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Research Article

Influence of GA_3 (gibberellic acid) and Ca(calcium) on root trait variation and osmotic potential of linseed (*Linum usitatissimum* L.) under chloride-dominated salinity

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Abstract
Linseed (*Linum usitatissimum*) is a versatile crop cultivated for its seeds, which are valuable source of ω-3 fatty acids. It adversely affected by soil salinity, as high salt levels can hinder their growth and reduce yields. To assess the potential for mitigating the adverse effects of high salinity concentrations, enhancing the resilience of three genotypes (Shekhar, Sheela, and Kartika) of linseed plants, this research aimed to find out the impact of Gibberellic acid (GA_3) and Calcium (Ca) on various aspects of root morphology, osmotic potential of linseed, under varying levels of Cl^- dominated salinity. The study employed three salinity levels (0, 5, and 10 dSm^{-1}) and exogenous application of 10^{-6} M GA_3 and/or 10 mg $CaCl_2$ kg^{-1} in potted plants.The findings indicated that increasing salinity stress significantly ($p \leq 0.05$) affected root parameters, including total surface area(43.45%), average diameter(42.06%), total projected area(44.45%), length per volume (66.23%), root length, total root volume (73.23%), tips, forks,fine roots, and osmotic potential(66.67%). Correlations among linseed genotypes were observed between various root morphology and osmotic potential parameters. The application of GA_3 and Ca effectively ameliorated the impact of salinity stress at its highest level (10 dSm^{-1}), resulting in increased root parameters while decreasing the osmotic potential (Ψs). Both GA_3 and Ca treatments significantly influenced root architecture and maintained optimal osmotic potential. The chloride-dominated salinity exerted inhibitory effects on all three genotypes' (Shekhar, Sheela, and Kartika) root growth parameters while applying GA_3 and Ca successfully mitigated these effects, enhancing root growth.

Keywords: Calcium, Gibberellic acid, Root architecture, Root length, Salinity

INTRODUCTION

Saline soils are prevalent worldwide, particularly in semiarid and arid regions. Hassania *et al.* (2020) estimated the total extent of saline soil to be 11.74 million km^2. Elevated salinity levels result in soil degradation,

characterized by poor soil structure and diminished nutrient content, ultimately contributing to desertification. Linseed (*Linum usitatissimum* L) is a diploid and self-pollinated annual crop. Modern cultivars of linseed are typically short, highly branched, and known for their abundant seed production (Deyholos, 2006). Linseed is among the earliest cultivated plants and cultivated historically for its best-quality bast fibers, cellulose-rich, and oil content (Huis *et al*., 2010; Zohary and Hopf, 2004). Linseed oil is a valuable dietary nutrition, renowned for its high omega-3 fatty acid and α-linolenic acid content. Furthermore, this oil is utilized to manufacture different industrial raw materials (Foster *et al*.,2009; Vaisey-Genser and Morris, 2003; McKenzie and Deyholos, 2011).While cotton and synthetic fibers have largely replaced linseed fibers in the textile industry, they continue contributing to the creation of premium linen products.Moreover, there is a rising trend in utilizing linseed fibers within biocomposite polymer matrices to improve their mechanical characteristics, marking an expanding field of application for this adaptable crop (Bodros *et al*.,2007; Chemikosova *et al*.,2006; Huis *et al*., 2010).

Linseed boasts a well-developed fibrous root system characterized by numerous lateral roots. However, these linseed roots are highly sensitive to the effects of salinity stress. Salt stress conditions can trigger phenotypic plasticity in plant roots by influencing the configuration of the root system (Julkowska *et al*.,2017; Korver *et al*.,2020; Li *et al*.,2021). The linseed's root system serves as the primary point of interaction with saline soil, and it displays a strong physiological reaction when confronted with adverse growth circumstances. When subjected to salt stress, plant roots tend to absorb excessive amounts of sodium ions (Na^+), leading to an disturbance in the Na^+/K^+ ratio.This discrepancy leads to the harmful effects of sodium ions and the initiation of osmotic stress, ultimately hindering roots' growth (Yang *et al*.,2008). This adverse impact was observed, where a reduction in overall root volume, root length, root count, and darkening of the root tip are depicted (Neves *et al*., 2010). Increased salinity in the soil reduces its water potential, exacerbating water scarcity and impeding water absorption by plant roots, which are already operating at reduced vigor. Additionally, the heightened salt levels within plant cells disrupt physiological metabolism, leading to a decline in the activity of carbon metabolism enzymes in the root system. The decrease in enzyme activity limits the ability to assimilate carbon (Nam *et al*., 2012), consequently impeding the growth of the root system.

Furthermore, signals associated with salt stress impact the production and transportation of a range of hormones, including abscisic acid (ABA) and gibberellic acid. Consequently, these hormonal changes significantly impact root system architecture (Osmont *et al*., 2007).Nonetheless, elevating the levels of gibberellic acid and other hormones in the roots has been shown to alleviate the impediments caused by salt stress on root differentiation, as illustrated by Galvan-Ampudia and Testerink (2011). These factors, when considered together, contribute to the difficulties encountered in differentiating and fostering the growth of crop roots in highly sodic soil. The external application of phytohormones helps mitigate the detrimental effects of salinity and promotes growth. Jia *et al*. (2020); Meena *et al*. (2016); Borsani *et al*.(2001) have also shown that saline stress significantly reduces plant growth and production. Gibberellic acid has a positive regulatory influence on plant stress tolerance under abiotic stress.

Calcium is known to play a significant role in maintaining the functional and structural integrity of plant membranes, stabilization of cell wall structure, regulation of enzyme activity, and signal transduction by acting as a secondary messenger (Sun *et al*., 2010; Song *et al*., 2008; Mahajan *et al*., 2008). Maeda *et al*. (2005) . The alleviating impact of Ca^{2+} on salt-induced damage in plants subjected to high salt levels involves the regulation of ion homeostasis within the stress-signaling pathway (SOS), as suggested by Zhu (2003). Additionally, it is proposed that calcium activation of the SOS_3/SOS_2 pathway contributes to enhanced vacuolar Na^+ sequestration facilitated by vacuolar antiporters (Na^+/H^+). Thus, the present study aimed to investigate the potential of gibberellic acid (GA_3) and calcium (Ca) in mitigating the adverse impacts of salinity stress on root architecture and osmotic potential in different linseed (*L. usitatissimum* L.) genotypes under environmental conditions.

MATERIALS AND METHODS

Conditions for growing plants

An experiment with sand culture pots was conducted in an atmosphere with ambient lighting. *Linum usitatissimum* L. healthy seeds of three genotypes (Shekhar, Sheela, and Kartika) were procured from NBPGR (National Bureau of Plant Genetic Resources) in New Delhi, India. Five kilograms of sand were placed inside the 12-inch clay pots that were coated with polythene bags (to prevent contamination). The sand was cleaned with 0.1 N HCl (to remove nutritional cations and fungal contamination), then with distilled water before being put into the pots. A straightforward randomised design with three replicates was used to place the pots. Seeds were surface sterilised with 1% NaClO for 10 minutes before to sowing, forcefully washed with DDW, and then planted in pots filled with sand and nutrient solution according to Raukura's method (Smith *et al*.,1983). Five healthy plants of the same size were kept in each container after two weeks of seeding and thinning. The plants received a salinity treatment when they had two

to three genuine leaves. Chloride-dominated salinity was created by adding different salts with different salt concentrations, viz., NaCl, $CaCl_2$, $MgCl_2$, and $MgSO_4$. These salts are mixed in the ratio such that Na : (Ca + Mg) ratio is 1:1, Ca : Mg is 1:3 and Cl: SO_4 ratio is 7:3 on meq basis. The desired salinity is 0, 5.0, and 10.0 dSm-1, which was applied to saturate the pot and maintain the same concentration. The dosage of calcium treatment was 10 mg Ca kg^{-1} sand in DDW(100 ml). Calcium chloride ($CaCl_2$) was used as the calcium source. GA3 (10-6 M) treatment was administered after a two-week chloride-dominated therapy. A 10^{-2} M stock solution of GA_3 was created by mixing GA_3 in ethyl alcohol and diluting it with DDW. DDW was used to create 10^{-6} M GA_3 from this standard solution. The only plants that received DDW were regarded as the control. The experimental pots received daily irrigations of DDW (50-100 ml) to maintain the sand wet. The salinity stress, $CaCl_2$, and GA_3 treatments are mentioned in Table 1. The plants were provided with Raukura's nutrient solution every two days, with 200 ml added per pot.

Root architecture

Following harvest, the freshly harvested root systems underwent a thorough tap water rinse and were then placed directly onto waterproof trays from Regent. To capture root system images, the study employed a highly optimized Epson Expression/STD 4800 scanner. Subsequently, we utilized the WinRHIZO software, which incorporates an automatic global thresholding method, for root system analysis. This software, developed by Regent Instruments Inc. in Quebec, Canada, is specifically tailored for the automatic and interactive analysis of various root morphological traits, including surface area, root length, average diameter, projected area and root volume. For each treatment, we scanned the roots of three seedlings (equating to single roots per pot), and the data presented here represent the average measurements derived from these three samples.

Osmotic potential

Osmolality was determined according to the method given by Cuin *et al*. (2009). Fresh shoot samples (1 g) were collected and frozen at −20 °C. Crushed samples were squeezed to extract the sap, and osmolality was measured with 5 µl of the sap using Vapor Pressure Osmometer (Model 5600, ELITech Group, Belgium) after calibrating it with the osmolality reference standards of NaCl (Wescor Inc, USA).

Table 1. Different treatments used in the experiment

Sr. No.	Treatment code	Chemical constituents in the treatment
1.	T_0	Control (0 dSm^{-1}+ 0 GA_3+ 0 $CaCl_2$)
2.	T_1	5.0 dSm^{-1}
3.	T_2	10.0 dSm^{-1}
4.	T_3	10^{-6} M GA_3
5.	T_4	5.0 dSm^{-1} + 10^{-6} M GA_3
6.	T_5	10.0 dSm^{-1} + 10^{-6} M GA_3
7.	T_6	$CaCl_2$
8.	T_7	5.0 dSm^{-1} + $CaCl_2$
9.	T_8	10.0 dSm^{-1} + $CaCl_2$

RESULTS AND DISCUSSION

Total root length and total projected area

Two-way ANOVA revealed chloride dominated salinity stress and GA_3, Ca had significant effects on total root length and total project area ($P < 0.05$; Fig.1).With increasing salinity stress (10 dSm^{-1}), the root length and total project area were significantly reduced (Fig. 2 A, B). At GA_3 supply, the 5 dSm^{-1} treatment reduced the root length by 25.26% (G1), 39.59%(G2) and 40.91% (G3), respectively, and the 10 dSm^{-1} treatment reduced the root length by 40.99%, 65.03% and 58.41%, in three genotypes 'Shekhar','Sheela' and 'Kartika' respectively, compared with control. At GA_3 supply, the 5 dSm^{-1} treatment increased total root length by 18.81%, 34.49% and 42.11%, respectively, and the 10 dSm^{-1} treatment increased total root length by 26.15%, 37.46% and 40.24%, respectively, compared with Control(T_0). The root length effective trends were similar to the root length in response to the calcium treatments. Compared to Control, the root length of 10 dSm^{-1} increased by 21.89%, 30.39%, and 32.53%, respectively, under the calcium treatments. Similarly, the root total projected area of 10 dSm^{-1} compared to that of control increased by 24.74%, 25.63%, and 27.18%, respectively, under the GA_3 treatments. Under GA_3 supply, the 5dSm^{-1} treatment increased the total projected area by 51.70%, 40.74% and 31.0%, respectively, and the 10 dSm^{-1} treatment reduced the root total project area by 44.41%, 42.72% and 41.45%, respectively, compared with Control (Fig. 1.,B).

Total surface area and average diameter

Total surface area and average diameter decreased obviously($p \leq 0.05$) by 37.69%,42.75% in 'Shekhar', 30.65%,37.48% in 'Sheela' and 43.45%,42.06% in 'Kartika'(Fig.2.A, B) in the 10 dsm^{-1} salinity stress plants respectively compared with control (Fig. 2 A, B). The linseed crop treated with GA_3 showed an increase in the total surface area up to 29.25% in variety 1, 62.05% in variety 2 and 50.25% in variety 3 concerning salinity stress. Similarly, a significant increase was observed in the total surface area of the root when supplementation of Ca was applied, upto 19.27%(G1), 54.93% (G 2) and 35.98%(G 3) compared to stress treated plants(Fig.2.A, B). Ca and GA_3 separately increased total surface area compared with plants grown under salinity stress. Stress related attribute average root diameter was recorded to be markedly($p \leq 0.05$)

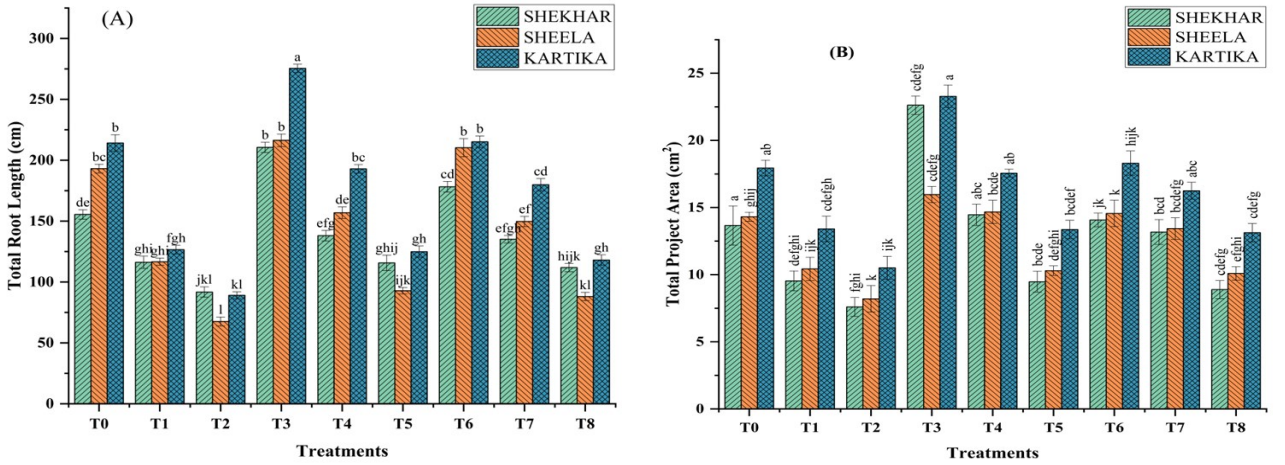

Fig. 1. *Effect of calcium and gibberellic acid on Linseed cultivated under chloride-dominant salinity stress on total root length (A) and total project area (B). Each value is the average across three replicates, with standard error calculated. Each bar with a distinct letter differed significantly across treatments (p≤ 0.05); means SE; two-way ANOVA; Tukey's post hoc test. [control (T_0);5.0 dSm^{-1} (T_1);10.0 dSm^{-1} (T_2);10^{-6} M GA_3 (T_3);5.0 dSm^{-1} + 10^{-6} M GA_3 (T_4);10.0 dSm^{-1} + 10^{-6} M GA_3 (T_5); $CaCl_2$ (T_6);5.0 dSm^{-1} + $CaCl_2$ (T_7);10.0 dSm^{-1} + $CaCl_2$ (T_8)]*

28.43%, 44.67% and 46.03%, respectively, compared with Control. The length per volume effective trends were similar to that of the length per volume in response to the calcium treatments. Compared to Control, the length per volume of 10 dSm^{-1} increased by 26.66%, 42.70%, and 37.94%, respectively, under the calcium treatments. Similarly, the total root volume of 10 dSm^{-1} compared to that of control increased by 42.17%, 64.47%, and 42.02%, respectively, under the GA_3 treatments. Under GA_3 supply, the 5dSm^{-1} treatment increased the total root volume by 46.60%, 52.15% and 37.63%, respectively, and the 10 dSm^{-1} treatment reduced the total root volume by 73.23%, 44.25% and 58.32%, respectively, compared with Control(Fig. 3.,B).Similar trends were obtained with Ca supplementation with 10 dSm^{-1}.Ca application with total root volume, plants under salt stress showed increased activity of 35.04%(G1),55.26%(G2), and 37.11%(G3) as compared to plants treated to chloride-dominated salinity 10 dSm^{-1}.However, the result indicated that out of three genotypes, Kartika performed significantly increased length per volume and total root volume compared to Sheela and Shekhar under saline conditions.

Tips and forks

The impact of different treatment (GA_3 & Ca) on root tips, forks and fine roots in Linseed varieties is presented in (Fig. 4 A,B,C).The GA_3 and calcium treatments observed a significant (p≤ 0.05) effect on tips,forks and fine roots compared with control C.At all salinity levels (5 dSm^{-1}, 10 dSm^{-1}), our analysis showed considerable improvements in tips,forks and fine of root architecture parameters (Fig. 4 A,B,C). The root tips number decreased as the salt stress level increased (Fig. 4 A),

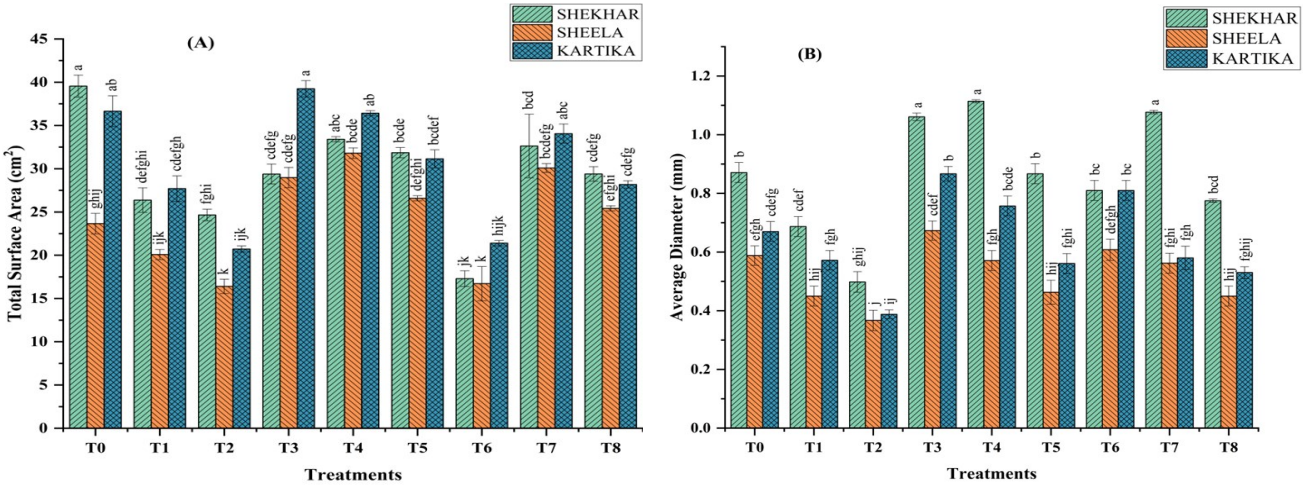

Fig. 2. *Effect of calcium and gibberellic acid on Linseed cultivated under chloride-dominant salinity stress on total surface area (A) and average diameter (B). Each value is the average across three replicates, with standard error calculated. Each bar with a distinct letter differed significantly across treatments (p≤ 0.05); means SE; two-way ANOVA; Tukey's post hoc test. [control (T_0);5.0 dSm^{-1} (T_1);10.0 dSm^{-1} (T_2);10^{-6} M GA_3 (T_3);5.0 dSm^{-1} + 10^{-6} M GA_3 (T_4);10.0 dSm^{-1} + 10^{-6} M GA_3 (T_5); $CaCl_2$ (T_6);5.0 dSm^{-1} + $CaCl_2$ (T_7);10.0 dSm^{-1} + $CaCl_2$ (T_8)]*

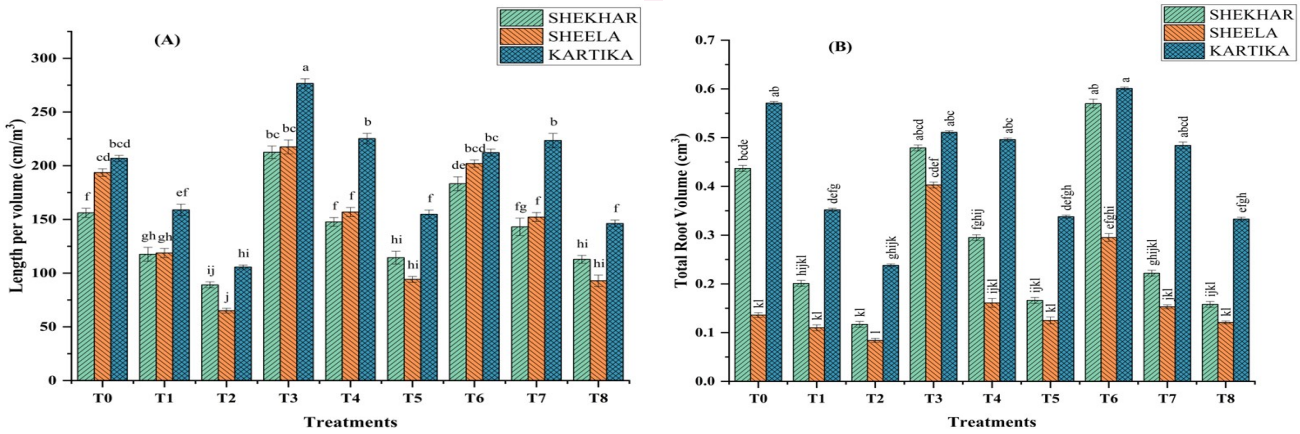

Fig. 3. *Effect of calcium and gibberellic acid on Linseed cultivated under chloride-dominant salinity stress on Length per volume (A) and total root volume (B). Each value is the average across three replicates, with standard error calculated. Each bar with a distinct letter differed significantly across treatments (p≤ 0.05); means SE; two-way ANOVA; Tukey's post hoc test. [control (T_0);5.0 dSm^{-1} (T_1);10.0 dSm^{-1} (T_2);10^{-6} M GA_3 (T_3);5.0 dSm^{-1} + 10^{-6} M GA_3 (T_4);10.0 dSm^{-1} + 10^{-6} M GA_3 (T_5); $CaCl_2$ (T_6);5.0 dSm^{-1} + $CaCl_2$ (T_7);10.0 dSm^{-1} + $CaCl_2$ (T_8)]*

increase by GA_3 by 74.12% (G 1),27.76%(G 2) and 44.67%(G3), respectively, compared with 10 dsm^{-1} salinity (Fig 2. A, B) Furthermore, the use of GA_3 caused the average root diameter parameter in stressed plants to increase even further, and the results were different from those in unstressed plants.The average diameter effective trends were similar to that of the total surface area in response to the calcium treatments. Compared to Control, the average diameter of 10 dSm^{-1} increased by 54.78%, 22.61%, and 36.72%, respectively, under the calcium treatments. This offered solid proof that GA_3 and Ca function better individually in correcting salt stress. Treatment with GA_3 and Ca increased the average root diameter and total surface area as GA_3 and Ca were applied, the effect of chloride-dominated salinity was neutralized, and the aforementioned metrics significantly improved compared to plants treated with salt. However, the result indicated that out of three genotype, Shekhar performed significantly increased average diameter compared to Sheela and Kartika under saline conditions.

Length per volume and total root volume

Two-way ANOVA revealed chloride dominated salinity stress and GA_3, Ca had significant effects on length per volume and total root volume ($P < 0.05$; Fig.1).With increasing salinity stress (10 dSm^{-1}), the length per volume and total root volume were significantly reduced (Fig. 3.A, B). 5 dSm^{-1} treatment reduced the length per volume by 24.73% (G1), 38.67%(G2) and 23.18 %(G3), respectively, and the 10 dSm^{-1} treatment reduced the length per volume by 42.95%, 66.38% and 48.76%, in three genotypes 'Shekhar','Sheela' and 'Kartika' respectively, compared with control. At GA_3 supply, the 5 dSm^{-1} treatment increased length per volume by 25.68%, 32.14% and 41.74%, respectively, and the 10 dSm^{-1} treatment increased length per volume by upto 60.86% in 'Shekhar', 79.86% in 'Sheela', and ,73.44% in 'Kartika' were observed at the highest salt stress (10 dSm^{-1}) compared to the control. The 10 dSm^{-1} salinity challenged root tips increased much more after GA_3 treatment. GA_3 was introduced to counteract the negative effects of salt on the plants' antioxidant defence mechanism.In addition foliar spray of GA_3 increased root tips up to 32.26% (G1),38.64% (G 2) and 56.23% (G 3) compared to highest salinity treatments (Fig 4.A). Ca application with root tip number under salt stress showed increased activity of 32.17% (G1), 34.89% (G2), and 50.69% (G3) as compared to plants treated with chloride-dominated salinity 10 dSm^{-1}.Similarly, results were observed with root forks. Its number decreased up to 50.32%(G1), 74.12% (G2), 67.76% (G3) at the highest salinity level 10 dSm^{-1}. But in contrast with GA_3 supplementation, the negative impact of salinity was counteracted by 24.21% in 'Shekhar',45.33% in 'Sheela' and 51.27% in 'Kartika'. Exogenously Ca supplementation in salinity treated plant, compared to highest salinity stress treatment. Similarly, results were obtained with forks, up to 22.81% in Shekhar,18.56% in Sheela and 36.21% in Kartika under the highest salinity stress. Fig. 4 A, B, C show increased values of tips, forks and fine roots in Ca, GA_3 and salinity-treated plants when compared to salinity treated, in contrast to the converse being true when Ca treated plants were compared to chloride dominated salinity treated plants. Results obtained from this study indicate that the fine root structure was decreased with increasing salinity up to 43.45% in G1, 64.67% in G2 and 60.88% in G3, but exogenous application of GA_3 increased by 24.57%, 41.075% and 49.72% in Shekhar, Sheela, Kartika genotype. Similarly, the results were obtained with Ca supplementation, and fine root diameter increased by 20.46%,30.94%, and 42.78%, respectively.In plants that were stressed

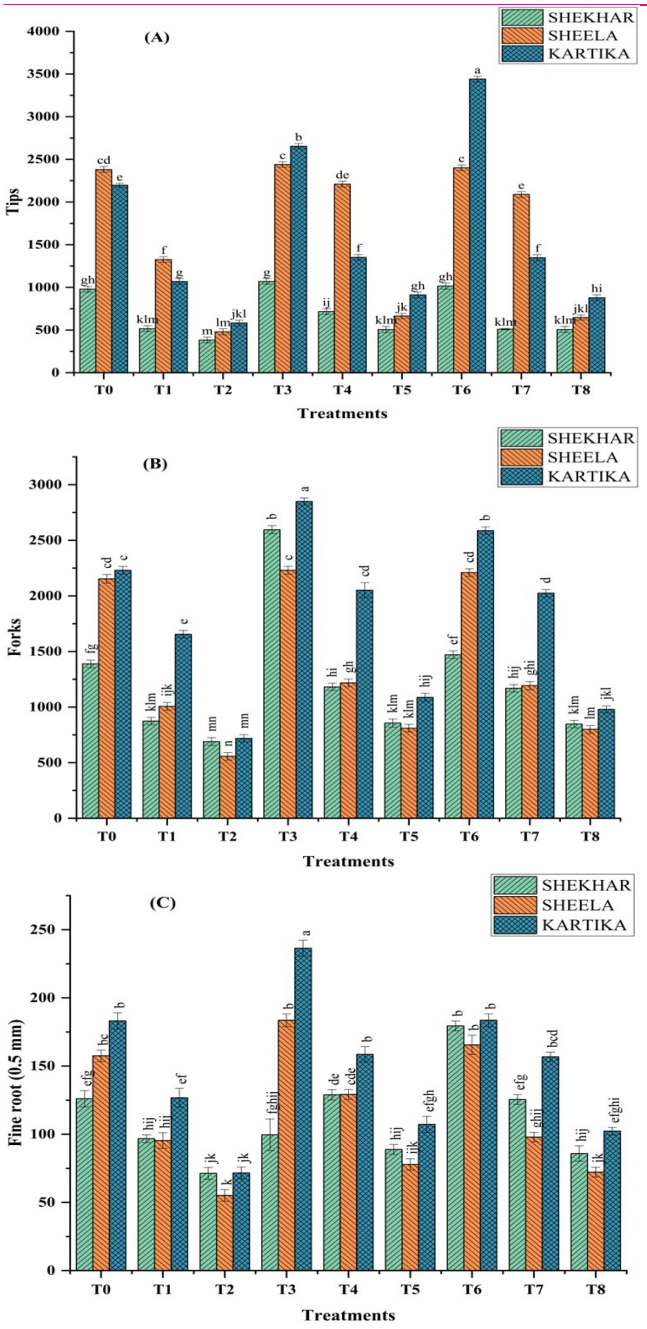

Fig. 4. *Effect of calcium and gibberellic acid on Linseed cultivated under chloride-dominant salinity stress on Tips (A), Forks (B) and Fine roots(C). Each value is the average across three replicates, with standard error calculated. Each bar with a distinct letter was significantly different across treatments (p≤ 0.05); means SE; two-way ANOVA; Tukey's post hoc test. [control (T_0);5.0 dSm^{-1} (T_1);10.0 dSm^{-1} (T_2);10^{-6} M GA_3 (T_3);5.0 dSm^{-1} + 10^{-6} M GA_3 (T_4);10.0 dSm^{-1} + 10^{-6} M GA_3 (T_5); $CaCl_2$ (T_6);5.0 dSm^{-1} + $CaCl_2$ (T_7);10.0 dSm^{-1} + $CaCl_2$ (T_8)]*

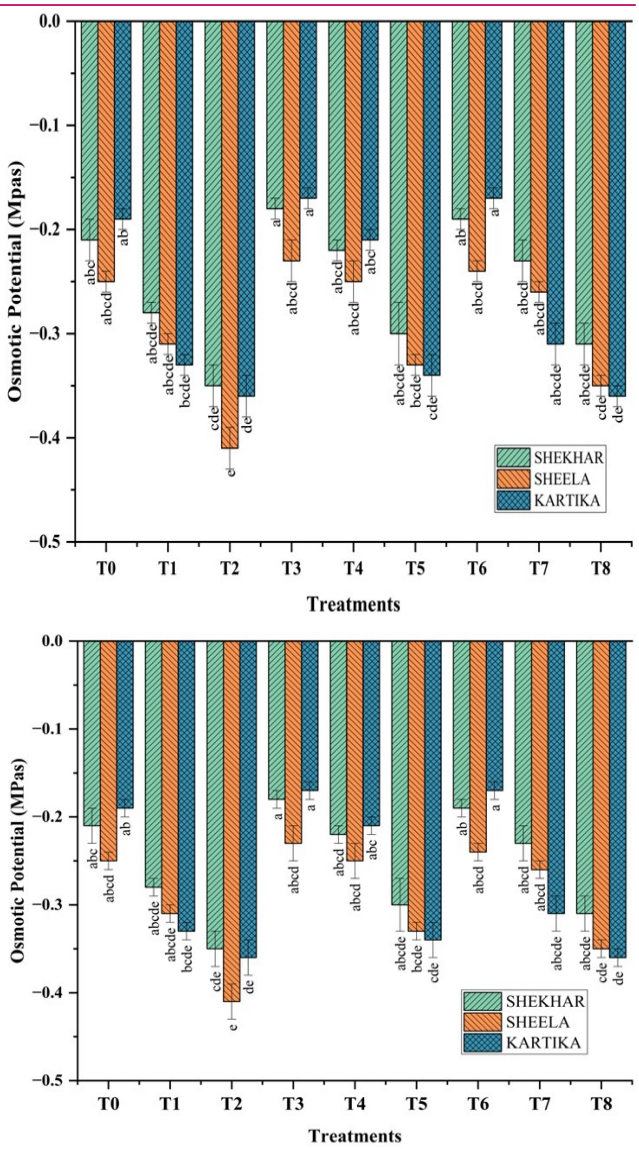

Fig. 5. *Effect of calcium and gibberellic acid on Linseed cultivated under chloride-dominant salinity stress on Osmotic potential of leaves (A) and roots (B). Each value is the average across three replicates, with standard error calculated. Each bar with a distinct letter differed significantly across treatments (p≤ 0.05); means SE; two-way ANOVA; Tukey's post hoc test. [control (T_0);5.0 dSm^{-1} (T_1);10.0 dSm^{-1} (T_2);10^{-6} M GA_3 (T_3);5.0 dSm^{-1} + 10^{-6} M GA_3 (T_4);10.0 dSm^{-1} + 10^{-6} M GA_3 (T_5); $CaCl_2$ (T_6);5.0 dSm^{-1} + $CaCl_2$ (T_7);10.0 dSm^{-1} + $CaCl_2$ (T_8)]*

by salinity, Ca and GA_3 were just as efficient in increasing root tips, forks and fine roots as Chloride salt alone.

Osmotic potential

Two-way ANOVA revealed chloride-dominated salinity stress and GA_3, Ca significantly affected the osmotic potential of leaves and roots ($P < 0.05$; Fig.6. A, B). Increasing salinity stress (10 dSm^{-1}) significantly reduced osmotic stress (Fig. 5. A,B). At moderate salinity level, the 5 dSm^{-1} treatment decreased the osmotic potential of leaves and roots by 33.33%,38.46% (G1), 24.00%,18.75%(G2) and 36.84%,27.78%(G3), respectively, and the 10 dSm^{-1} treatment reduced the osmotic potential by 66.67%,69.23% in 'Shekhar', 64.00%,56.25% in 'Sheela' and 68.42%,66.67% in 'Kartika', all three genotypes respectively, compared with Control. At GA_3 supply, the 5 dSm^{-1} treatment in-

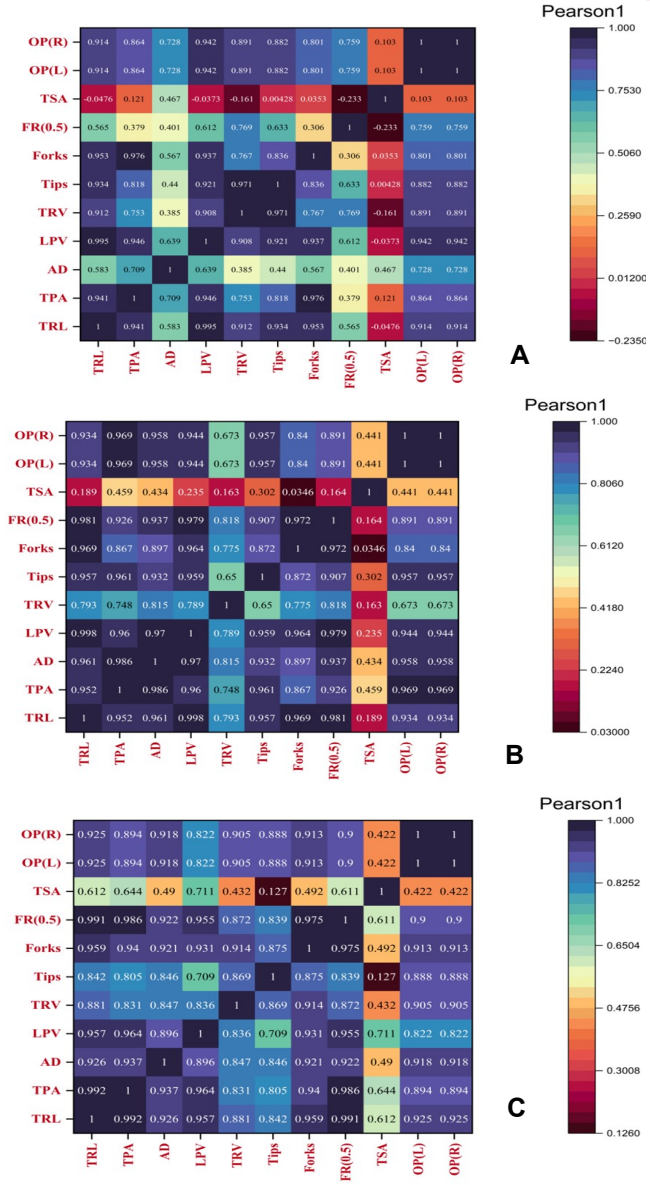

Fig. 6. *A Pearson's correlation graph among various root architecture related parameters and osmotic potential of Linseed under salinity, GA_3, Ca conditions with three genotypes Shekhar* ***(A),*** *Sheela* ***(B)*** *and Kartika* ***(C)****. In this study, the mean values of the various variables were normalised and grouped together. The colour scale shows the strength of the normalised mean values of different factors. Abbreviations -TRL:Total root length; TPA:Total project area; AD:Average diameter; LPV:Length per volume; TRV- Total root volume; FR-Fine roots; OP-Osmotic potential;L-Leaves;R-Roots. The lines emerging out of the central region of the biplots show negative or positive correlations with various parameters, and how close they are to each other displays how strong the correlation is between that parameter and that line*

creased osmotic potential by 21.43%(G1), 16.67%(G2) and 21.74%(G3), in leaves respectively, and the 10 dSm^{-1} treatment increased osmotic potential by 14.29%, 19.51% and 12.50%, of leaves respectively, compared with Control (Fig.5. A, B). The osmotic potential effective trends were similar in response to the calcium treatments. Compared to the Control, the osmotic potential of 10 dSm^{-1} increased by 11.43%, 14.63%, and 15.63% in leaves and 13.64%,16.00%, 20.00% in roots, respectively, under the calcium treatments.

Correlation

A Pearson's correlation graph was constructed to examine the associations among various root trait parameters, encompassing total projected area, total root length, length per volume, total root volume, average diameter, tips, forks, fine roots, and osmotic potential (both in leaves and roots) of Linseed genotypes (Fig. 6. A, B, C). Among the three genotypes, namely Shekhar, Sheela, and Kartika, positive correlations were observed between all these parameters. However, these parameters exhibited a negative correlation with the total surface area in the case of the "Shekhar" genotype, and conversely.

Furthermore, leaves subjected to GA_3 and Ca treatments in the presence of salinity exhibited an elevated osmotic potential, indicating greater solute accumulation compared to the control. Salinity stress negatively affected the root morphological structure including primary and secondary lateral roots, number of forks, root diameter, root length and root volume compared to control (Fig. 7A-B). Fig. 7 C,D showed that the exogenous application of GA_3 and Ca increased these parameters under salinity stress.

Previous studies (Jia *et al*. 2020; Meena *et al*. 2016; Borsani *et al*. 2001) show that saline stress significantly reduced plant growth and production. Gibberellic acid has a positive regulatory influence on plant stress tolerance under abiotic stress. Salt stress conditions can trigger phenotypic plasticity in plant roots by influencing the configuration of the root system (Julkowska *et al*.,2017; Korver *et al*.,2020; Li *et al*.,2021). The present study observed that chloride-dominated salinity at 10 dSm^{-1} exerted inhibitory effects on root system growth and induced changes in root architecture (Fig.1-4). Elevated salinity stress has been observed to impede the lignification process and the development of transport tissues (Gowda *et al*., 2011). In particular, the cortical tissue, a vital component of fine roots, occupies a substantial portion of the root cross-section, influencing both root absorption and radial transport (Gowda *et al*., 2011).

In typical crops, root growth parenchyma cells in the cortex naturally die, causing radial cell walls to come together and create air-filled cavities, forming aerenchyma.The paerenchyma serves as a conduit for the efficient transport of oxygen during root aerobic respiration, thus minimizing potential harm to the plant, as highlighted in the study by Zhang *et al*. (2016). Some of the oxygen in the paerenchyma moves toward

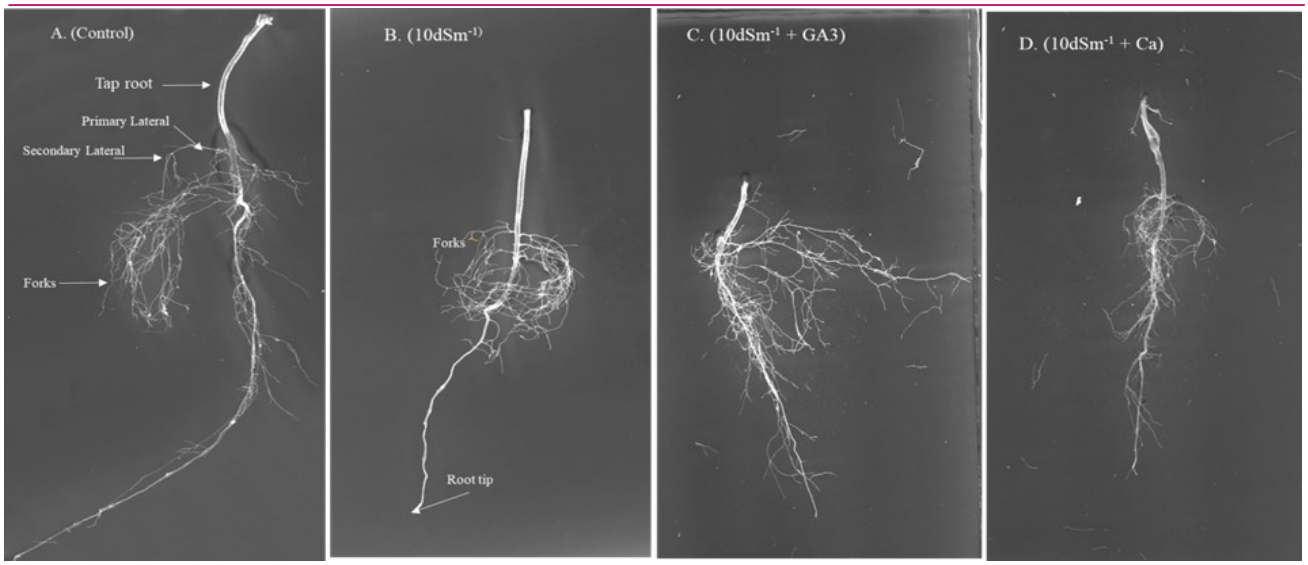

Fig. 7. *Showing the morphological and architectural traits of the root system under different salinity conditions: Control (A), Salinity(B),10 dSm^{-1} +GA$_3$(C), 10 dSm^{-1} + Ca (D), including length, diameter, tips, forks, root formation, root volume and surface area.*

the root tip, gradually reaching the surrounding soil. This creates an oxygen-rich environment that supports rhizospheric microbes, preventing damage from salt stress in root cells (Akcin *et al*., 2015).Root morphological characteristics, such as root volume, surface area, root length, and the number of root branches and tips, play a vital role in a plant's adaptation, overall well-being, and productivity (Huang *et al*., 2019).

As a highly responsive component of the plant system for sensing soil conditions, root tissue undergoes remarkable morphological alterations to minimise metabolic demands while maximizing nutrient acquirement (Mishra *et al*., 2017). Under stressful conditions, root growth is frequently hindered, and the inhibition of root elongation is often the first sign of adverse environmental factors (Munzuroglu and Geckil, 2002). In present study, the Linseed genotypes exhibited a reduction in various root parameters, including root volume(Fig. 3A,B), root surface area(Fig.2 A),total root length(Fig.1 A), root branches number and tips,forks (Fig. 4 A,B) with increasing soil salinity levels. A similar pattern was observed by Huang *et al*. (2019), who noted that Pb toxicity in soils led to decreased root length, root diameter, and root surface area in *Robinia pseudoacacia* seedlings. Additionally, Spagnoletti and Lavado (2015) reported that elevated soil arsenic concentrations had a detrimental effect on morphological traits in G. max seedlings, including a reduction in root length.However, Srivastava *et al*. (2009) reported a slight increase in root density but no significant difference in root length for *Brassica juncea* seedlings under arsenic stress in soils. These alterations in root characteristics provide direct evidence of stress-induced damage to roots in the soil environment and are likely linked to a compromised plant metabolism Chen *et al*.,2007). In the present study, Linseed genotypes exhibited higher root diameter (Fig.2B), root surface area (Fig. 2A), total root length(Fig.1 A), root volume (Fig.3 B), and the number of root branches and tips with GA$_3$ and Ca application compared to salinity levels at 10 dSm^{-1}. These findings align with the conclusion drawn by Wu *et al*. (2011) and Huang *et al*. (2019).

Lu *et al*. (2013) found that the increase in the number of root tips enhances the plant's ability to access resources and nutrients from the soil. In the current investigation, the root tips number (Fig.4A) in Linseed genotypes 'Shekhar,' 'Sheela,' and 'Kartika' decreased as soil salinity levels increased from 5 dSm^{-1} to 10 dSm^{-1}. However, it was observed that Kartika genotypes had a higher root tip number than Shekhar and Sheela, suggesting that Kartika could alleviate the reduction in lateral root growth and improve the plant's ability to acquire resources in saline soil. This, in turn, facilitated the uptake of soil nutrients and water by increasing the active root length, and the number of root forks and tips within the root system of Linseed genotypes.In this particular investigation, Shekhar and Sheela exhibited notably lower root length (Fig.1 A) percentages when compared to the Kartika genotypes. As a result, Kartika exhibited an enlarged root diameter under different salinity treatment conditions, which could be associated with promoting larger parenchyma cells and enhanced cortical tissues, potentially influenced by AM symbiosis (Sheng *et al*., 2009). A similar outcome was observed in a pot experiment involving *Robinia pseudoacacia* seedlings inoculated with either *Glomus versiforme* or *Rhizophagus irregularis* under standard growth conditions, as reported by Zhang *et al*. (2016).

Plant growth regulators, specifically phenolic hormones recognized as stress hormones, are pivotal in enhancing plant stress tolerance by modulating a wide array of physiological and metabolic responses(Kim *et al*., 2017;

Wu *et al*.,2018). In the present investigation, the exogenous application of GA_3 had a profound positive impact on root growth, including enhancements in root volume (Fig.3 B), surface area (Fig.2A), total root length (Fig.1A), root tips (Fig.4A), and root diameter(Fig.2 B). This contrasts a prior study involving Eucalyptus(Liu *et al*.,2018), which reported a decrease in average root diameter under non-saline conditions (NS). This suggests that GA_3 may alleviate salinity stress's adverse effects on root growth. Additionally, in wheat seedlings subjected to salt stress, the levels of certain compounds in the roots were significantly elevated following treatment with JA. Similar findings were reported by Kim *et al*.(2017), who observed a notable increase in SA levels in cucumber leaves after exposure to salt stress, with even higher SA levels in cucumber plants pretreated with exogenous SA under stress conditions.
Furthermore, Iqbal *et al*. (2006) had noted a considerable rise in SA concentrations in the leaves of hexaploid wheat when pretreated with $CaCl_2$ under saline conditions. Previous studies have consistently demonstrated that elevated levels of plant growth regulators (PGRs) contribute to stomatal closure, reducing transpiration and helping to maintain a balanced water status in plants, ultimately mitigating the effects of salt stress, as supported by research conducted by Wu *et al*. (2018) and Bharath *et al*. (2021). Following the application of plant growth regulators, a substantial rise in root diameters was observed, with distinct differences between the regulators across various root diameter groups. This leads to improved water and nutrient uptake and increased tolerance to stress (Guo *et al*.,2017; Werner *et al*.,2001; Wang *et al*.,2021). Calcium ions (Ca^{2+}) play a crucial role in the protein phosphorylation/ dephosphorylation cascades that link the perception of salt stress to signal transduction. The present findings indicate that supplementing with Ca^{2+} at a salinity level of 10 dSm^{-1} resulted in increased root parameters compared to the highest salinity level. Preserving an elevated cytosolic K^+/Na^+ ratio is a critical characteristic linked to salt tolerance in plants (Wu and Zou, 2009; Chinnusamy *et al*.,2005).The present study observed that the osmotic potential of both leaves and roots decreased as salinity increased to 10 dSm^{-1}.These findings align with previous research in *Crocus sativus* L. and *Zea mays* L. (Hajlaoui *et al*., 2010), which established a clear correlation between potentials, solute accumulation, and salt stress.

Conclusion

This study demonstrated that an appropriate GA_3 and Ca supply effectively relieved chloride-dominated salinity stress in linseed(Linum usitatissimum L.). The results show significant correlations between root morphology and GA_3 and Ca concentrations. With increased salinity stress, the root architecture parameters decreased and osmotic potential also decreased. However, at the highest salinity stress condition (10 dSm^{-1}), the GA_3 and Ca supply had a significantly effect on root architecture, including total surface area, average diameter, length per volume, total projected area, root length, total root volume, tips, forks, fine roots, and osmotic potential in Linseed genotypes. The results suggest that gibberellic acid and calcium supply can reduce salinity stress by enhancing the root growth of linseed.

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Conflict of interest

The authors declare that they have no conflict of interest.

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