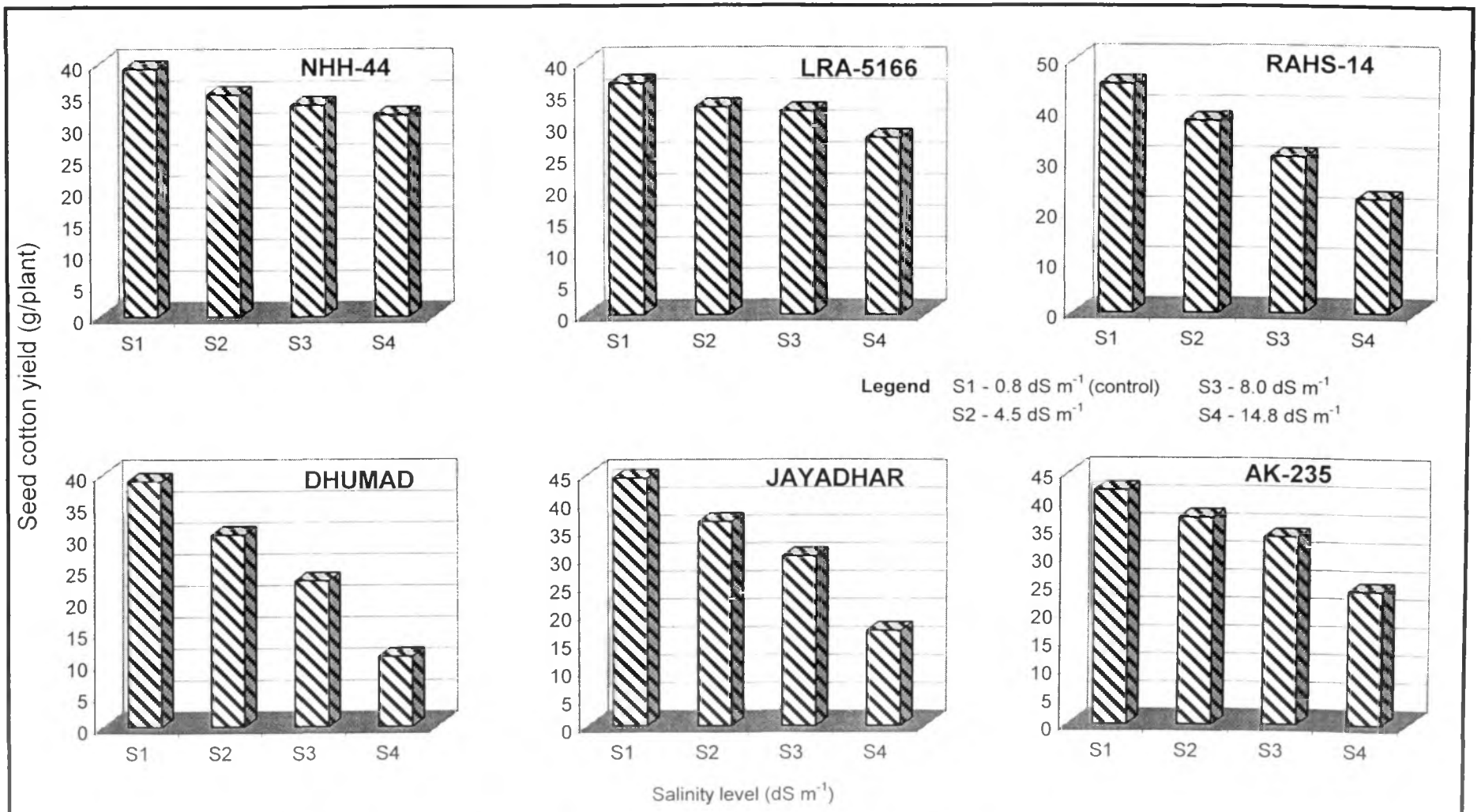


**Figure 8. Effect of salinity on seed cotton yield (g/plant) in cotton genotypes**

With increase in salinity level from $S_1$ to $S_4$ total dry matter decreased from 120.3 to 68.5 g/plant. $S_1$ salinity level recorded maximum dry weight of 120.3 g/plant, whereas, $S_4$ salinity level had significantly lower dry matter yield (68.5).

The maximum dry matter production was noticed in NHH-44 at $S_1$ level (129.9) and least was observed in Dhumad at $S_4$ salinity level (55.3).

### 4.13.3 Harvest Index

Significant differences in harvest index were observed for genotypes, salinity levels and interaction.

Among genotypes LRA-5166 had higher harvest index of 0.356 and was on par with RAHS-14 (0.348) and Jayadhar (0.345). Lowest harvest index (0.301) was noticed in Dhumad.

In general, salinity increased the harvest index up to $S_3$ salinity level and later decreased at $S_4$ level. $S_3$ level had significantly higher harvest index and $S_4$ level had significantly lowest harvest index.

The highest harvest index was noticed in LRA-5166 at $S_4$ salinity level (0.410), whereas, lowest harvest index was recorded in Dhumad at same salinity level of $S_4$.

### 4.13.4 Total number of bolls picked per plant

The observation on number of bolls indicated significant differences among salinity levels, genotypes and their interaction (Table 22).

The genotype RAHS-14 (16.62) recorded significantly higher number of bolls over other genotypes and was on par with Jayadhar (16.50). Least

Table 22. Effect of salinity on number of bolls per plant and boll weight (g/boll) in cotton genotypes

| Genotypes | No. of bolls /plant | | | | | Boll weight | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Salinity levels | | | | | Salinity levels | | | | |
| | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean | $S_1$ | $S_2$ | $S_3$ | $S_4$ | Mean |
| **NHH-44** | 11.63 | 10.66 | 10.63 | 10.40 | 10.83 | 3.37 | 3.30 | 3.15 | 3.07 | 3.22 |
| **LRA-5166** | 11.50 | 10.54 | 10.51 | 09.30 | 10.46 | 3.19 | 3.12 | 3.07 | 3.02 | 3.10 |
| **RAHS-14** | 20.87 | 18.30 | 15.67 | 11.63 | 16.62 | 2.17 | 2.08 | 1.98 | 1.95 | 2.05 |
| **DHUMAD** | 18.27 | 14.90 | 12.00 | 06.87 | 13.01 | 2.13 | 2.04 | 1.92 | 1.61 | 1.92 |
| **JAYADHAR** | 20.63 | 18.27 | 15.55 | 11.54 | 16.50 | 2.15 | 2.00 | 1.95 | 1.47 | 1.89 |
| **AK-235** | 18.53 | 16.83 | 15.47 | 11.00 | 15.46 | 2.26 | 2.20 | 2.17 | 2.17 | 2.20 |
| **Mean** | 16.91 | 14.92 | 13.31 | 10.12 | 13.82 | 2.55 | 2.46 | 2.37 | 2.22 | 2.40 |
| **For comparing** | | **S.Em±** | | **CD at 5%** | | | **S.Em±** | | **CD at 5%** | |
| **Genotypes (G)** | | 0.327 | | 0.906 | | | 0.075 | | 0.208 | |
| **Salinity (S)** | | 0.258 | | 0.893 | | | 0.094 | | 0.325 | |
| **Interaction (GxS)** | | 0.655 | | 1.816 | | | 0.150 | | 0.416 | |

$S_1$ : 0.8 dS $m^{-1}$, $S_2$ : 4.5 dS $m^{-1}$, $S_3$ : 8.0 dS $m^{-1}$, $S_4$ : 14.8 dS $m^{-1}$

number of bolls was observed in LRA-5166 (10.46) and was on par with NHH-44 (10.83).

Lower salinity of $S_1$ (0.8 dS $m^{-1}$) had higher bolls/plant (16.91). With increase in salinity, number of bolls/plant decreased from $S_1$ (16.91) to $S_4$ salinity level (10.12). $S_1$ salinity level had 67 per cent more bolls/plant compared to $S_4$.

Interaction effect was found to be significant with higher number of bolls/plant in RAHS-14 (20.87) followed by Jayadhar (20.63), whereas, the least number of bolls/plant was observed in Dhumad at $S_4$ (06.87).

**4.13.5 Boll weight (g/boll)**

The data showed significant differences among salinity levels, genotypes and interaction (Table 22).

Significantly higher mean boll weight was observed in NHH-44 (3.22) followed by LRA-5166 (3.10). The genotype Jayadhar that had the lowest boll weight of 1.89 g was 41 per cent lower than the highest boll weight in NHH-44. With increase in salinity, there was decrease in boll weight. Salinity level of $S_1$ recorded highest boll weight of 2.55 and least boll weight was observed with $S_4$ salinity level (2.22).

Interaction effect was found significant with NHH-44, which had significantly higher boll weight at $S_1$ level (3.37) and was followed by itself at $S_2$ salinity level (3.30). However, significantly lower boll weight (1.47) was observed in Jayadhar at $S_4$ (14.8 dS $m^{-1}$) salinity level.

# Discussion

# V. DISCUSSION

The predominant occurrence of saline and alkaline soils in arable lands of arid and semi arid regions has been a matter of great concern to all those involved in maximising food and oil seed production. Only limited number of varieties have been developed with improved tolerance to salt based on agronomic characters such as yield or survival under saline conditions and also based on physiological characters to some extent.

An understanding of the physiological basis along with anatomical adaptations is an another approach to salinity problem. Eventhough there are many mechanical and chemical methods for the reclamation of saline soils, they are expensive. Hence, selection of crop varieties to salt tolerance and breeding for salt tolerance might be more successful for the proper use of waste saline areas.

Cotton is one of the most important salt tolerant crops among the field crops. The present investigation deals with the response of some cotton genotypes to varying salinity levels and their relative salt tolerance. The relative performance of these genotypes to certain morpho-physiological, biochemical and anatomical characters and cause for yield difference are discussed in this chapter.

A pot experiment was carried out during the year 2001-02 to study the influence of salinity on different physiological, biochemical, anatomical and yield and yield parameters of six cotton genotypes. Correlation coefficients were worked out between physiological, biochemical parameters and yield and total dry matter at harvest.

**Table 23. Correlation coefficients between morpho-physiological, biophysical, anatomical parameters, total dry matter and seed cotton yield at increasing salinity levels from 0.8 to 14.8 dS $m^{-1}$**

| Parameters | TDM 30DAS | TDM 60 DAS | SL | RL | SVI | RVI | SD (abaxial) | SD (adaxial) | LA 30 DAS | LA 60 DAS | PS 30 DAS | PS 60 DAS | SC 30 DAS | SC 60 DAS | TR 30 DAS | TR 60 DAS | MT | TDM harvest | Seed cotton yield |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TDM 30 DAS | - | 0.902* | 0.726* | 0.783* | 0.810* | 0.832* | -0.582 | -0.550 | 0.868* | 0.715* | 0.873* | 0.816* | 0.864* | 0.813* | 0.884* | 0.920* | 0.512 | 0.541* | 0.617* |
| TDM 60 DAS | | | 0.715* | 0.816* | 0.798* | 0.850* | -0.594 | -0.572 | 0.794* | 0.786* | 0.743* | 0.861* | 0.701* | 0.725* | 0.742* | 0.792* | 0.538 | 0.701* | 0.759* |
| SL | | | - | 0.862* | 0.895* | 0.824* | -0.625 | -0.634 | 0.789* | 0.734* | 0.756* | 0.863* | 0.794* | 0.765* | 0.803* | 0.831* | 0.586 | 0.614* | 0.625* |
| RL | | | | - | 0.873* | 0.896* | -0.725 | -0.701 | 0.796* | 0.838* | 0.631* | 0.799* | 0.621* | 0.643* | 0.639* | 0.695* | 0.621 | 0.810* | 0.796* |
| SVI | | | | | - | 0.906 | -0.619 | -0.611 | 0.898* | 0.778* | 0.776* | 0.883* | 0.825* | 0.810* | 0.838* | 0.862* | 0.701 | 0.648* | 0.693* |
| RVI | | | | | | - | - | -0.742 | 0.829* | 0.870* | 0.664* | 0.815* | 0.654* | 0.680* | 0.669* | 0.710* | 0.593 | 0.832* | 0.834* |
| SD (abaxial) | | | | | | | | 0.935 | -0.581 | -0.778 | -0.471 | -0.641 | -0.415 | -0.501 | -0.428 | -0.503 | 0.732 | -0.661 | -0.672 |
| SD (adaxial) | | | | | | | | - | -0.601 | -0.742 | -0.492 | -0.638 | -0.450 | -0.488 | -0.478 | -0.518 | 0.561 | -0.601 | -0.660 |
| LA 30 DAS | | | | | | | | | - | 0.692 | 0.874* | 0.908* | 0.881* | 0.850* | 0.821* | 0.819* | 0.721* | 0.572* | 0.618* |
| LA 60 DAS | | | | | | | | | | - | 0.516* | 0.698* | 0.482* | 0.534* | 0.648* | 0.601* | 0.420 | 0.896* | 0.901* |
| PS 30 DAS | | | | | | | | | | | - | 0.924* | 0.906* | 0.864* | 0.948* | 0.926* | 0.621* | 0.398* | 0.424* |
| PS 60 DAS | | | | | | | | | | | | - | 0.902* | 0.899* | 0.910* | 0.937* | 0.395 | 0.543* | 0.632* |
| SC 30 DAS | | | | | | | | | | | | | - | 0.915* | 0.964* | 0.960* | 0.482 | 0.340* | 0.372* |
| SC 60 DAS | | | | | | | | | | | | | | - | 0.972* | 0.928* | 0.398 | 0.351* | 0.435* |
| TR 30 DAS | | | | | | | | | | | | | | | - | 0.978* | 0.496 | 0.325* | 0.401* |
| TR 60 DAS | | | | | | | | | | | | | | | | - | 0.506 | 0.361* | 0.458* |
| MT | | | | | | | | | | | | | | | | | - | 0.513 | 0.548* |
| TDM harvest | | | | | | | | | | | | | | | | | | - | 0.864 |
| Seed cotton yield | | | | | | | | | | | | | | | | | | | - |

* Significant at 0.05 level
TDM : Total Dry Matter
SL : Shoot Length
RL : Root Length
SVI : Shoot Vigour Index
RVI : Root Vigour Index
SD : Stomatal Density
LA : Leaf Area
PS : Photosynthesis
SC : Stomatal Conductance
TR : Transpiration Rate
MT : Mesophyll Thickness

## 5.1 GERMINATION PERCENTAGE, SHOOT LENGTH AND ROOT LENGTH

Seed germination and seedling growth differ from one crop to other under saline conditions and also a variation was recorded among the cultivars of the same crop (Ahmed *et al.*, 1991, Khan *et al.*, 1995 and Qadir and Shams, 1997). Initial stages of plant growth are most critical as later plant development and crop yield depends on effective germination and seedling growth.

Germination per cent decreased with an increase in salinity level from 0.8 dS $m^{-1}$ to 14.8 dS $m^{-1}$. Genotypes differed significantly with respect to the extent of reduction in germination under salinity stress. Among genotypes, AK-235 (24%) followed by LRA - 5166 (27%) had lower reduction in germination per cent at highest salinity levels (14.8 dS $m^{-1}$). Germination was not much affected at lower salinity level (0.8 dS $m^{-1}$). NHH-44 had comparatively higher germination per cent in lower salinity level of 0.8 dS $m^{-1}$. Dhumad had higher per cent reduction of germination (31%) followed by Jayadhar (29%) and RAHS –14 (28 %). Reduction in germination has been reported in different crops by several workers (Mishra *et al.*, 1996 in *Brassica juncea* and Dash and Panda, 2000 in wheat). Sharma *et al.* (1991), Varghese *et al.* (1995) and Gill *et al.* (1999) reported a decrease in germination per cent of cotton with an increase in salinity level and genotypes with least reduction in germination were considered to be tolerant (Khan *et al.*, 1995, Phogat *et al.*, 2001 and Ashraf *et al.*, 2002). The reduction in germination under saline conditions could be due to decreased water potential of soil water resulting in decreased absorption of water by the seeds (Chazen and Neumann, 1994).

Genotypes differed to a large extent in the reduction of shoot and root length under salinity stress. Shoot length was found to be more sensitive than root length. Eventhough NHH-44 had higher reduction in shoot length (48%) after Dhumad (57%), it recorded comparatively higher shoot length at all salinity levels. However, lower reduction in root length was observed in AK-235 (23%) and Jayadhar (25%) and maximum reduction in root length was noticed in RAHS-14 (34%) and LRA-5166 (32%). The results indicated that increasing salinity had greater influence on shoot growth than root growth. In general, genotypes with lower reduction in shoot and root length are found to be tolerant with higher dry matter accumulation at higher salinity levels.

Correlation studies indicated that shoot length had significant positive correlation with total dry matter at 30 DAS (0.726) and 60 DAS (0.715) and seed cotton yield (0.625). Similarly, root length had a strong association with total dry matter at 30 and 60 DAS and seed cotton yield (0.783, 0.816 and 0.796 respectively). Thus, results showed that the genotypes having higher shoot and root length at early stages of crop growth would produce more dry matter and yield under salt stressed environment.

## 5.2 ROOT TO SHOOT RATIO, SHOOT VIGOUR INDEX AND ROOT VIGOUR INDEX

Root to shoot ratio increased with an increase in salinity level from 0.8 dS $m^{-1}$ to 14.8 dS $m^{-1}$. Genotypes differed significantly with respect to the increase in root to shoot ratio under salinity stress. Among genotypes, RAHS-14 (18%) had lower per cent increase in root to shoot ratio at

highest salinity level (14.8 dS $m^{-1}$). Genotype Dhumad had comparatively higher per cent increase in root to shoot ratio (61%) with highest root to shoot ratio at highest salinity level (14.8 dS $m^{-1}$). Increase in root to shoot ratio with the increase in salinity level is mostly due to decrease in growth rate of shoot as compared to root (Varghese *et al.*, 1995). Root to shoot ratio indicates the decline in the growth rate of shoot. Similar increase in root to shoot ratio was reported by Meloni *et al.*, (2001).

Genotypes differed to a large extent in the reduction of shoot and root vigour indices under salinity stress. Shoot vigour index was found to be more sensitive than root vigour index. Dhumad had higher reduction in shoot vigour index (73%). NHH-44 recorded comparatively higher shoot vigour index at all salinity levels. Lower reduction in root vigour index was observed in AK-235 (44%) and the maximum reduction in root vigour index was noticed in Dhumad (56%) along with RAHS-14 (55%). The results indicated that increasing salinity had greater influence on shoot growth than root growth. In general, genotypes with lower reduction in shoot and root vigour indices recorded better dry matter accumulation and yield at higher salinity levels.

Correlation studies indicated that shoot vigour index had significant positive correlation with total dry matter at 30 DAS (0.810) and 60 DAS (0.798) and seed cotton yield (0.693). Similarly, root vigour index had a strong association with total dry matter at 30 and 60 DAS and seed cotton yield (0.832, 0.850 and 0.834 respectively). Thus, results showed that the genotypes having higher shoot and root vigour indices at early stages of crop growth would produce more dry matter and yield under salt stressed environment.

## 5.3 TOTAL DRY MATTER

Total dry matter at 30 and 60 DAS differed significantly among genotypes and salinity levels. The genotype NHH-44 maintained significantly higher total dry matter at both stages at all the salinity levels. However, the genotypes AK-235 (39%) and RAHS–14 (41%) at 30 DAS and AK-235 (39%) and NHH-44 and RAHS-14 (45% each) at 60 DAS had lower reduction of dry matter at highest salinity level (14.8 dS $m^{-1}$). Whereas, the genotype Dhumad had the highest reduction of dry matter at both the stages. Similarly, salt tolerant cotton genotypes were found to have lower reduction in dry matter yield (Khan *et al.,* 1995). In general, genotypes, which recorded comparatively smaller reduction in dry matter, recorded higher yields.

Total dry matter at 30 and 60 DAS had a significant positive correlation with total dry matter at harvest and seed cotton yield. This indicates that the genotypes, which had higher dry matter at early stages, are tolerant to salt stress.

## 5.4 LEAF AREA

Salinity affect leaf expansion before photosynthetic process (Munns *et al.,* 1982). The genotypes NHH-44 and LRA-5166 maintained higher leaf area at all salinity levels at both the stages (30 and 60 DAS), whereas, Dhumad recorded lower leaf area at both the stages, The genotypes AK-235 at 30 DAS (42%) and NHH-44 at 60 DAS (31%) recorded lower reduction in leaf area at highest salinity level over control, whereas, Jayadhar at 30 DAS (53%) and Dhumad at 60 DAS (53%) had maximum reduction. The genotypes, which showed lesser reduction in leaf area also, recorded a lower decrease in dry matter both at 30 and 60 DAS.

Correlation studies showed a significant positive correlation of leaf area both at 30 and 60 DAS with total dry matter at harvest and yield indicating that the early differences in leaf expansion resulted in differences in final dry matter production.

The decrease in leaf area was attributed to reduction in cell size rather than cell number (Curtis and Lauchi, 1987) due to reduction in cell osmotic potential of leaf cells (Patil *et al.*, 1996).

## 5.5 STOMATAL DENSITY, STOMATAL LENGTH AND STOMATAL BREADTH.

The effect of few characteristics on yield and total dry matter production is usually small and depends on genetic constitution of genotype and environmental condition. So it is very difficult to determine the effect of such characteristics on yield and total dry matter production. Stomatal behavior is one of such characters, which influence transpiration rate, stomatal conductance and photosynthesis. Stomatal density and size changes under salt stress mainly due to changes in leaf area (Curtis and Lauchli, 1987).

The data indicated that both abaxial and adaxial stomatal density increased with salinity. The number of abaxial stomata was more than the number of adaxial stomata regardless of salinity. The genotype LRA-5166 had maximum number of stomata both on abaxial and adaxial leaf surfaces. Genotype NHH-44 showed smaller increase in stomatal density on both surfaces (10% on lower and 18% on upper surfaces respectively) along with AK-235 (10%) on lower surface and LRA-5166 (18%) on upper surface, whereas, Dhumad (14%) and Jayadhar (13%) on lower surface

and Dhumad (27%) on upper surface had maximum increase in stomatal density.

Stomatal length and breadth both on abaxial and adaxial leaf surfaces decreased with increasing salinity, but to a smaller extent. However, significant reduction in stomatal length and breadth was noticed at higher salinity level (14.8 dS $m^{-1}$). On lower leaf surface, maximum reduction in stomatal breadth was observed in AK-235 and Jayadhar. Dhumad had minimum reduction in stomatal breadth on lower leaf surface and recorded comparatively higher reduction in stomatal length. Similarly, on upper leaf surface, minimum reduction in stomatal breadth was observed in LRA-5166, but it had maximum reduction in stomatal length. Genotypes having higher reduction in stomatal breadth had lower reduction in stomatal length and vice versa.

An increase in stomatal frequency under salt stress has been reported by many workers (Curtis and Lauchli, 1987 and Rajgopal, 1999), whereas, Patil *et al.*, (1996) found no significant differences among salinity levels for stomatal density in salt stressed maize. However, Jafri *et al.* (1995) noted that the stomatal density decreased in cotton under salt stress and this was followed by an increase in stomatal size whereas, Buttery *et al.* (1992) reported an increase in stomatal density as a result of moisture stress in soybean and they presumed that this was brought about by a decrease in leaf expansion. In general, the present study indicated that the genotypes having higher reduction in leaf area recorded larger increase in stomatal density. Similarly Jones (1977) reported negative relationship between stomatal density and leaf size under stress conditions.

## 5.6 PHOTOSYNTHESIS, STOMATAL CONDUCTANCE AND TRANSPIRATION RATE

The Photosynthetic rate, Stomatal conductance and transpiration rate decreased under stress conditions in cotton (Leidi *et al.*, 1993).

In the present study, the photosynthetic rate, stomatal conductance and transpiration rate decreased with an increase in the salinity level at both stages. Among the genotypes, species belonging to *Gossypium hirsutum* had comparatively higher values for these characters. NHH-44 had maximum photosynthetic rate at all salinity levels at both stages and a lesser reduction in photosynthesis (20%) along with AK-235 (23%) which showed least effect of salinity on photosynthesis Maximum reduction in photosynthesis was observed in Jayadhar at both stages (32% and 39% respectively). Similar results of decrease in photosynthetic rate under saline conditions were reported by Leidi *et al.* (1993), Patil *et al.*, (1996) and Jhu and Frederick (1999).

In the present experiment, the salt tolerant genotypes viz., NHH-44, AK-235 which recorded lower reduction in leaf area, maintained higher photosynthetic rate even under saline conditions.

In the present study, stomatal conductance and transpiration rate decreased with increase in salinity level. Genotypes of species *Gossypium hirsutum* recorded higher stomatal conductance and transpiration rate but these species showed maximum reduction in stomatal conductance and transpiration rate compared to other genotypes. The genotypes NHH-44 and LRA-5166 had comparatively higher stomatal conductance and transpiration rate at all salinity levels, whereas, AK-235 recorded minimum reduction of these attributes.

Reduction in stomatal conductance and transpiration rate under saline conditions has been reported by Dong *et al.* (1996) and Ashraf and O'leary (1996) which resulted in smaller reduction in total dry matter at harvest and seed cotton yield.

Photosynthetic rate, stomatal conductance and transpiration rate had significant positive correlation with total dry matter both at 30 and 60 DAS, total dry matter at harvest and seed cotton yield. These characters had significant negative correlation with stomatal density of both abaxial and adaxial leaf surfaces.

## 5.7 BIOCHEMICAL PARAMETERS

### 5.7.1 Chlorophyll content

A reduction in chlorophyll content under saline conditions has been reported by many workers (Saha and Gupta, 1993 and Singh *et al.*, 1994), which influence photosynthetic rate and in turn influence growth and yield of cotton (Garg and Garg, 1985).

In the present study, chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of leaf decreased with increase in salinity at both stages, whereas, it increased from 30 DAS to 60 DAS. The genotype NHH-44 had significantly higher chlorophyll 'a', chlorophyll 'b' and total chlorophyll content at both stages. In general, the genotypes LRA-5166, RAHS-14 and AK-235 had lower reduction in chlorophyll 'a' content, whereas, genotypes Dhumad and NHH-44 showed more reduction. Similarly, genotypes NHH-44 and RAHS-14 showed lesser reduction in chlorophyll 'b' content, whereas, Dhumad and AK-235 showed higher reduction. The data on total chlorophyll content indicated that, it was high in genotypes NHH-44, RAHS-14 and AK-235. These genotypes

maintained higher chlorophyll content at higher salinity levels. Lower total chlorophyll content was observed in Dhumad and Jayadhar.

In all genotypes, relatively higher rate of depletion was found with chlorophyll 'a' than chlorophyll 'b' (Sudhakar *et al.*, 1991 and Ramanujalu *et al.*, 1993). The reduction in chlorophyll under salinity was attributed to destruction of chlorophyll and instability of pigments and proteins (Somani, 1991). Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content at both stages had significant positive correlation with total dry matter at harvest and seed cotton yield.

**5.7.2 Free proline content**

The proline is known to accumulate rapidly in a variety of crop plants in response to salt stress (Gill and Sharma, 1999). Eventhough its precise role is not clear, proline accumulation was correlated with salt tolerance and also served as a source of solute for intercellular osmotic adjustment under saline conditions (Stewart and Lee, 1974).

In the present study, the proline content in leaves of different cotton genotypes increased with increase in salinity levels and genotypes differed significantly in their ability to accumulate free proline under salinity stress. Genotypes LRA-5166 and NHH-44 at 30 DAS and NHH-44, RAHS-14 and LRA-5166 at 60 DAS contained more proline at higher salinity levels. The genotypes Jayadhar and Dhumad at 30 DAS and Dhumad, Jayadhar and AK-235 at 60 DAS accumulated less proline at higher salinity levels. Proline accumulation increased from 30 to 60 DAS also. Increased accumulation of proline imparts certain degree of tolerance against salinity.

Accumulation of proline during salinity stress has been reported by many workers (Patil *et al.*, 1974, Plunneke and Johan, 1972, Chandrashekhar and Sandhyarani, 1996 and Lal-Hussain *et al.*, 1999). Chu *et al.*, (1976) suggested that the accumulation of proline under salt stress followed as a consequence of reduction in cell osmotic potential.

### 5.7.3 Sugar content

Solute accumulation was considered as a suitable screening parameter for salinity tolerance (Ashraf *et al.*, 1991). Osmoregulation through accumulation of soluble sugars in roots and leaves are characteristics of salinity stressed plant (Rathert *et al.*, 1983).

Soluble sugar content increased with increasing salinity level. Higher rate of sugar accumulation was observed between $S_2$ (4.5 dS $m^{-1}$) and $S_3$ (8.0 dS $m^{-1}$) salinity levels at both stages. Prakash and Prathapasenan (1989), Ding et al. (1995) and Rajgopal (1999) have also reported increase in sugar content. In contrast to this, decrease in sugar content with increase in salinity level has also been reported by several authors (Dubey, 1987 and Salam and Awadalla, 1989).

Among the genotypes, RAHS-14 and Jayadhar had higher soluble sugar content at 30 DAS, whereas, RAHS-14 and LRA-5166 at 60 DAS. AK-235 showed lower sugar content at both stages. AK-235 and Dhumad showed higher per cent increase in sugar content with increase in salinity levels.

In general, no relationship was found between accumulation of sugar and differential genotypic response to salt stress (Saiz and Leidi, 1994). Further, Rathert (1983) reported that, metabolic osmotic adjustment through soluble sugars alone is doubtful.

The effect of anatomical adaptations due to salinity on yield and total dry matter production is negligible. So it is very difficult to identify the effect of such characters on yield and its components. However, mesophyll surface area increases with increase in salinity (David *et al.*, 1979).

The data indicated that mesophyll thickness, pallisade thickness and spongy layer thickness increased with salinity. The spongy layer thickness was more as compared to pallisade layer thickness. The genotype NHH-44 had maximum mesophyll thickness and spongy layer thickness, whereas, genotype Dhumad recorded maximum pallisade thickness. Genotype NHH-44 showed smaller per cent increase in mesophyll thickness (6%), pallisade thickness (4%) and spongy layer thickness (7%), whereas, Dhumad showed maximum per cent increase for mesophyll thickness (17%), pallisade thickness (20%) and spongy layer thickness (14%).

Widths of pallisade and spongy cells increased with increase in salinity. Similar results were obtained by David *et al.*, (1979). Genotype NHH-44 had maximum width for both pallisade and spongy cells. Maximum per cent increase in pallisade cell width was observed in Dhumad (18%) and minimum per cent increase was observed in case of AK-235 (9%). In case of spongy cell width, Dhumad showed maximum per cent increase of 20 per cent, while, RAHS–14 showed minimum per cent increase (4%). Increase in mesophyll thickness under salinity was reported by many workers (Jafri *et al.*, 1995 and Singh and Mohan, 1999).

Increase in the area of mesophyll cells available for gas exchange was attributed to increase in leaf thickness has been proposed as a mechanism by which plants might offset the deleterious effects of salinity in cotton (Longstregth and Nobel, 1979).

## 5.9 SEED COTTON YIELD AND ITS COMPONENTS

The data on seed cotton yield and its components indicated significant genotypic differences at all the salinity levels. A linear reduction in yield with increase in salinity levels was noticed. Reduction of seed cotton yield due to salinity have been reported by EL-Saidi (1973), Ahmad *et al.* (1991), Munk and Roberts (1995), Vulkan *et al.* (1998) and Phogat *et al.* (2001).

A superior genotype with better salt tolerance can be evaluated by identifying the genotype, which can perform its growth and development, and having lesser yield reduction with higher yield potential under saline conditions.

In the present study, among six genotypes tested, NHH-44 recorded maximum yield and differed significantly from others. RAHS-14 and AK-235 were also recorded comparatively higher yield. The genotypes NHH-44 and LRA-5166 recorded a lesser per cent reduction in their yields over control.

Similar to seed cotton yield, yield components also showed significant variations among different genotypes. Among the genotypes, RAHS-14 (16.62) and Jayadhar (16.50) recorded higher number of bolls but NHH-44 (11%) and LRA-5166 (19%) recorded least reduction in boll

number at highest salinity level compared to control whereas, NHH-44 (3.22) and LRA – 5166 (3.10) recorded maximum single boll weight as well as least per cent reduction in boll weight (9% and 6% respectively) after AK-235 (4%). Decrease in the number of bolls and single boll weight with increase in salinity was also reported by Janardhan *et al.* (1979), Latif and Khan (1976), Sharma *et al.* (1991) and Subbaiah *et al.* (1995).

# Summary

# VI. SUMMARY

Salinity has become a major agricultural treat to large areas of land world wide, especially in irrigated agriculture. Eventhough cotton is recognised as most salt tolerant of all the field crops, it has not received the proper attention to investigate most salt tolerant genotypes with respect to physiological, biochemical and anatomical characters and yield and yield attributes. Hence, the present investigations were carried out with six cotton genotypes, two belonging to *Gossypium hirsutum* (NHH-44 and LRA-5166), three to *Gossypium herbaceum* (RAHS-14, Dhumad and Jayadhar) and one to *Gossypium arboreum* (AK-235) under four salinity levels (0.8, 4.5, 8.0 and 14.3 dS $m^{-1}$) during 2001–02. It was intended to study the changes in the growth, physio–biochemical, biophysical, anatomical and yield potential of cotton genotypes under saline conditions to know the salt tolerance characters in different genotypes.

1. In the present investigations, seed germination, shoot length, root length, shoot vigour index and root vigour index at 30 DAS and leaf area and total dry matter at 30 and 60 DAS decreased with increase in salinity levels. The genotypes AK-235 and NHH-44 recorded comparatively lower reduction in these characters at higher salinity levels, whereas, Dhumad had comparatively higher reduction. These characters had significant positive relationship with seed cotton yield and total dry matter at harvest.

2. The genotypes differed significantly for stomatal density, stomatal breadth and stomatal length on both the leaf surfaces. Salinity increased the stomatal density but reduced stomatal breadth and length.

3. Photosynthetic rate, stomatal conductance and transpiration rate decreased with an increase in the salinity. The genotypes NHH-44 and LRA-5166 had comparatively higher photosynthetic rate, stomatal conductance and transpiration rate at all the salinity levels. However, RAHS-14 had lower reduction in these characters at different salinity levels. Maximum reduction in photosynthetic rate was observed in Jayadhar. These characters had significant positive correlation with seed cotton yield and total dry mater at harvest.

4. Chlorophyll 'a', chlorophyll 'b' and total chlorophyll contents decreased with an increase in salinity. In general, RAHS-14 and AK-235 showed lower reduction in chlorophyll 'a' content, whereas, Dhumad showed higher reduction in both chlorophyll 'a' and chlorophyll 'b' content. RAHS-14 and NHH-44 showed lower reduction in total chlorophyll content.

5. Free proline content increased in all the genotypes with an increase in the salinity. The genotypes NHH-44 and LRA-5166 accumulated more proline, while Jayadhar at 30 DAS and Dhumad at 60 DAS accumulated less proline, particularly at higher salinity levels.

6. The sugar content increased due to salinity and the genotypes RAHS-14 and Jayadhar had higher sugar content at 30 and 60 DAS respectively. In general, AK-235 recorded lower sugar content at all the salinity levels.

7. The genotypes differed significantly for mesophyll thickness, pallisade thickness and spongy layer thickness and also for size (width) of pallisade and spongy cells. Salinity increased mesophyll surface area

(pallisade layer + spongy layer) and also cell size (pallisade and spongy cells). AK-235 and NHH-44 showed comparatively minimum increase in these characters.

8. Salinity reduced the seed cotton yield to the extent of 25 and 45 per cent at 8.0 and 14.8 dS $m^{-1}$ salinity levels respectively as compared to control. Among the genotypes, NHH-44 had the highest yield followed by RAHS-14. The per cent reduction in seed cotton yield was least in NHH-44 and highest in Dhumad.

9. Total dry matter at harvest was reduced significantly due to salinity. The genotypes NHH-44 and AK-235 recorded higher total dry matter at all the salinity levels. While, NHH-44 and LRA-5166 had lower per cent reduction in dry matter at higher salinity level. Maximum reduction in total dry matter at harvest was observed in Dhumad.

10. Yield components such as, number of bolls and boll weight were also affected by salinity. RAHS-14 and Jayadhar had higher boll number at all the salinity levels, while, Dhumad had higher reduction in boll number. Boll weight also decreased with increase in salinity.

Based on the information generated from the present investigation, it can be concluded that genotypes differed widely in their response to salinity and different genotypes may have different adaptations against salinity stress. Thus information obtained would be useful in breeding cotton for salt tolerance. Based on this study, the genotypes NHH-44, RAHS-14 and AK-235 would be better suited for salinity stress. LRA-5166 had moderate values for different physiological, biochemical, biophysical and anatomical parameters that found to be physiologically and

biochemically efficient in salt stressed environments. From this, it can be concluded that the following important characters serve as tool in identifying the salt tolerance,

1. Lower reduction in seed germination, shoot growth and root growth at higher salinity levels.
2. Higher shoot and root vigour indices at higher salinity levels.
3. Lower reduction in leaf area and total dry matter at highest salinity levels.
4. Maintenance of higher chlorophyll content.
5. Higher proline accumulation.
6. Maintenance of higher photosynthetic rate, stomatal conductance and transpiration rate at higher salinity levels.

The plant improvement involving only yield and yield related characters does not sufficiently explain the salt tolerant characters. So it is necessary to make use of other physiological, biophysical, biochemical and anatomical characters in breeding for salt tolerance of crop plants.

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**MESO-STRUCTURE OF LEAF IN RELATION TO PHOTOSYNTHESIS AND PRODUCTIVITY IN COTTON (*Gossypium* spp.) UNDER SALT STRESS**

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## ABSTRACT

Investigation on meso-structure of leaf in cotton was made with six cotton was made with six cotton genotypes, belonging to *Gossypium hirsitum* (NHH-44 and LRA-5166), *Gossypium herbaceum* (RAHS-14, Dhumad and Jayadhar) and *Gossypium arboreum* (AK-235) under four levels of salinity in pot experiment. The objective was to study the changes in meso-structure of leaf, biochemical and biophysical parameters, yield potential and their relationship under varying salinity levels and finally to find out the mechanism of salt tolerance in relation to meso-structure of leaf in cotton.

The results showed that seed germination, shoot length, root length, shoot vigour index, root vigour index, leaf area and total dry matter (30 and 60 DAS) decreased with increase in salinity level. The relative decrease was lower in NHH-44 and AK-235 as compared to other genotypes.

Salinity increased stomatal density while decreased stomatal size, stomatal conductance, transpiration rate, photosynthetic rate in all the genotypes. The genotypes RAHS-14 and AK-235 showed lower reduction in these characters whereas maximum reduction was observed in genotypes Jayadhar and Dhumad.

In general, chlorophyll 'a', chlorophyll 'b' and total chlorophyll content decreased with increase in salinity, whereas, free proline content and sugar content increased in all the genotypes.

Mesophyll surface area increased with increase in salinity, AK-235 and NHH-44 showed comparatively minimum increase in this character.

Seed cotton yield and yield components such as, number of bolls and boll weight were reduced with increase salinity.

Based on the study, the genotypes NHH-44, AK-235 and RAHS-14 were found to be tolerant to salinity stress. Lesser reduction in germination, shoot and root growth, shoot and root vigour indices, leaf area, total dry matter and chlorophyll content and proline accumulation can serve as selection criteria for identification of salinity tolerance. In addition anatomical character mesophyll surface area can also be considered as one of the important criteria for salinity tolerance in cotton.