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ALLEVIATION OF CHANGES IN GROWTH AND PHYSIOCHEMICAL PARAMETERS IN SALT (NACL) STRESSED OF TWO SESAME (*SESAMUM INDICUM L)* VARIETIES

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ABSTRACT

Present research was carried out with the aim of identifying effect of salt stress (NaCl 0(Control), 40, 80, and 120 mM) on growth and physiochemical parameters of sesame *(Sesamum indicum* L.) variety CO-1 and KRR-2. Various parameters such as height of the plant, leaf area, fresh weight of the whole plant and dry weight of whole plant and decreased the content of physiochemical parameters were analyzed. All the parameters were recorded at 30th Days after Treatment (DAT). Analysis revealed a significant reduction in all parameters with increased salt concentration. This experiment carried out fully pot culture methods. Severity of salinity stressed condition were also observed with increased salinity concentration level at the same time decrease in all growth parameter were non-significant in stressed plant as compared two sesame varieties CO-1 and KRR-2. CO-1 in the sesame varieties at 120mM high salt concentration increased with lower decreased was observed in height of the plant, Leaf area, fresh weight of the whole plant and dry weight of whole plant and decreased the content of physiochemical parameters compared to other sesame varieties KRR-2 under salt stress condition. The results indicated that plants of sesame variety CO-1 exhibited higher adaptive potential under salinity stress as judged by higher growth and higher content of physiochemical parameters when compared to variety KRR-2.

Keywords: Salinity, Growth, Pigments, Sesame, Protein. Foliar nitrogen

INTRODUCTION

The total area of salt-affected soils in the world is 831 million hectares which includes 397 and 434 million hectares of saline and sodic soils, respectively (FAO, 2021). The agricultural land is decreasing constantly due to population pressure, adverse environmental condition, continuously increasing natural calamities, and global climate change. More than 45 million hectares of irrigated land are affected by salt which account for 20% of total land and 1.5 million ha of land are taken out of production each year owing to high salinity levels, if it continues in such way, 50% of cultivable lands will be lost by the 21st century (FAO, 2021). Effect of salinity stress on carbohydrate, lipid peroxidation and proline contents of two horse gram varieties (Kanagaraj and Sathish, 2017). Alkalinity and acidity in saline soil are two major factors that inhibit the growth and development of higher plants in both halophyte and glycophyte species (Yang *et al*., 2008). Salinity alters a wide array of metabolic processes in growing plants and induces changes in contents and activities of many enzymes (Khan and Panda, 2008). The reduction in yield of many crops by salinity is well documented (Khalida and Da Silva, 2010). Farhoudi (2010) showed salt stress declined root length, shoot length and seedling dry weight of although increased seedling electrolyte leakage and catalase (CAT) and peroxidase (POD) activity. (Kandil *et al.*, 2012) found that increasing salinity concentrations from 0 to 1.75% NaCl significantly decreased final germination percentage (FGP%), germination rate (GR) and speed germination index (SGI). The growth of plants may be reduced under salt stress because of (a) an osmotic stress due to lowering of the external water potential or (b) effects of specific ions on metabolic processes ranging from the absorption of nutrients to enzyme activation or inhibition (Zhang *et al.,* 2010; Qin *et al.,* 2011). Osmotic adjustment in two ways under saline condition i) make capable plants to uptake water under saline condition and ii) keep stomata open by maintaining turgor of the plant cell. So, plant species/varieties tolerant to high level of salt essential for the utilization of the highly salt affected soils. The metabolic imbalances due to ionic toxicity, osmotic stress and nutritional deficiency may lead to oxidative stress (Baatour *et al.,* 2010). Salt stress affects all major processes including photosynthesis, protein synthesis, lipid and energy metabolism (Desingh and Kanagaraj, 2007).

Sesame (*Sesamum indicum* L.) belongs to the Pedaliaceae family, and Africa is considered its center of origin due to the large number of species from the *Sesamum* genus on that continent (Sousa *et al.,* 2014). The oil extracted

from its seeds can be used in the manufacturing of pies, margarine, perfumes, lubricants, medicines and soap. *Sesamum indicum* L. is the major commercial source of sesame seeds and is primarily grown in Burma, India, China, Ethiopia and Sudan with 9.398.770 ha under sesame cultivation worldwide, producing 4.76 million tones (FAO, 2013). Sesame seeds contain high oil and protein accounting to about 50% and 25% respectively. Sesame protein is rich in arginine, leucine and the sulpher containing amino acids cysteine and methionine but slightly low in the essential amino acid reflects linolic acid (47%), oleic acid (43%), palmitic (11%) and steric acid (10%) with trace amount of leinoleic acid (Latif and Anwar, 2011). Sesame oil is valuable edible oil with a composition that provides good health benefits including high level of unsaturated fatty acids and antioxidants (Bist *et al*., 1998). In India, Sesame (*Sesamum indicum* L.) has a wide geographic distribution extending over a range of environmental conditions. However, as other crops in India, Sesame is also subjected to environmental stresses, particularly salinity. Although much information is available on the agronomics aspects of Sesame very little is known about the effects of salinity on physiological and biochemical aspects of sesame. The present study was undertaken to evaluate the salinity responses of two Sesame varieties (*Sesamum indicum* L.) usually used for cultivation.

MATERIALS AND METHODS

The certified sesame (*Sesamum indicum* L.), seeds (Variety: CO-1, KRR-2) were procured from Coimbatore district in Tamilnadu Agricultural University and Karur district. Seeds with inform size were selected and the plants were raised in pots containing red and clay soil and pH of the soil was 7.2 with EC of 0.2 dsm^{-1}. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. Plants were grown under natural climatic conditions. The maximum irradiance (PAR, 400-700nm) available during growth was 1800-2000 µmol $m^{-2}s^{-1}$ on a clear day. Daily maximum and minimum temperatures were 29-33^{0}C and 20-22^{0}C, respectively. Plants were watered for the first 20 days after germination. The seedlings were divided into four groups. One group of seedlings was maintained under non-salinized conditions which served as control plants. The watering solution for control plants consists of tap water and one-fourth strength of Hoagland nutrients (Hoagland and Arnon, 1950). Other three groups were salinized by irrigation daily to soil capacity (500 ml d^{-1}) with the nutrient medium containing 40 mM, 80 mM and 120 mM NaCl. 40mM consider as a low salinity level, 80mM consider as a medium salinity level and 120mM salinity consider as a high salinity level. All the plants used in this study were of comparable size. Sodium chloride used in this study was Laboratory AR grade Assay 99.8%, (Universal Laboratories Pvt. Ltd. Mumbai). Salt treatment was continued until each plant received the required mM NaCl. Care was taken for individual plants in each group received the pre-calculated concentrations of NaCl in full. Additional pots with plants were also maintained for control, as well as each salinity treatment for need of plant material. Young and fully matured leaves were taken from control and salinity treated plants on 30th After Treatment (DAT) for all the experiments described below.

GROWTH COMPONENTS

Height of the plant

The height of the plant was measured with a measuring tap on 30th DAT.

Leaf area

The leaf area was calculated multiplying the length and breadth of the broadest regions of the leaf.

$$\text{Leaf area} = \text{length} \times \text{breadth}$$

Fresh weight of the whole plant

Mature plants were carefully uprooted. The roots were washed blotted and whole plant was weighed.

Dry weight of the whole plant

Mature plants were carefully uprooted and the roots were washed, blotted and the whole plant was dried in an oven at 75-80^0 for 40 hours until a constant weight was obtained.

PHOTOSYNTHETIC PIGMENTS

Total Chlorophyll

The total chlorophyll content of the leaves was estimated according to Arnon, (1949).One gram of leaf samples was cut into small pieces and macerated with 80% (V/V) acetone, with little sand and a pinch of calcium carbonate. The homogenate was centrifuged at 3000g for 10 minutes and the supernatant was made up to a known volume with 80% acetone. The optical density of green supernatant was determined at 645 nm and 663 nm in spectrophotometer, against 80% acetone blank. All the procedures were carried out in dim light.

The total chlorophyll content was calculated using the following formula:

$$\text{Total chlorophyll} = \frac{(20.0\times A645)+(802\times A663)}{1000\times w\times a}\times V(\text{mg/g fw})$$

Chlorophyll-a

Chlorophyll-a content was estimated according to Arnon, (1949).

The same extract, as indicated above was used for the estimation. The formula used:

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

Chlorophyll-b

Chlorophyll-b content of the leaf was also estimated according to Arnon, (1949) using the formula:

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

Protein Content

Total leaf protein content was estimated by Lowry's method (1951) using Folin-Ciocalteu reagent. Extraction Leaf was macerated with 10 ml of 20% trichloroacetic acid (TCA) using mortar and pestle. The mixture was incubated in darkness for 30 minutes and the blue color was read at 660 nm in spectrophotometer. Optical density of a known concentration of Bovine Serum Albumin (BSA) was determined by following the same procedure and a standard graph was prepared using different concentrations of a

standard protein. The values are expressed as mg/gfw.

Foliar Nitrogen Conent

The nitrogen content of the leaves was estimated according to Kjeldahl method using the KJEL PLUS System (Pelican, India).The method involves three stages: 1. Digestion 2.Distillation 3.Titration *Reagents* Hydrochloric acid (0.1 N): 0.82 ml of concentrated hydrochloric acid was added to 99.18 ml of distilled water. *Digestion activator*: 25 gm of potassium sulphate, 5 gm of copper sulphate and 0.5 gm of selenium were mixed. Sodium hydroxide (40%): 40 gm of sodium hydroxide was dissolved in 100 ml of distilled water. Mixed indicator: 30 mg of bromocresol green and 20 mg of methyl red were dissolved in 40 ml of 90 % ethanol *Digestion* Leaves were dried, powdered after removing the midribs. 500mg of the powder and 3 g of digestion activator were weighed and added to the digestion tube of the KJEL PLUS Digestion Block System. To this 10 ml of concentrated sulphuric acid was added. The tubes were loaded on to the KJEL PLUS Digestion Block System and the temperature set at 350 °C. The samples were digested for 1 hour. *Distillation* The digestion tube was placed inside the KJEL PLUS DISTIL-M chamber through the alkali hose. The alkali hose at the back panel was immersed in to the bottle containing 40% alkali solution and the volume of the alkali was fixed. The receiver end of the hose was immersed into a conical flask containing 20 ml of boric acid and 2 to 3 drops of the mixed indicator. 30 ml of the alkali was added to the digestion tube. The distillation time was fixed at 6 min and the distillation process started. Titration the solution collected in the conical flask was titrated against 0.1N Hydrochloric acid. The titration value was noted. The percentage of Nitrogen was calculated using the following formula: The Nitrogen content was expressed as percentage of nitrogen per gram d.w.

$$\%\ \text{of}\ N_2 = \frac{(\text{Titrate value}) \times (\text{Normality of HCl}) \times (\text{Nitrogen factor})}{\text{Weight of the sample}}$$

Statistical Analysis

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

RESULTS AND DISCUSSION

Worldwide, more than 45 million ha of irrigated land have been damaged by salt and 1.5 million ha are taken out of production each year as a result of high salinity levels in the soil (Munns and Tester, 2008). The high salt content lowers osmotic potential of soil water and consequently the availability of soil water to plants. The salt–induced water deficit is one of the major constraints for plant growth in saline soils. In addition, many nutrient interactions in salt–stressed plants can occur that may have important consequences for growth. In the present study, the values of plant height was lowered by increasing salinity and were more pronounced using the highest concentration of NaCl (120 mM) compared to untreated control plants of sesame varieties. Plant height was decreased with increasing salinity levels (40 mM, 80 mM and 120 mM) in all two sesame varieties on the sampling days (30^{th} DAT (Days After Treatment) and it was Maximum plant height was recorded in the variety CO-1 (44.11 cm) under high salinity (120 mM) on 30^{th} DAT relative to control plants (78.49 cm respectively) while minimum plant height was recorded in KRR-2 (39.65 cm) over the control plants (70.11 cm respectively). However, lower reduction in plant height was observed CO-1 with high salinity on all the sampling days (30^{th} DAT), while significantly higher reduction was recorded in KRR-2 on all the sampling days under salinity stress. Reduction in growth under salinity has been reported in various plant species e.g. rice (Demiral and Turkan, 2006), tomato (Kaya *et al.*, 2001), cotton (Kanagaraj and Desingh, 2009), Finger millet (Manikandan and Desingh, 2009a). Salinity stress causes extensive oxidative damage, affecting several physiological processes which results in significant reduction of different growth parameters such as germination capacity, radicle and plumule lengths, fresh and dry mass, yields, seed nutritional quality**,** productivity, chlorophyll, protein and sugar content, antioxidative enzymes activity as well as nodulation (Asadi, 2009).Our data on plant height suggest that variety CO-1 maintained its better height on all the sampling days under varying salinity levels compared to other varieties, indicating substantial salt tolerance. Salt stress increased to significantly reduction of growth and physiological parameters such as germination percentage shoot and root length, shoot-root ratio, number of leaves and branches, vigour index, fresh and dry weight, moisture content, relative water content and photosynthetic pigments (Neelesh Kapoor and Veena Pande, 2015). In our study, all varieties of sesame plants showed reduction in surface area of the leaves on exposure to salinity. Among the two varieties, CO-1 exhibited lower reduction of leaf area under salinity stress even on 30^{th} DAT relative to control plants, while comparatively higher reduction of leaf area was observed in KRR-2 under salinity stress. Salt stress, like other abiotic factors, affects leaf size through a decrease of cell expansion (Ticha, 1982) and cell division (Zhu, 2001). Moreover, cellular differentiation is also affected (Bird and Gray, 2003), altering the spatial relations between the different types of leaf cells. Leaf area was measured in salinity treated and control plants of two sesame varieties on two sampling days. On 30^{th} DAT, significantly higher reduction of leaf area was measured in the variety KRR-2 (32.97 cm^2) over the control plants (54.11 cm^2 respectively) with 120 mM salinity stress, while lower reduction of leaf area was observed in the variety CO-1 (29.11 cm^2) compared to control plants (54.01 cm^2 respectively). The results on leaf area clearly indicated that under all salinity levels, CO-1 recorded higher leaf area in the plants on all the sampling days, which is relative to higher photosynthetic rates. Mathur *et al.* (2006) reported, that the stress of the moth bean plant with increasing concentrations of sodium chloride, led to a decrease in leaf area. This decrease was inversely proportional to the concentrations.

The results of the biomass (Fresh and Dry weight of the whole plant) indicated that applied NaCl (40, 80 and 120 mM) inhibited the growth of two varieties on all the sampling days (30^{th}) than control plants. Fresh and Dry weight of the whole plant was decreased with increasing salinity levels in all sesame varieties. However, higher fresh and dry weight of the whole plant was recorded in PAIYUR-1 under salt stress

on all the sampling days (30th DAT). The lowest fresh and dry weight of the whole plant was noted in KRR-2 under all the levels of salt stress. This may be related to the effect of salt stress which resulted in the limitation of water absorption and biochemical processes (Parida and Das, 2005). In addition, a decline in the rates of net photosynthesis occurs, due to adverse affect on CO_2 assimilation, which leads to a decrease in nutrient uptake and finally growth of plants (Aragao *et al.,* 2005). Fresh weight of the whole plant was decreased with increasing salinity concentrations on all the sampling days in two sesame varieties. On 30th DAT, the significantly higher decrease in fresh weight of the whole plant was observed in KRR-2 by 37% (36.19gram) with 120mM salinity relative to control plants (20.56 gram respectively), while lowest decrease in fresh weight of the whole plant was recorded in CO-1 by 25% (18.25gram) over the control plants (35.65 gram respectively). In spite of the fact that many studies have pointed to the positive effect of sodium chloride on fresh and dry weight, there are contrary results, as well, pointing to the negative effect of salt stress on fresh and dry weight and these include a study by (Jamil *et al.,* 2007 b) on radish plants. The present data on biomass revealed that sesame variety CO-1 recorded higher biomass even under higher salinity level compared to other varieties which is directly related to growth and yield of the plant. The variation of the dry weight of the whole plant under salinity stress and it was decreased with increasing salinity levels on all the sampling days. Maximum reduction of dry weight of the whole plant was recorded in the variety KRR-2 and it was (8.74 gram respectively) compared to control (15.90 gram respectively) on 30th DAT with 120mM salinity, while minimum reduction of dry weight of the whole plant was noticed in the variety CO-1 and it was (7.61 gram respectively)relative to control (16.22 gram respectively).

Plant pigments content were determined in different tolerant and sensitive plant varieties at wide range of salt concentration, reduced in chl a, chl b and carotenoid are main photosynthetic pigments and they play important role in photosynthesis. The changes in amount of pigments system were evolved as the changes in photosynthesis (Sarwat and El-Sherif, 2007). Changes of pigment system contents under salt stress are used as parameter for selection of tolerant and sensitive cultivars in crop plants (Eryilmaz, 2007). Chl-a, Chl-b, chl a\b and carotenoid contents showed increase and decrease and depending on exposure time of NaCl exposure in many plants (Pinherio *et al.,* 2008). In the current study, the total chlorophyll content of leaves averaged over two varieties indicated that it decreased significantly with the increase in salt concentration**.** Effect of salinity on total chlorophyll content was studied in two sesame varieties and it was decreased with increasing salinity levels on all the sampling days. On 30th DAT highest total chlorophyll content was recorded in the variety CO-1(0.83 mg/gfw) over to control plants (1.28 mg/gfw, respectively) under 120mM salinity stress, whereas low total chlorophyll content was observed in the variety KRR-2 (0.75 mg/gfw) relative to controls (1.27 mg/gfw, respectively). Varieties differed significantly under salt stress treatments. However, highest total chlorophyll content under salinity stress was recorded in CO-1 on all the sampling days (30th DAT) even at high salinity concentrations, whereas low level of total chlorophyll content was observed in KRR-2 under salinity stress. The reduction of Chl-a and Chl-b amounts with NaCl application was reported in many plants such as *Zea mays*, *Carthamus tinctorius*, *Bean* and *Paulownia imperiallis* that this due to increase of destructive enzymes called chlorophyllase (Rahdari *et al.,* 2012). Pigments system reduction is attributed to a salt induced weakening of protein-pigment-lipid complex are increased chlorophyllase enzyme activity (Turan *et al.,* 2007). Also, increase in pigment in content was observed in salinity stressed plant such as rice (Doganalar *et al.,* 2010). In our study, salinity stress led to a decrease in chlorophyll 'a' and 'b' on all the sampling days (30th DAT) and this effect increased consistently with increasing salinity levels as compared to non- stressed treatment. However, higher reduction of chlorophyll 'a' and 'b' was observed in KRR-2 and lowest reduction was noticed in the variety CO-1 on all the sampling days with varying salinity levels.

On all the sampling days, Chlorophyll 'a' and 'b' content was decreased with increasing salinity levels in all the sesame varieties. On 30th DAT, with 120 mM salinity treatment, lowest chl 'a' and chl 'b' content was observed in the variety KRR-2 and it was 0.25 mg/gfw and 0.47 mg/gfw, respectively, over the controls (0.45 mg/gfw and 0.79 mg/gfw, respectively), while highest chl 'a' and chl 'b' content was recorded in the variety CO-1 and it was 0.27 mg/gfw, 0.54 mg/gfw, respectively, compared to control plants (0.47mg/gfw and 0.83 mg/gfw, respectively). Photosynthesis depends on leaf chlorophyll content and stomatal conductance, and is thus linearly correlated with the nitrogen content of the leaves (Dingkuhn *et al.,* 1992). The present study on the pigment composition clearly showed that the variety CO-1 maintained high pigment content on all the sampling days than other sesame varieties when subjected to salt stress. Found that salt tolerant and salt sensitive accessions of safflower did not differ significantly in leaf soluble proteins there are reports of decrease in soluble protein content in response to salinity. In higher plants, osmotic stress, induce several proteins in vegetative tissues, which are related to late- embryogenesis-abundant (LEA) proteins. The correlations between (LEA) protein accumulation in vegetative tissues and stress tolerance indicates its productive role under dehydration stress (Ingram and Bartels 1996).

Protein content was decreased in leaves of all the two sesame varieties with increasing salinity level on all the sampling days. Under 120mM salinity stress, on 30th DAT, protein content was highly decreased in KRR-2 by 50% (30.24 mg/gfw) over the control plants (61.51 mg/gfw, respectively), while low decrease of protein content was recorded in the variety CO-1 by 26% (34.49 mg/gfw) relative to control plants (62.21 mg/gfw, respectively). In this investigation, when NaCl concentration increased, a soluble protein in Sesame two varieties was significantly changed. Comparatively, lower decrease of soluble protein content was observed in the leaves of sesame variety CO-1 on all the sampling days even with high salinity levels as compared to controls. More decrease of soluble protein content was observed in the variety KRR-2 on all the sampling days compared to control plants. Several possible roles have been attributed to supra-optimal level of proline, including osmoregulation under salinity, stabilization of proteins, and prevention of heat denaturation of enzymes and conservation of nitrogen and energy for a post-stress period (Ashraf *et al.,* 2008).

The effects of salinity on plant nitrogen metabolism studied to date revealed increased protein degradation,

inhibition of protein synthesis and non accumulation and/or depletion of protein and non-protein amino acids in a small group of dicots and monocots (Teixeira and Fidalgo, 2009). Under salinity stress, foliar nitrogen content decreased on all the sampling days in two sesame varieties. On 30th DAT with 120mM salinity stress, higher nitrogen content was recorded in the variety CO-1(20.56 mg/gdw) and (26%) followed by relative to control plants (0.26 mg/gdw) (34% respectively), while lowest nitrogen content was monitored in variety KRR-2 (0.14 mg/gdw and (13%) over the control plants (0.20 mg/gdw) (28% respectively). Higher reduction was observed in the variety KRR-2 all salinity levels on the sampling stages 30th DAT.

Table 1: Influence of salinity stress on growth and physiochemical content in two sesame varieties on 30th DAT subject varying levels of salinity concentration. Each value represents mean ±of five independent determination

Varieties and parameters	control	40mM	80mM	120mM
Height of the plant(cm)				
CO-1	78.49 ±3.98	64.87±3.11	51.33±2.67	44.11±2.25
KRR-2	70.11±3.86	62.46±3.32	46.54±2.46	39.65±2.38
Leaf area (cm^2)				
CO-1	54.11±2.10	41.50±2.03	36.2±1.53	32.97±1.25
KRR-2	54.01±1.73	40.11±1.64	34.56±1.46	29.11±1.13
Fresh weight of the while plant				
CO-1	36.19±1.99	29.60±1.48	22.26±1.22	20.56±1.13
KRR-2	35.65±1.95	27.70±1.31	19.90±1.11	18.25±0.97
Dry weight of the whole plant				
CO-1	16.22±1.22	12.10±1.18	11.25±1.12	8.74±1.09
KRR-2	15.90±1.18	11.24±1.14	10.51±1.07	7.91±1.03
Total chlorophyll (mg/gfw)				
CO-1	1,28±0.023	1.11±0.019	1,00±0.017	0.83±0.014
KRR-2	1,27±0.019	1.o8±0.017	0.90±0.015	0.75±0.012
Chlorophyll-a (mg/gfw)				
CO-1	0.45±0.012	0.36±0.012	0.34±0.011	0.27±0.009
KRR-2	0.43±0.011	0.34±0.010	0.28±0.009	0.25±0.008
Chlorophyll-b(mg/gfw)				
CO-1	0.83±0.013	0.76±0.012	0.66±0.011	0.54±0.09
KRR-2	0.79±0.012	0.74±0.011	0.61±.0.09	0.47±0.08
Protein(mg/gfw)				
CO-1	62.21±	49.11±	43.61±	34.49±
KRR-2	61.51±	46.22±	40.61±	30.24±
Foliar nitrogen content (%)				
CO-1	0.26±0.018	0.20±0.017	0.19±0,016	0.17±0,014
KRR-2	0.25±0,016	0.18±0,014	0.17±0.012	0.14±0.011

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