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Salinity Induced Changes in Growth, Enzyme Activities, Photosynthesis, Proline Accumulation and Yield in Linseed Genotypes

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Abstract: An out door pot experiment was carried out to investigate the influence of salt stress on performance of four linseed (*Linum usitatissimum* L.) genotypes namely, Laxmi-27, Parvati, Rashmi and Shubhra in terms of growth, carbonic anhydrase (CA: E.C.4.2.1.1) and Nitrate Reductase (NR: E.C.1.6.6.1) activities, net photosynthetic rate (P_N), chlorophyll content, Electrolyte Leakage (EL), Leaf Relative Water Content (LRWC) and proline content, studied at 60 and 75 days after sowing and fibre content and yield parameters at harvest. Fifteen days old plants were subjected to four levels of salinity viz. 0, 50, 100 and 150 mM NaCl. Salinity affected all the considered parameters. The plants fed with highest level of salinity (150 mM) showed a considerable decrease in all the growth parameters such as plant height, leaf area and root and shoot dry weight of plants but root/shoot ratio exhibited a significant increase particularly in 60 days old plants. These changes were associated with a decrease in the LRWC, P_N, total chlorophyll content and activities of CA and NR but proline content and electrolyte leakage were found to increase in the leaves of all of the tested genotypes. The proline was found to keep pace with the increasing values of electrolyte leakage in salinized plants; it indicates that proline was involved in the osmotic adjustment of salinized plants. At harvest the entire yield attributes such as capsule number, seed yield, biological yield and Harvest Index (HI) decline except seed number capsule^{-1}, which showed a non-significant effect. The genotype Shubhra proved comparatively more tolerant whereas Rashmi, less tolerant. The effect of salinity on genotypes was evident particularly in 60 days old plants submitted to highest level of salinity (150 mM).

Key words: *Linum usitatissimum* L. . carbonic anhydrase activity . electrolyte leakage . net photosynthetic rate . nitrate reductase activity . proline content . salinity

INTRODUCTION

Abiotic stresses, such as salinity, chemical toxicity, drought, extreme temperatures and oxidative stress are serious threats to agriculture and result in the deterioration of environment. Salinity is a worldwide problem; it limits growth and development of plants by affecting different metabolic processes, such as CO_2 assimilation, chlorophyll (chl) content, death of cells and tissues, dehydration, membrane permeability, phytohormone turnover, protein synthesis, respiration, turgor loss and uptake of mineral nutrients [1-3]. Several studies have shown that activities of CA and NR decrease in plants under salt stress [4, 5]. The enzyme CA is used in the efficient functioning of photosynthetic CO_2 fixation facilitated through Rubisco enzyme [6], whereas, NR is the enzyme that catalyzes the first step of nitrate assimilation which appears to be a rate limiting process in the acquisition of nitrogen [7]. Salinity affects the crop during vegetative and reproductive stage and therefore, causes reduction in dry mass, crop yield [8], chl content and photosynthesis which contributed to increase rate of leaf senescence [1]. Plants can cope to some degree with the excess of Na^+ by its active uptake and compartmentation in the vacuole, restriction of Na^+ influx and active Na^+ efflux [9, 10] through osmotic adjustment facilated by accumulation of compatible solutes such as glycinebetaine (GB), proline (Pro) and polyols [11, 12]. Thus, these parameters may be used to assess the extent of tolerance of plants to salinity.

Linseed (*Linum usitatissimum* L.) is one of the most important oil crops for the extraction of oil (from seeds) and fibres (from plants' stems). About 80% of the linseed oil goes for industrial purpose and remaining 20% is used for edible purpose. However, little is known about the response of linseed to salt stress. It was, therefore, decided to evaluate the effect of different levels of salinity on linseed genotypes at different stages of plant growth to provide information

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on the significance of pro, CA and NR activities in response to salt stress and to determine the effect of salinity on growth, net photosynthetic rate (P_N) and yield characteristics.

MATERIALS AND METHODS

Plant materials and growth conditions: A pot experiment was conducted according to factorial experiments in randomized complete design at the department of Botany, Aligarh Muslim University, Aligarh, India. Authentic seeds of linseed (*Linum usitatissimum* L.) genotypes, viz. Laxmi-27, Parvati, Rashmi and Shubhra were obtained from the Division of Oilseed Crops, CSA University of Agriculture and Technology, Kanpur, India.

The healthy and uniform seeds of each genotypes were selected and sown directly 2 cm deep in earthen pots (25 cm height x 25 cm diameter) containing 4 kg homogenous mixture (3:1) of soil and farmyard manure. The soil mixture had texture-sandy loam and available 96 mg kg^{-1} N, 5.3 mg kg^{-1} P and 138 mg kg^{-1} K (the equivalent of 215.0 kg N, 11.9 kg P and 311.1 kg K ha^{-1}). Prior to sowing a uniform recommended dose of fertilizer (90 kg N+30 kg P+30 kg K ha^{-1}) was applied. The sources for nitrogen phosphorus and potassium were urea, sodium dihydrogen orthophosphate and muriate of potash respectively. Ten plants pot^{-1} were maintained. Four levels of sodium chloride (NaCl), viz. 0, 50, 100 and 150 mM were applied. There were four replicates for each treatment. Salinity treatments were started 15 Days After Sowing (DAS). To avoid osmotic shock, NaCl concentration was increased gradually by 25 mM every two days until the desired concentration was achieved. The plants were watered as and when required. To study the effect of NaCl on growth, physiological and biochemical parameters, the plants were sampled at 60 and 75 DAS, whereas yield characteristics including fibre content were studied at harvest.

Measurement of growth characteristics: The effect of salinity on growth parameters was studied in terms of plant height, leaf area, dry weight of root $plant^{-1}$ (dry wt r), dry weight of shoot $plant^{-1}$ (dry wt s) and root/shoot ratio. Leaf area $plant^{-1}$ (LA) was measured by outlining about 10% of leaves on a graph paper and dry wt of these leaves was recorded. LA was determined using leaf dry wt $plant^{-1}$ and dry wt of those leaves for which the area was estimated [13]. Dry wt was recorded by drying the plants at 80°C for 24 hours. The results were expressed as the relative reduction of yield in comparison to the control using the formula adopted by Ghoulam *et al*. [14].

Relative reduction (%) = [1-(Salinized/Control)]x100

Determination of physiological and biochemical parameters

Leaf relative water content: LRWC was measured by adopting the method of Yamasaki and Dillenburg [15]. Leaves were collected from mid section of plant in order to minimize age effect. For each treatment 10 leaves were taken. To obtain their fresh weight (fr wt), the leaves were weighed just after removal from the stem. In order to determine turgid wt (t wt), the leaves were kept in Double Distilled Water (DDW) inside a closed petridish for four hours. After gentle wiping the water from the leaf surface with tissue paper, the leaves were weighed. To determine dry wt the leaf samples were dried at 80°C for 48 h. Values for fr wt, t wt and dry wt were used to calculate LRWC using the equation below.

LRWC (%) = [(fr wt - dry wt)/(t wt - dry wt)] x100

Carbonic anhydrase activity: The activity of CA enzyme was determined by the method of Dwivedi and Randhava [16]. The leaf samples were cut into small pieces and suspended in cystein hydrochloride solution. The samples were incubated at 4°C for 20 min. The pieces were blotted and transferred to the test tubes containing phosphate buffer (pH 6.8) followed by the addition of alkaline bicarbonate solution and bromothymol blue indicator. The test tubes were incubated at 5° for 20 min. After addition of 0.2 mL of methyl red indicator, the reaction mixture was titrated against 0.05 N HCl. The results were expressed as μ mol CO_2 kg^{-1} leaf fr wt s^{-1}.

Nitrate reductase activity: NR activity was estimated by the intact tissue method of Jaworski [17]. Fresh leaf samples were weighed and transferred to plastic vials. To each vial, 2.5 mL phosphate buffer (pH 7.5), 0.2 M potassium nitrate and 5% isopropanol solutions were added. Each vial was incubated for 2 h. in the dark at 30°C. To the incubated mixture, 1% sulphanilamide and 0.2% NED-HCl (N-1-naphthylethylene-diamine dihydrochloride) were added. The reaction mixture was kept for 20 min. for colour development. The absorbance was read at 540 nm and was compared with that of the calibration curve . The activity of NR was expressed as μ mol NO_2 $h^{-1}g^{-1}$ leaf fr wt.

Net photosynthetic rate and chlorophyll content: P_N in intact leaves was measured with the help of a LI-6200 portable photosynthesis system (LI-COR Lincoln, NE, USA).

Fresh leaf sample taken from the youngest fully expanded leaves was extracted with 80% acetone and the absorbance was read spectrophotometrically at 663 and 645 nm. The chl was determined by using the formula of Arnon [18].

$$[(A_{645}x28.2)+(A_{663}x8.3)]x[(v/1000)xW]$$

Electrolyte leakage: EL is used to assess membrane permeability as described by Lutts *et al.* [19]. Samples were washed three times with DDW to remove surface contamination. Young leaf discs from each sample were taken. Leaf discs were placed in a closed vial containing 10 mL of DDW and incubated on rotatory shaker for 24 h. subsequently the electrical conductivity of solution (EC_1) was determined. Samples were then autoclaved at 120°C for 20 min. and last electrical conductivity (EC_2) was noted after cooling the solution at room temperature. The electrolyte leakage was calculated as

$$EL\ (\%) = (EC_1/EC_2)x100$$

Proline content: The proline content was determined spectrophotometrically adopting the ninhydrin according to method of Bates *et al.* [20], 300 mg fresh leaf samples were homogenized in sulphosalicylic acid. To the extract, 2 mL each of acid ninhydrin and glacial acetic acid were added. The samples were heated at 100°C. The mixture was extracted with toluene and the free toluene was quantified spectrophotometrically at 528 nm using L- proline as a standard.

Determination of yield characteristics: At harvest, 3 plants from each replicate were uprooted randomly and were used for computing yield parameters namely capsules plant^{-1}, seeds capsule^{-1}, seed yield plant^{-1}, biological yield plant^{-1}, HI and fibre content plant^{-1}. After counting the capsules plant^{-1} they were crushed and cleaned to assess seeds capsule^{-1} and seed yield plant^{-1} (economic yield). The remaining plant material was sun-dried and weighed for biological yield. The HI was calculated using the following formula.

$$HI\ (\%) = (Economic\ yield/Biological\ yield)x100$$

Fibres were extracted by water retting process. 12 plants were taken from each treatment and tied to make a bundle. Bundles were dipped into fresh water tank for 10-15 days for complete degradation of cell wall material due to microbial action. After complete retting, the fibres were separated from woody material by means of an operation known as scutching.

Statistical analysis: Each pot was treated as one replicate and all of the treatments were repeated four times. The data was analyzed by two-way analysis of variance (ANOVA), by adopting the procedure described by Gomez and Gomez [21]. The LSD was calculated at 5% level.

RESULTS

Growth characteristics: The effect of salinity treatments and their interaction with genotypes, on all growth parameters studied were significant at both stages (60 and 75 DAS). The reduction was greater at highest NaCl concentration (150 mM) (Fig. 1).

Plant height decreased in the plants supplemented with NaCl and their rate of loss was proportional to the concentration of the NaCl. Maximum reduction was noticed in the plants receiving highest concentration (150 mM NaCl), which decreased plant height by 11.6 and 10.2% at 60 and 75 days plants old, respectively. The effect was to some extent was not much severe at latter growth stage (75 days old plants). A considerable varietal response was also recorded, where Shubhra proved the least affected genotype, followed by Laxmi-27; whereas Rashmi was the most affected among the tested genotypes (Fig. 1).

The percent reduction in leaf area was greater in 60 days old plants exposed to 150 mM NaCl and maximum reduction was obtained in 60 days old plants of genotype Rashmi where it reached around 32.8% and 26.7% at 60 and 75 days plants old, respectively at 150 mM NaCl than the water treated control, whereas the genotype Shubhra showed least reduction in leaf area at both the growth stages.

Dry mass gradually decreased with increasing levels of NaCl for both root and shoot (Fig. 1). In comparison with the water treated control, 150 mM NaCl decreased root dry mass by 17% and shoot dry mass by 25% at 60 days plants old. 150 mM NaCl concentration exhibited a considerable reduction in shoot dry mass for the genotype Rashmi, in which it attained 25.2 and 22.7% of the control at 150 mM NaCl at 60 and 75 days plants old, respectively. However, a decrease of 17.3 and 13.5% of the control at 150 mM was also recorded for root dry mass at 60 and 75 days plants old, respectively in the same genotype.

The values for root/shoot ratio were increased with increasing concentrations of NaCl and reached at its

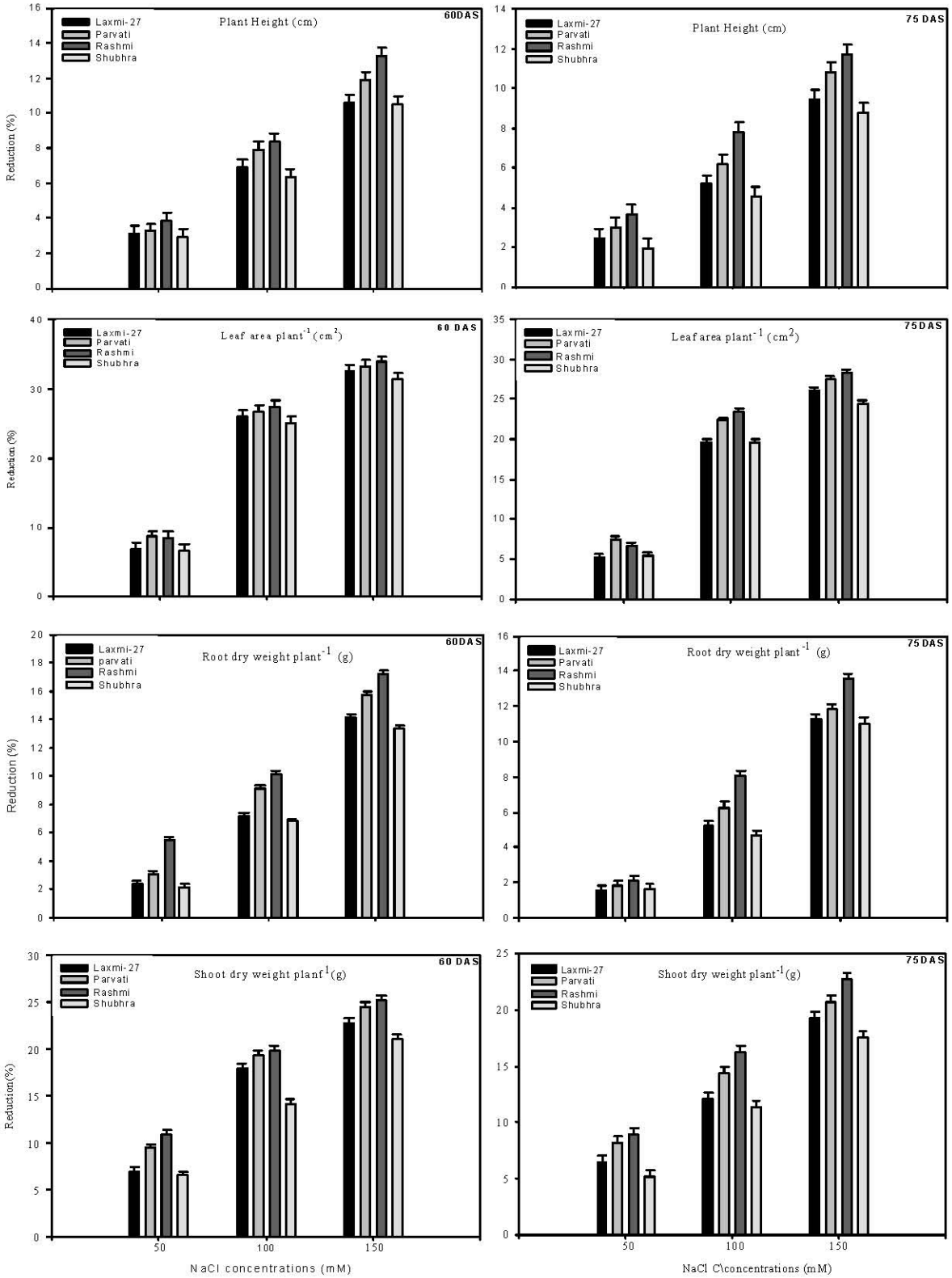

Fig. 1: Changes in plant height, leaf area, root and shoot dry weight in 60 and 75 days old plants of four linseed genotypes subjected to NaCl treatments. Results are means of three replicates and expressed as reduction percentage of controls. Error bars (-) show LSD at 5% level

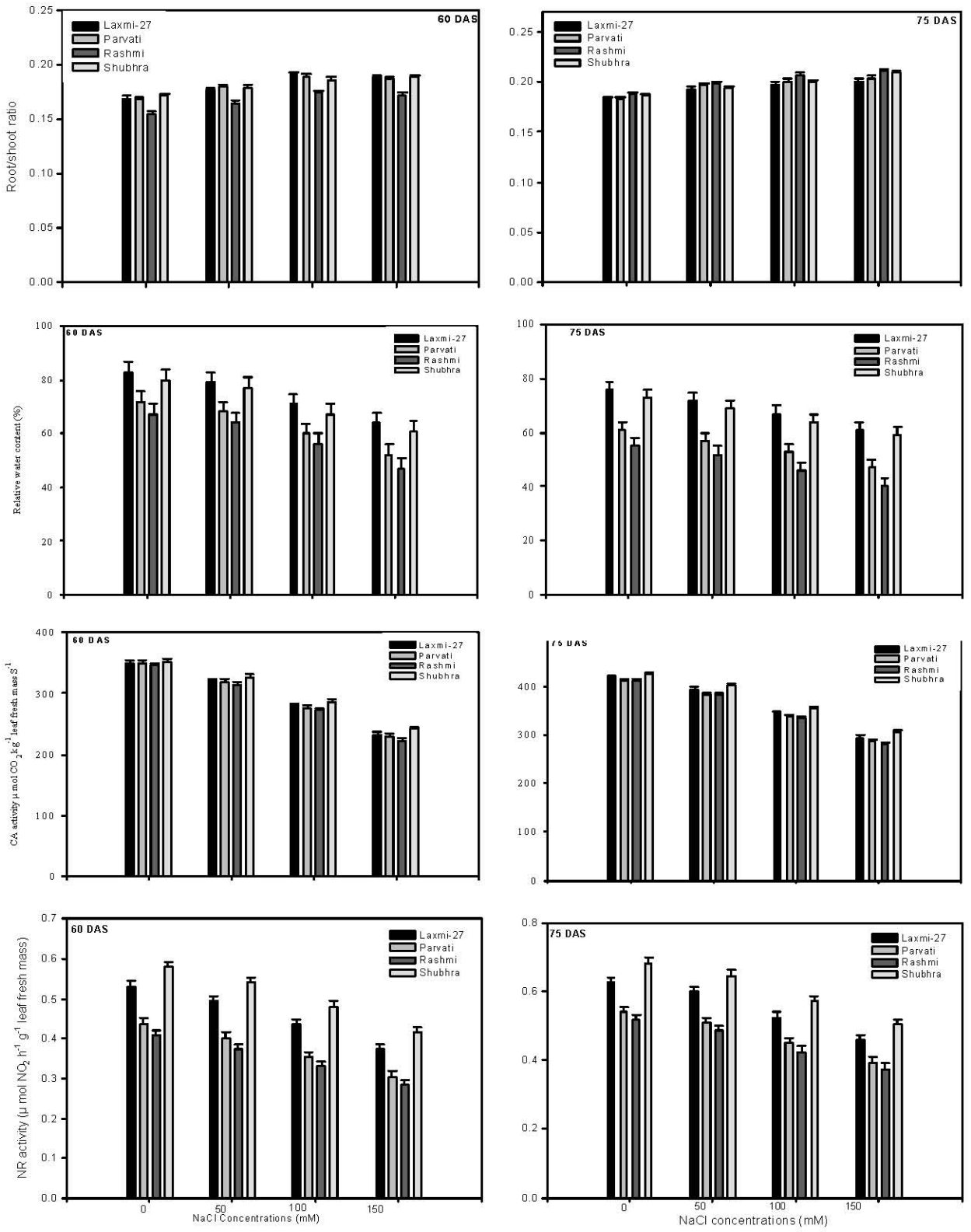

Fig. 2: Effect of NaCl on root/shoot ratio, relative water content, CA and NR activities of 60 and 75 days old plants of linseed genotypes. Results are means of three replicates. Error bars (-) show LSD at 5% level

maximum at 150 mM NaCl at both 60 and 75 days plants old (Fig. 2). At 60 days plants old, 100 and 150 mM NaCl showed equal effects however, 150 mM NaCl concentration registered the highest values at 75 days plants old. Among genotypes Laxmi-27, Parvati and Shubhra being at par gave higher values than Rashmi at 60 DAS.

Based on these growth attributes, Rashmi was found the most affected genotype by salt stress whereas Shubhra was the least affected one and all the four genotypes were more prone to salinity at earlier growth stage.

Physiological and biochemical parameters: Low values of LRWC were recorded in NaCl treated plants in comparison to controls (Fig. 2). At lower concentration of NaCl (50 mM), the plants are able to adjust osmotically, leading to maintenance of LRWC. However, at highest concentration (150 mM NaCl), the linseed plants showed a significant decrease, which reached at its maximum in Rashmi viz. 47 and 40% at 60 and 75 days plants old, respectively at 150 mM NaCl. The less important change was recorded in Laxmi-27 with 83% and 76% for the control, whereas 64% and 61% for 150 mM NaCl at 60 and 75 days plants old, respectively (Fig. 2)

The application of NaCl to linseed plants adversely influenced the CA activity in the leaves of all of the genotypes tested. CA activity was lower as salt concentration increased and reached the minimum values at 150 mM NaCl at both sampling stages (Fig. 2) The varietal effect on this parameters was significant and comparison among the tested genotypes at two higher levels of NaCl (100 and 150 mM), indicated that Laxmi-27 and Shubhra were less affected, whereas Parvati and Rashmi were more affected by salt treatment at both stages.

NR activity was significantly inhibited by NaCl treatments at both sampling stages (Fig. 2). The inhibition increased progressively with an increase in NaCl concentration from 50 to 150 mM. The inhibition was more pronounced at earlier growth stage in four tested genotypes. Among genotypes, inhibition was more pronounced in Parvati and Rashmi, in which the NR activity was reduced from nearly 0.408 for control to 0.284 μmol $NO_2^{?}h^{-1}g^{-1}$ FW at 150 mM NaCl at 60 DAS. Shubhra appeared to be the less affected genotype and showed a little reduction at 150 mM NaCl in comparison to control at both the growth stages

It is clear from Fig. 3 that both P_N and chlorophyll content were decreased, if the plants were supplied with NaCl. The maximum loss at both the growth stages was recorded in the plants receiving highest concentration (150 mM) of NaCl. Among the genotypes, Rashmi was more affected and Shubhra was less affected. The interactive effect of NaCl and genotypes was also significant and the lowest values of P_N and chlorophyll were recorded for Rashmi with 150 mM NaCl at both stages, whereas Shubhra was less affected among four tested genotypes.

Salt treatment caused a high significant increase in electrolyte leakage in these genotypes at both stages and low values for electrolyte leakage were recorded in the control. Electrolyte leakage was greater as salt concentration increased and reached the maximum values at 150 mM NaCl. The varietal effect on this parameter was significant and comparison among the genotypes at 150 mM NaCl indicated that Shubhra was less affected and Rashmi was more affected by salt stress (Fig. 3).

The results revealed a significant effect of salinity and genotypes and their interaction on proline content. The salt treatment caused an increase in proline content at both stages in all the genotypes tested, except Rashmi, in which the proline content did not change significantly. Although, the genotypes Parvati showed the highest proline content for the control and Shubhra showed the lowest, but genotypes Shubhra gave the highest amount of proline at 100 and 150 mM NaCl (Fig. 3). The lowest values for proline content were recorded in Rashmi. Although, an increase in proline content was noticed as growth progressed from 60 to 75 days, but the percent increase in proline content was lesser in 75 days old plants as compared to at 60 days plants old (Fig. 3).

Yield characteristics: Capsule number $plant^{-1}$, seeds $capsule^{-1}$, seed yield, biological yield and fibre content $plant^{-1}$ and HI were used to assess the effect of NaCl on yield attributes. The plants supplemented with NaCl exhibited a significant reduction in capsule number, seed yield, biological yield and HI (Table 1). Whereas, a non-significant effect was noticed on seed number $capsule^{-1}$. The highest reduction in seed yield at highest level of NaCl (150 mM) was obtained for the Rashmi where it reached around 25.5%. The reduction in seed yield for Shubhra did not exceed 16.02% at 150 mM NaCl.

The plants exposed to highest concentration of NaCl (150 mM), exhibited a significant reduction in fibre content $plant^{-1}$. However, the plants of all the four genotypes tested, are shown to adjust themselves at lower concentration (50 mM NaCl) and did not show a significant reduction in this attribute (Table 1). The highest reduction was recorded at 150 mM NaCl for the Rashmi, which proved the most affected genotype,

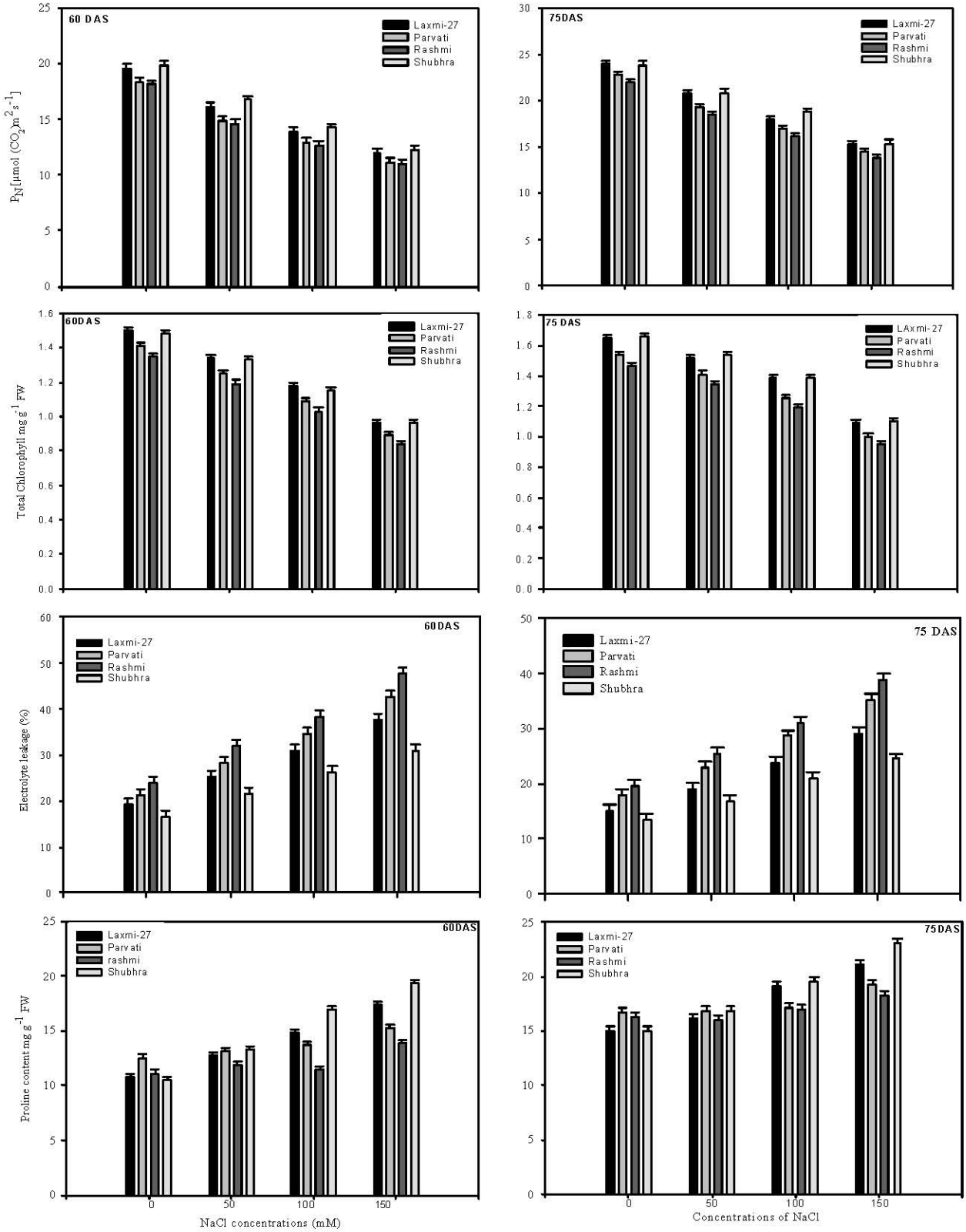

Fig. 3: Effect of NaCl on net photosynthetic rate (P_N), chlorophyll content, electrolyte leakage and proline content of 60 and 75 days old plants of linseed genotypes. Results are means of three replicates. Error bars (-) show LSD at 5% level

Table 1: Effect of NaCl concentrations on yield attributes of four linseed genotypes

Genotypes (G)	NaCl conc. (mM) (S)	Capsules plant^{-1}	Seeds capsule^{-1}	Seed yield plant^{-1} (g)	Biological yield plant^{-1} (g)	Harvest index (%)	Fibre content plant^{-1} (g)
Laxmi -27	0	66.67	10.00	2.59	9.19	28.17	1.28
	50	64.33	10.25	2.53	9.01	28.09	1.25
	100	61.67	09.75	2.32	8.38	27.70	1.20
	150	56.00	09.00	2.21	8.14	27.16	1.15
	Mean	62.17	09.75	2.41	8.68	27.78	1.22
Parvati	0	59.33	08.75	2.47	9.35	26.42	1.19
	50	59.00	08.25	2.39	9.08	26.32	1.15
	100	56.00	08.00	2.16	8.34	25.91	1.10
	150	52.67	08.00	1.98	7.79	25.40	1.04
	Mean	56.75	08.25	2.25	8.64	26.01	1.12
Rashmi	0	62.00	09.00	2.36	9.06	26.05	1.23
	50	61.67	08.75	2.27	8.74	25.96	1.18
	100	54.67	08.00	2.08	8.15	25.53	1.13
	150	51.00	08.25	1.88	7.47	25.03	1.06
	Mean	57.34	08.50	2.15	8.36	25.64	1.15
Shubhra	0	71.00	09.75	2.68	9.51	28.16	1.36
	50	69.33	09.75	2.61	9.28	28.11	1.33
	100	65.00	09.00	2.45	8.82	27.79	1.29
	150	59.67	09.00	2.31	8.49	27.21	1.25
	Mean	66.30	09.38	2.51	9.03	27.82	1.31
LSD at 5% level	G	1.21	NS	0.10	0.26	0.32	0.05
	S	1.10	NS	0.08	0.19	0.24	0.03
	S x G	2.31	NS	0.19	0.52	NS	0.10

NS = Non Significant

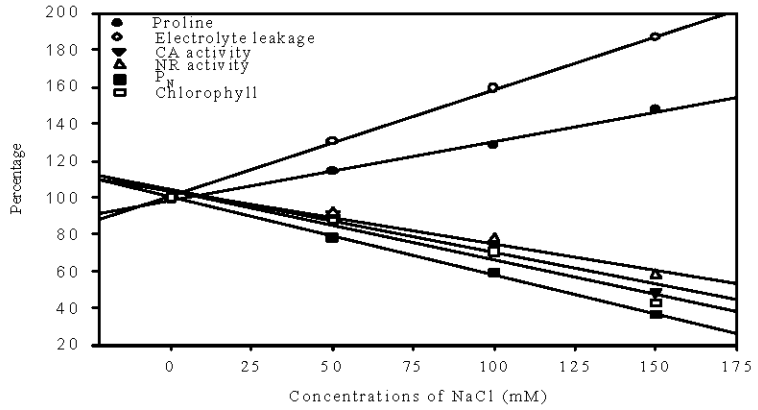

Fig. 4: Relationship of proline, electrolyte leakage, CA and NR activities, net photosynthetic rate (P_N) and chlorophyll content with different salinity levels in 60 days old plants of linseed. Each point represents the mean of four measurements

whereas Shubhra exhibited least reduction and expressed itself as more tolerant genotype by producing highest amount of fibre even under highest salinity level (Table 1).

DISCUSSION

The data (Fig. 1) revealed that plant height and LA were decreased with increasing of salinity levels. The reduction in plant height might be responsible for improper orientation of leaves for harvesting solar radiation, hence poor dry wt as noted in treated plants. The decrease in LA might be on two counts (i) lesser number of leaves and (ii) decreased area leaf^{-1}. The lowest values for these parameters could also be responsible for poor dry matter production. These results are in accordance with the findings of Gouia *et al.* [5], Mc Cue and Hanson [22] and

Wang *et al.* [23], who working on different crops also reported adverse effect of salinity on various growth parameters. The explanation regarding the increase in root/shoot ratio (Fig. 2) resulting from the application of the salinity treatments could be based on their differential effect on root and shoot i.e. in comparison with root dry wt, shoot dry wt was affected comparatively more (Fig. 1). Meloni *et al.* [24, 25], working on gossypium and *Prosopis alba*, also found that salinity inhibited shoot growth more than root growth.

The observed decrease in LRWC (responsible for turgidity), CA and NR activities, R_N and chl content resulting from the salinity treatments (Fig. 2 and 3) could be ascribed to the reduction in water potential of soil solution as the addition of NaCl results in a decrease in water potential. Lower water potential of soil solution is responsible for decreased absorption of water by plant. Thus, the resultant limited supply of water in plant would naturally decrease LRWC, CA and NR activities, R_N and chl content as these directly or indirectly dependent on water content of the cell. Moreover, salinity may retard uptake of nitrate which is the substrate and an inducer of NR [26]. Solmonson and Barber [27] and Croser *et al.* [28] also reported similar results on various physiological and biochemical parameters.

Enhancement in solute leakage due to salinity suggests a disturbance (damage) in permeability of membranre. Lutts *et al.* [29], working on rice Kaya *et al.* [30, 31] on tomato and strawberry also found an increase in solute leakage due to salinity.

The observed increase in proline content under salinity might be caused by the induction or activation of proline biosynthesis or decrease in oxidation of proline to glutamate or decrease in its utilization in protein synthesis or enhancement in protein turnover. The parallel increase in proline content with the electrolyte leakage suggested that proline might be involved in the osmotic adjustment of salinized plants. An increase in proline content was also observed by Delauny and Verma [32]. Thus proline may be the major source of energy and nitrogen during immediate post stress metabolism and accumulated proline apparently supplies energy for growth and survival thereby inducing salinity tolerance. The results in the present study suggest that salt-induced proline synthesis and accumulation might have served as a compatible solute and thus helped plant tissues to tolerate stress. This judgment is agreed with the finding of Gzik[33].

The reduction in RWC leads to flaccidity responsible for stopping of cell division as a cell has to occupy a requisite size before entering in the cell cycle. The decrease in cell division, CA and NR activities, P_N, chl content and solutes (due to leakage) would ultimately have adverse effect on growth leading to poor dry wt of root and shoot of treated plants (Fig. 1).

The deleterious effect of salinity on one of the important yield parameters namely capsules plant^{-1} (Table 1) was not surprising. As mentioned earlier, salinity treatments decreased plant height, LA, LRWC, CA and NR activities, chl content and EL (Fig. 1-3). The lowest values for these parameters of treated plants would have cumulative adverse effect on P_N hence less dry mass production. The observed lower values for shoot dry wt plant^{-1} of stressed plants (Fig. 1) strengthened this proposition. The limited synthesis of photosynthates would adversely affect their partitioning to developing sink and thus resulting in lower values for capsules plant^{-1} and HI. As seed number capsules^{-1} was not affected by salinity, the lowest value of capsules plant^{-1} would naturally be responsible for decreasing seed yield. Ram *et al.* [34] also registered the productivity of chick pea plants was decreased under saline conditions. The adverse effect of salinity on fibre content might be resulted from the decrease in plant height of treated plants which enabled them to bear poor fibres. The overall superiority of genotypes Shubhra in terms of salinity tolerance could be ascribed to its superior genetic constitution responsible for its better performance under saline conditions.

CONCLUSION

The assessment of the effect of salinity on performance of linseed genotypes allows us to conclude that increasing levels of salinity decrease most of the growth, physiological and biochemical and yield parameters linearly. Proline exhibited a parallel increase with the EL under salt stress, which indicates that the synthesis of compatible solutes (proline in the present study) may act as protective agents by adjusting water potential of salinized plants. Data also revealed that in comparison with the respective control, there was less difference in the values of salinity treatments for various growth and physiological parameters at later growth stage (75 DAS) than at earlier growth stage (60 DAS). Among genotypes tested, Shubhra was the most tolerant and Rashmi the least.

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