Research Article

Enhancing salinity stress tolerance in chickpea (*Cicer arietinum* L.) genotypes through foliar application of spermidine

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Abstract

Chickpea (*Cicer arietinum*), the 2nd most important legume after dry beans, known for its protein content, is an important crop agronomically as well as economically. The present study aimed to determine the effect of foliar application of spermidine (spd) on various aspects of chickpea genotypes under salt stress. The chickpea genotypes were cultivated under 4 and 8 dSm^{-1} Cl^- dominated salinity followed by the spermidine application of 0.5 and 1.0 mM. Salinity stress inhibited the plant height and root length, reducing plant biomass (fresh and dry weight). Furthermore, it also decreased the chlorophyll and nitrogen content of the plant. Results obtained from spermidine (spd) application indicated that both concentrations of spermidine are good in enhancing the tolerance in chickpea genotypes against salt stress. Spermidine application increased the plant's height as well as biomass. It also enhanced the chlorophyll content (32.93%), increasing the Nitrogen Balance Index (77.37%) at 1.0 mM. It further increased the flavonoid and anthocyanin content in plant leaf. In addition, the application of spermidine retained more moisture (25.81%) and increased seed fiber (13.30%) in all chickpea genotypes at 1.0 mM. It reduces the Cl^- ion accumulation and maintains the ionic balance in chickpea seeds. The effect of spermidine application (0.5 and 1.0mM) was more pronounced, but 1.0 mM had a more positive effect in salt-sensitive chickpea genotypes.

Keywords: Anthocyanin, Chickpea, Flavonoid, Nitrogen Balance index, Salinity, Spermidine

INTRODUCTION

Salinity stress is one of the major abiotic stresses in agriculture that affects crop productivity worldwide, especially in arid and semi-arid areas. It affects the crop plant negatively at both vegetative and reproductive stages of development and causes serious economic loss (Shanko *et al*., 2017). Salinity can be natural and anthropogenic, resulting in salt deposition in soil. High salt concentration in the soil reduces the osmotic potential inside the plant root cell, which influences water and ion uptake. High concentration in the soil reduces the osmotic potential inside the plant root cell, which influences water and ion uptake, known as the osmotic effect. The plant faces water deficit conditions or physiological drought condition. High salt concentration results in the accumulation of sodium (Na^+) and chloride (Cl^-) ions in the cytoplasm, which create ion toxicity and affect various metabolic processes inside the plant cell, including ionic stress and adversely affect various metabolic processes inside plant cells. This phenomenon gives rise to ion toxicity, triggering the production of reactive oxygen species.

Consequently, oxidative stress occurs, which damages the plasma membrane and increases permeability. About 15% of the total land area worldwide is salt-

affected and is increasing by 1-2% every year. In India, about 6.7 mha of land are saline salt-affected (saline and sodic), out of which 80% is contributed by Gujarat, Uttar Pradesh, Maharashtra, West Bengal, Rajasthan and Tamil Nadu (Sahab *et al.*, 2021).The effect of salt stress depends upon the growth stage, plant species salt concentration and time duration of salinity (Mann *et al.,* 2019; Kaur *et al.,* 2021, 2022a, 2022b).

Chickpea (*Cicer arietinum* L.) is a salt-sensitive crop cultivated in various climatic conditions, especially as a food grain in arid and semi-arid regions worldwide. Traditionally, chickpea was cultivated in saline soil and marginal areas, especially in arid and low rain-fall areas. It is more sensitive under Cl⁻ Cl-dominated salinity. It is the 2nd most important legume after dry beans. Chickpea is grown in 54 countries, with nearly 75.03% of its production covered by India alone (FAOSTAT, 2021). It is a low input requiring protein-rich crop in a cereal-based diet, especially for poor population in developing countries. It is cultivated in rotation with cereal crop because 70% of nitrogen in the soil is fixed biologically in a symbiotic association with rhizobium bacteria in its nodules, improving soil fertility (Ladha *et al.*, 2022). The foliar application of substances is an effective and simple method to lessen the salt stress effects. Among these, polyamines are low molecular weight aliphatic nitrogen-containing amino groups present ubiquitously in all organisms and have high biological activities in plant growth and development processes and also under adverse environmental conditions, especially under abiotic stress tolerance(Hai *et al.*, 2022). The most common forms of polyamines in plants are diamine putrisine, tri-amine spermidine and tetra-amine spermine. The distribution of polyamine is tissue-specific. Putrisine is found mainly in plant leaves, while spermidine and spermidine occur abundantly in the rest of the plant organ. Polyamine plays an essential and wide variety of roles in plant metabolism, such as cell proliferation, embryogenesis, dormancy termination, flower development, senescence, fruit maturation and influence tolerance (ElSayed *et al.*, 2022). Exogenous application of polyamine increases the endogenous polyamine level that initiates the various metabolic functions to ameliorate salt stress (Raziq *et al.*, 2022). Among the three forms, spermidine (spd) is more effective in enhancing the stress response. Spermidine plays an important role in regulating plant growth and development, such as germination, membrane integrity, cell division, enzyme activity, and nutrition acquisition (Bouabdallah *et al.*, 2022).

Exogenously applied spermidine decreases the Na^+ ion content in the leaves. The effectiveness of spermidine application depends upon various factors such as species developmental stage, concentration of spermidine, intensity of stress and treatment duration. Salt stress inhibits the germination, plant height and biomass in *Vigna angularis* (Al-Mushhin, 2022). Under salt stress, Cl- ion antagonises N_2 uptake and reduces plant nitrogen content (Ashraf *et al.*, 2018). However, the salinity enhance the flavonoid and anthocyanin content in *Phasolous vulgaris* (Taibi *et al.,* 2016) and decrease the chlorophyll content in chickpea salinity enhances the flavonoid and anthocyanin content in *Phaseolus vulgaris* (Taibi *et al.*, 2016) and decreases chickpea's chlorophyll content (Dadasoglu *et al.*, 2022). The exogenous application of spermidine mitigates salt stress. It enhances the plant height, root length, biomass, and chlorophyll content of Vigna angularis (Al-Mushhin, 2022), enhancing crop yield and seed quality. Therefore, the present study aimed to investigate the effect of exogenous spermidine on chickpea genotypes under salt stress by investigating the various plant growth parameters, chlorophyll, flavonoid and nitrogen content and seed quality parameters.

MATERIALS AND METHODS

Plant material and treatments
Four genotypes of chickpea seeds were procured from the Pulses Section, CCS Haryana Agricultural University Hisar and were surface sterilized with 0.2% $HgCl_2$. Seven seeds of each genotype were raised in pots lined with polythene bags filled with 7.0 Kg of dune sandplaced in natural environment condition in natural condition. Before sowing, the chickpea seeds were inoculated with *Rhizobium* strain.

The desired Cl⁻ dominated salinity 0, 4.0, and 8.0dSm⁻¹ were applied at the seedling stage to saturate the pot and were created by adding different concentrations of salt, viz., $MgSO_4$, $CaCl_2$, $NaCl$, and $MgCl_2$. The soil was saturated with its field capacity of soil, i.e. 30% of 200ml H_2O/Kg soil.The foliar application of Spermidine (Spd) was applied on the foliage part at 55th DAS in two different concentrations of 0.5 and 1.0 mM (milli Molar). The chickpea is a rabi crop and is sown in October to mid-November. The temperature ranges from 18-25°C and humidity ranges between 85- 70% approximately. Sampling was done after 15 days of polyamine treatments. The desired levels of treatments were given in the pots, which comprised the following:

T_0 = 0 dSm⁻¹ + 0 mM Spd
T_1 = 4 dSm⁻¹ + 0 mM Spd
T_2 = 4 dSm⁻¹ + 0.5 mM Spd
T_3 = 4 dSm⁻¹ + 1.0 mM Spd
T_4 = 8 dSm⁻¹ + 0 mM Spd
T_5 = 8 dSm⁻¹ + 0.5 mM Spd
T_6 = 8 dSm⁻¹ + 1.0 mM Spd

Growth parameters
Plant height and Root length
The plant height was measured from the base to the tip (shoot apex) of the plant with the help of a ruler in cm.

Similarly, the length in cm from the base to the root apex of the main tap root of the plant was measured.

Fresh and Dry weight

The fresh and dry weight was determined after 15 days of spermidine application. The plants were removed from the soil and washed thoroughly to remove soil. The water from the plant was removed with the help of tissue paper, and then the root and shoot were separated. The root and shoot from each plant were weighed on an electric balance and the reading was observed. To measure the dry weight, the root and shoot of each plant were oven-dried at 75^0C for 72 hrs and then weighed using an electronic balance.

Biochemical parameters
Chlorophyll content

The chlorophyll content was determined using the SPAD 502 plus instrument (Shibaeva *et al*., 2020). The measurement was done by inserting the leaf in the measuring head in real time without removing the leaf from the plant. The SPAD value was recorded which indicated the total chlorophyll present in the leaf.

Nitrogen Balance Index (NBI)

Similar to the chlorophyll content, the NBI was determined using the Dualex 4 Scientific (Dx4) analyzer by measuring the absorbance of the leaf in real-time (Cerovic *et al*., 2012).

Flavonoid and anthocyanin content

The flavonoid and anthocyanin content were also determined using the Dualex 4 Scientific (Dx4) analyzer by inserting the leaf and closing the measuring head. It gives a real-time SPAD value, a time value proportional to the leaf's flavonoid and anthocyanin content.

Seed quality parameter

The seed quality parameters (moisture, ash, fibre, and Cl⁻ ion)were determined by using Near-Infrared Spectroscopy (NIRSystems 6500 FOSS©) without crushing the seeds (Tomar*et al*., 2021).

Statistical analysis

Results obtained from the experiment were analyzed using Complete Randomized Design (CRD) for two factors and compared using critical difference (CD) at a 5% level of significance and graphical presentation was conducted using Origin pro, 2022 and GGEwabiplot.

RESULTS AND DISCUSSION

Plant height and root length

The plant height and root length of all the chickpea genotypes were significantly decreased under both salinity levels (4-8 dSm⁻¹) in all chickpea genotypes as compared with the control (Fig. 1). The maximum decrease for plant height and root length was observed in genotype HC 5 and minimum in genotype RSG 931at high salinity level. Compared with T4 (8dSm⁻¹), Spd application (0.5 and 1.0mM) increases the plant height and root length in all chickpea genotypes significantly with T4 (8dSm⁻¹), Spd application (0.5 and 1.0mM) significantly increases the plant height and root length in all chickpea genotypes. The maximum increase was recorded in genotype HC 5 of 14% and 22.99%, respectively, at 1.0mM Spd.

Fresh weight

The results clearly determined that each chickpea genotype showed a significant decrease in their biomass (shoot and root) with an increase in salinity level over their respective control (T0). The maximum decrease was observed in genotype HC 5 and the minimum in genotype RSG 931 at high salinity levels (Fig. 2). Foliar application of spermidine (0.5mM and 1.0mM) significantly increased the plant biomass at both salinity lev-
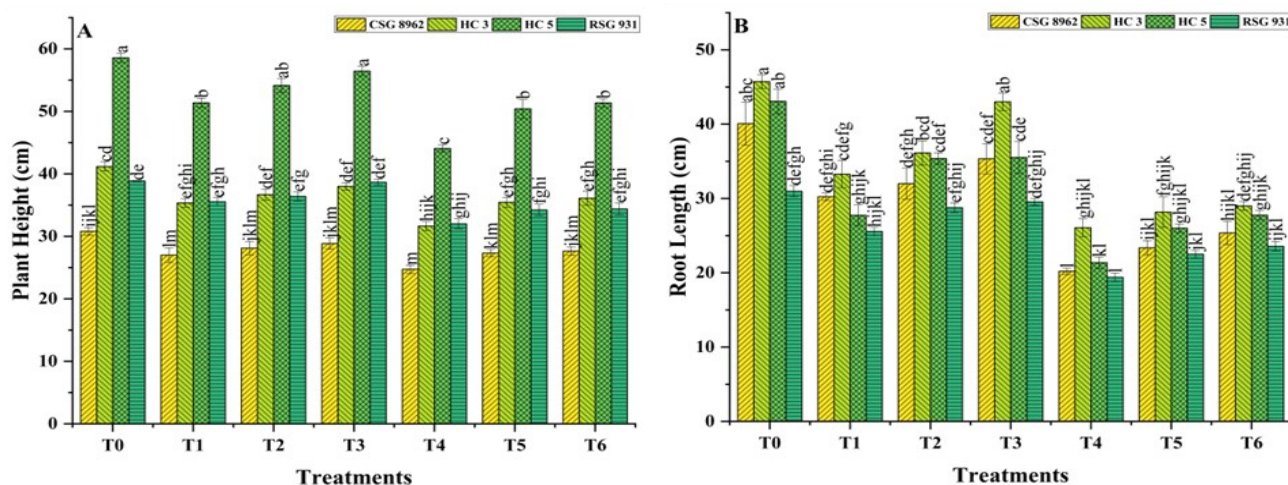

Fig. 1. *Effect of spermidine application on plant height (A), and Root Length (B) in chickpea genotypes under salt stress. Bars represent M ± SE. ;Different letters show significance (p ≤ 0.05) among the treatments according to Tukey's HSD Test*
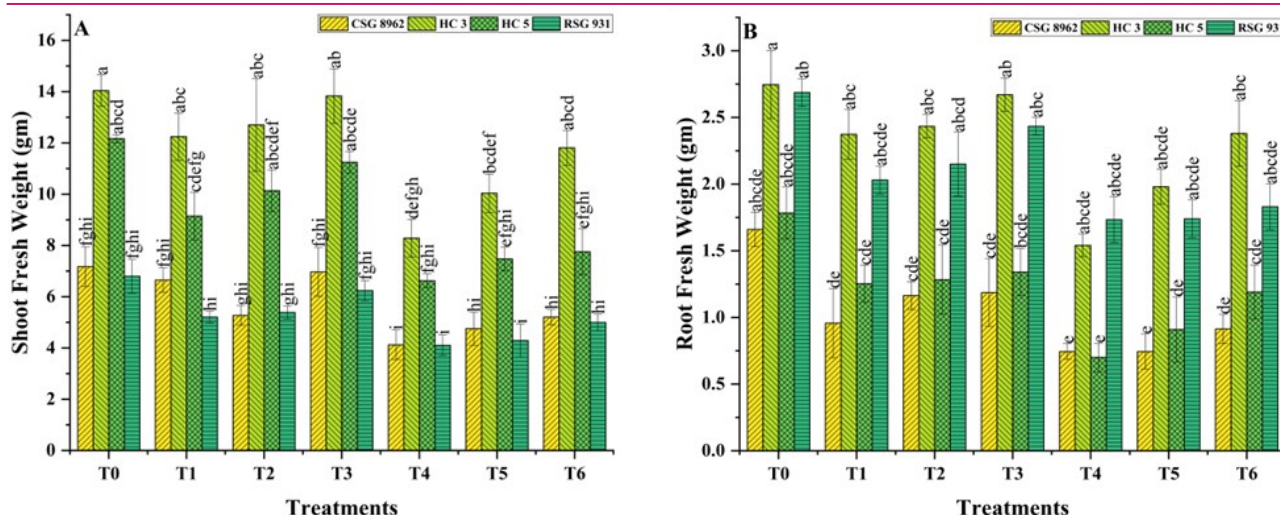
Fig. 2. *Effect of spermidine application on shoot fresh weight (A), Root fresh weight (B) in chickpea genotypes under salt stress.Bars represent M ± SE. Different letters show significance (p ≤ 0.05) among the treatments according to Tukey's HSD Test*

els. The increase in fresh weight at high salinity was highest in genotype HC 3 at 29.86% and in genotype HC 5 at 41.17% in shoot and root, respectively, at 1.0mm Spd level.

Dry weight
Compared with control T0, salt stress (4-8 dSm^{-1}) led to a significant decrease in the plant dry weight (shoot and root) in all chickpea genotypes over their respective control (Fig. 3). The maximum decrease was observed in genotype HC 5 and minimum in genotype RSG 931. Spd application significantly increases the dry weight in all chickpea genotypes at both salinity levels. The maximum increase at high salinity was observed in genotype HC 5 of 33.87% followed by RSG 931 (24.23%), HC 3 (21.20%) and CSG 8962 (12.77%) in shoot and in root the maximum increase was observed in genotype HC 5 of 33.5% followed by RSG

931 (27.46%), HC 3 (24%) and CSG 8962 (8.66%) respectively, at 1.0mM Spd.

Chlorophyll content and Nitrogen Balance Index (NBI)
In each chickpea genotype, the chlorophyll content and NBI under 4 and 8dSm^{-1} salinity were significantly decreased relative to control (T0) (Fig. 4A-B). The maximum decrease in chlorophyll and NBI was reported in genotype HC 5 of 54.02% and 86.20% and minimum in genotype RSG 931 of 38.95% and 52.41%, respectively, at high salinity level. The spd application increased the chlorophyll and NBISpd application increased the chlorophyll and NBI. Compared with 8dSm^{-1}, Spd application of 0.5 mM and 1.0mM enhances the chlorophyll and NBI in plant leaves at both salinity levels significantly mM significantly enhances the chlorophyll and NBI in plant leaves at both salinity levels. The maximum increase in chlorophyll and NBI at high salinity
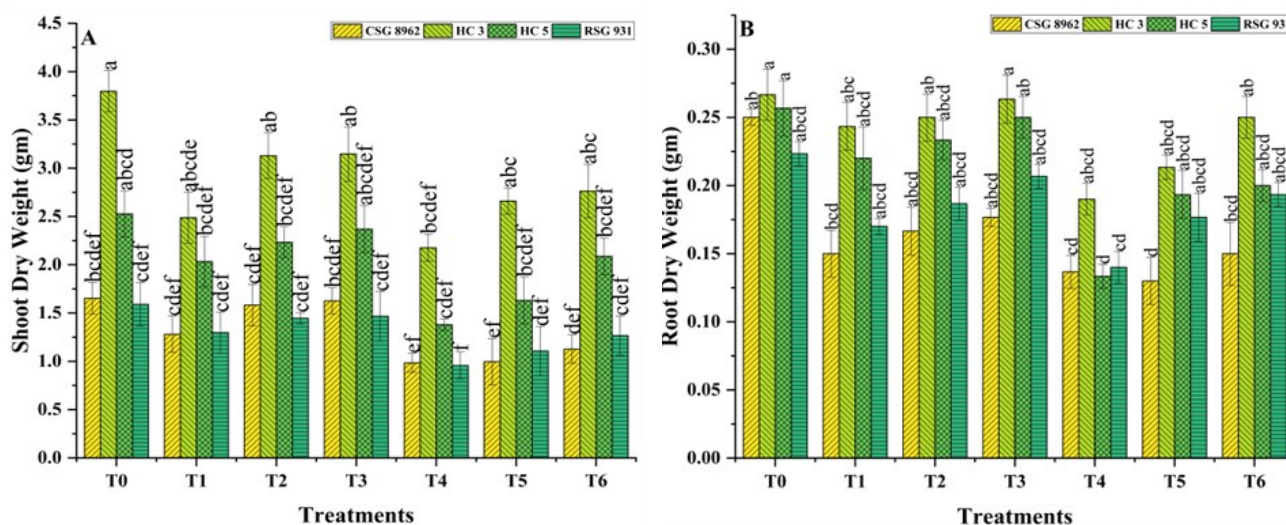

Fig. 3. *Effect of spermidine application on shoot dry weight (A), root dry weight (B) in chickpea genotypes under salt stress.Bars represent M ± SE. Different letters show significance (p ≤ 0.05) among the treatmentsaccording to Tukey's HSD Test.*
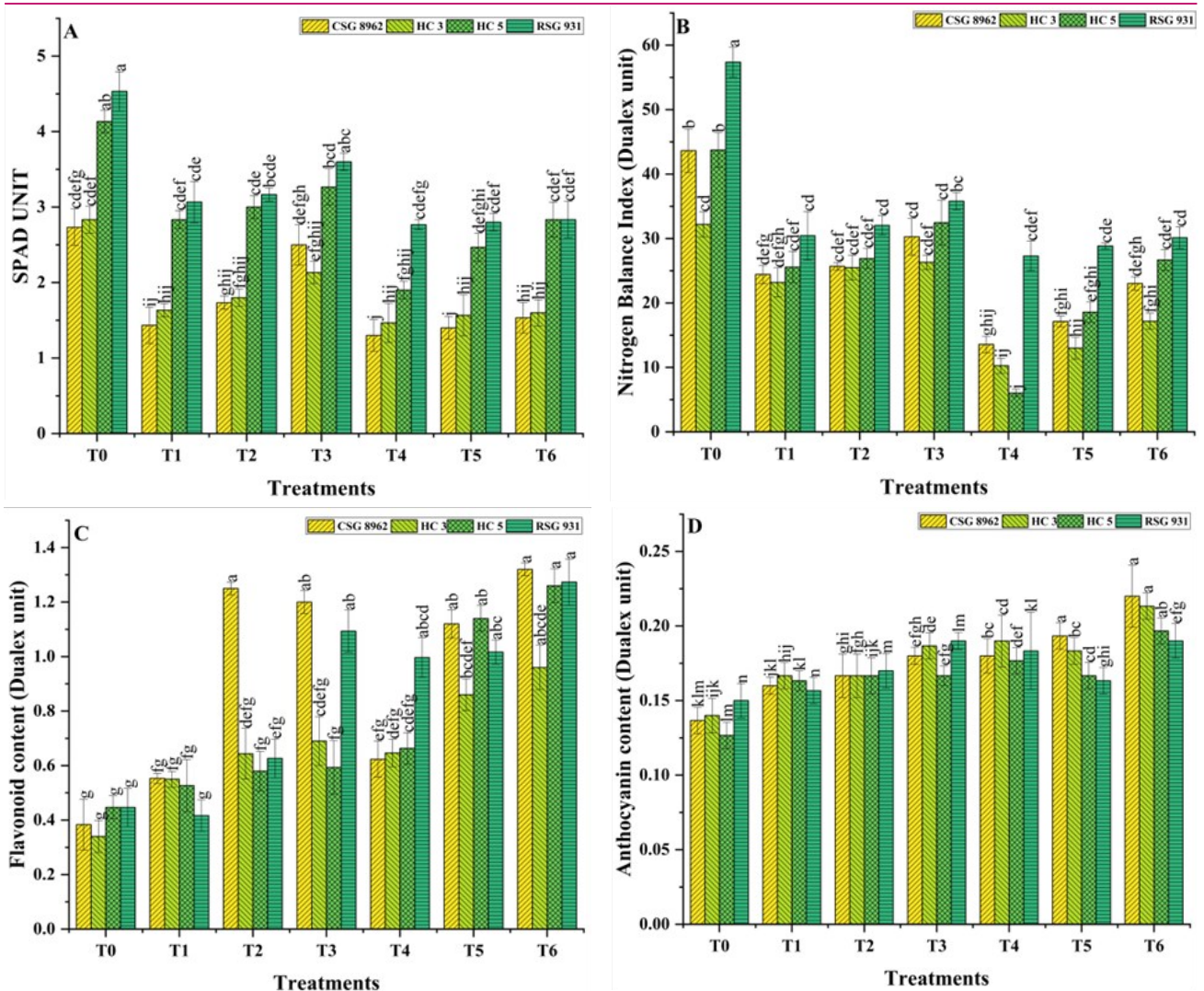
Fig. 4. *Effect of spermidine application on chlorophyll (A), NBI (B), flavonoid (C), anthocyanin (D) in chickpea genotypes under salt stress. Bars represent M ± SE. Different letters show significance (p ≤ 0.05) among the treatments according to Tukey's HSD Test.*

level was observed in genotype HC 5 of 32.93% and 77.37%, respectively, at 1.0mMSpd.

Flavonoid and anthocyanin content

In each genotype**,** plant leaves' flavonoid and anthocyanin content significantly increased at both salinity levels over their respective control (Fig. 4 C- D). These were intrinsic responses of the chickpea genotype against salt stress. The maximum increase for flavonoid content was observed in genotype RSG 931 at high salinity level. However, the maximum increase for anthocyanin content was observed in genotype HC 5 over their respective in anthocyanin content was observed in genotype HC 5 over their control. These were further increased by Spd application (0.5 and 1.0mM). Compared with 8dSm, Spd application led to a significant increase in flavonoid and anthocyanin content in all chickpea genotypes and the maximum increase was observed in genotype CSG 8962 of 52.80% and 18.18%, respectively.

Compared with the control (T0), salt stress led to a significant decrease in seed quality. It enhances the ash and Cl⁻ ion content and decreased the fiber and moisture content in seed of all chickpea genotypes over their respective control. The decrease in seed quality was maximum in genotype HC 5 and minimum in genotype RSG 931. Spd spraying of 0.5 and 1.0 mM improves the seed quality in all chickpea genotypes at both salinity levels. Compared to 8dSm^{-1}, Spd application of 1.0mM significantly increased the fiber and moisture content and decreased the ash and Cl⁻ion content in seeds of all chickpea genotypes (Fig. 5). The maximum increased in fiber and moisture content was observed in genotype HC 5 at 13.35% and 25.81%, respectively, and maximum decrease in ash and Cl⁻ ion was reported in genotype HC 5 at 13.96%, 9.64%, and 17.20%, respectively.

Response of various parameters to the treatments

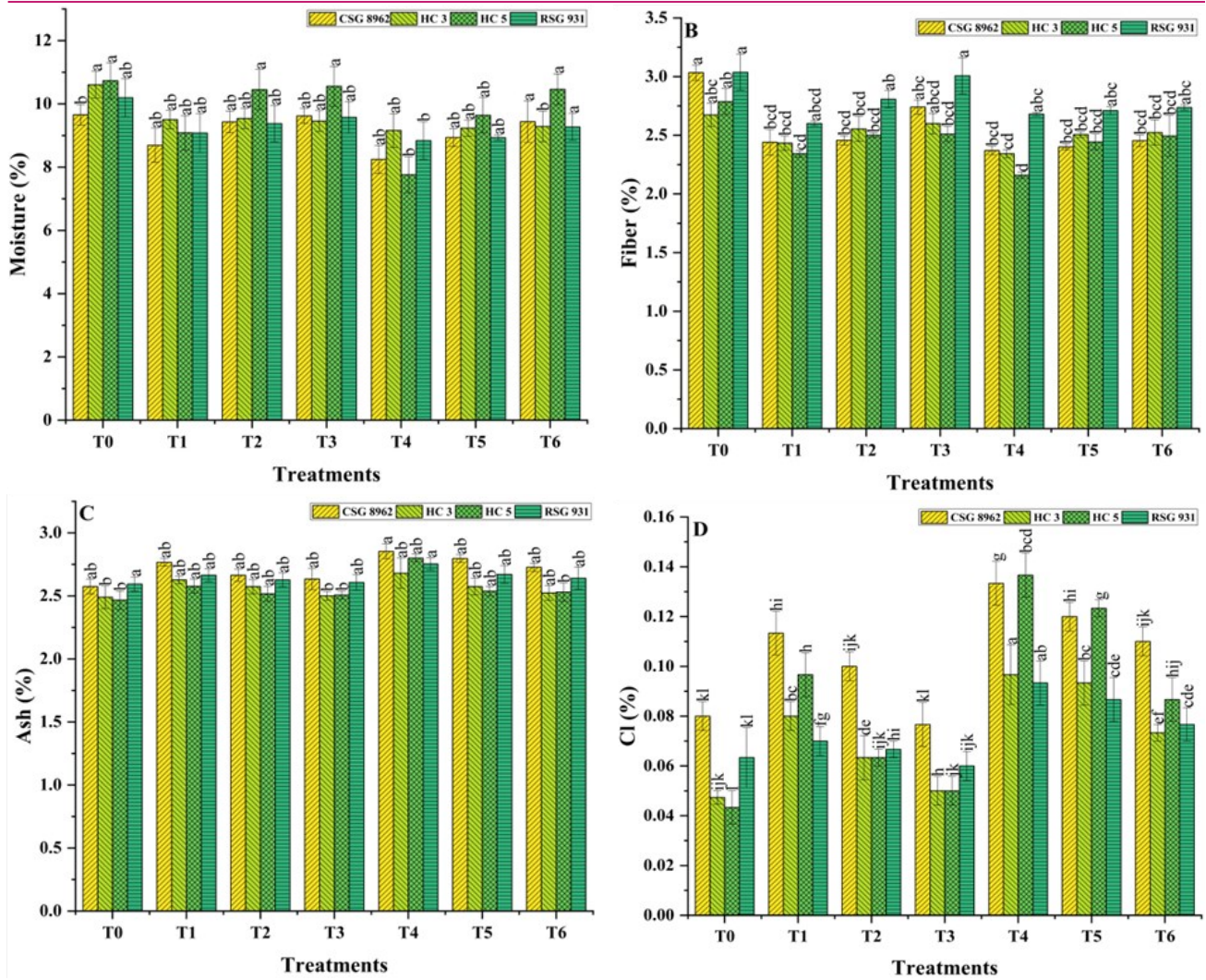The biplot (Fig. 6) showed the values of various param-

Fig. 5. *Effect of spermidine application on moisture (A),fiber (B), Ash (C), and chloride (D) content in chickpea genotypes under salt stress. Bars represent M ± SE. Different letters show significance (p ≤ 0.05) among the treatments according to Tukey's HSD Test.*

eters under different genotypes- treatment combinations. In this biplot, PC 1 explained 48.25% and PC 2 explained 19.56%, a total sum of the square of the variations about 67.81%. The plot biplot presents the following points presents the following points:

PC 1 divides the biplot into two groups (stressed and non-stressed): the non-stress treatments (T0) for each genotype on the right side and the stress treatments (T1, T2, T3, T4, T5, and T6) for each genotype on the left side of the biplot. However, the stress treatments (T2 AND T3) are present on the right side of the biplot, representing the tolerance against salt stress.

Each genotype (HC 3, HC 5 and RSG 931) under non-stress conditions (T0) showed differences as they were placed far from each other on the biplot characterized by relatively high levels of PH, RL, SFW, SDW, RFW, RDW, CHL, NBI and Fiber content and relatively low levels for those placed on the left side (ASH, FLV, Cl⁻, ANTH and Moisture) of the biplot. The opposite can be said for the salt stress treatments (T1 and T4). However, for treatments T2 and T3, the genotype CSG 8962

showed a high difference from other genotypes as it placed on the left side of the biplot, characterized by relatively high ASH, FLV, Cl-, and ANTH levels. Therefore, the salt-treated and non-treatment chickpea seedlings showed contrasting states in the two sets of indicators.

PC 2 separates the four genotypes under salt-stress conditions. HC 3, HC 5, and CSG 8962 genotypes under T1, T4, T5 and T6 show little differences as they are placed on the upper left of the biplot, characterized by relatively high levels of ASH, FLV, Cl⁻, ANTH and Moisture. In contrast, RSG 931 is placed in the lower left of the biplot for the treatments (T1, T4, T5, and T6). This suggested that the high levels of flavonoid and anthocyanin in the leaf and low levels of chloride ion and ash content in the seed are the indicators of intrinsic or Spd-induced salt tolerance.

The present study evaluated salinity's effects on plant growth and biochemical process in chickpea genotypes, directly affecting the seed quality. Salt stress first causes osmotic stress, creating water deficit condi-
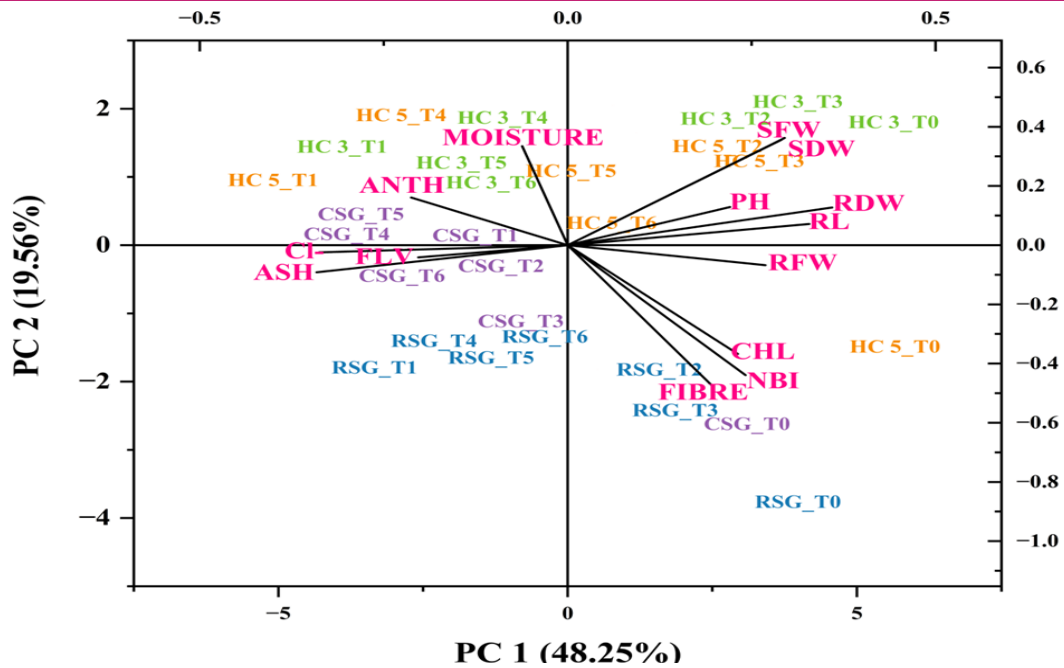
Fig. 6. Biplot showing the relative level of each of the genotype –treatment combination for each of the determined parameters.

tions and then accumulating ions in cell cytoplasm causes ionic stress. The water deficit conditions generate ROS production, such as superoxide (O_2^-)and hydrogen peroxide (H_2O_2), disturbing the plant metabolic activities. These cytotoxic active free oxygen radicals seriously affect the metabolic process through oxidative stress. All these effects inhibit plant growth and biomass production (Mamta *et al.*, 2020). The present results on chickpea show salt sensitivity at high salinity levels but can withstand salt stress up to 4dSm^{-1}. The chickpea growth is limited by osmotic stress under salinity. One of the major effects of salinity on chickpea was the reduction in plant growth in the form of plant height and root length, fresh and dry weight (Fig. 1,2,3). The reason for the reduction in plant height and biomass under osmotic stress conditions is that the plant cell is unable to take up water because of theless osmotic potential of soil present around the root cell, which results in reduced metabolic activities related to growth and photosynthesis (FilSantos Filho *et al.*, 2022). The reduction in growth is also attributed to the inhibition of cell elongation under salt stress. The result was consistent with the previous study under salinity stress in *Vigna angularis* (Al-Mushhin, 2022) and chickpea (Atieno et al., 2017; Abd-Allaet al., 2019).The foliar application of spermidine reduced the osmotic effect under salt stress in chickpea genotypes. Spd regulates the accumulation of various osmolytes in the plant cell cytoplasm under stress condition. The accumulation of osmolytes in the cytoplasm enhances the water uptake through roots and reduces the osmotic stress created by salinity (Santos Filho*et al.*, 2022). The results are in agreement with Hai *et al.*(2022) in oat, Raziq *et al.* (2022) in tomato, Saleethong*et al.*(2011) in *Oryza sati-*

va under salinity.

Chlorophyll is an important photosynthetic pigment present in chloroplast. In the present experiment, the total chlorophyll content in the leaf of chickpea genotypes reduced (Fig. 4A) significantly under salt stress, which is in agreement with the previous results in chickpea (Garg and Singla *et al.*, 2009; Singh *et al.*, 2018; Dadasoglu*et al.*, 2022).The reduction in chlorophyll molecules is attributed to the oxidative stress generated under salt stress, which damages the membrane structure and alters is attributed to the oxidative stress generated under salt stress, which damages the membrane structure and alters its permeability. The increase in membrane permeability led to the accumulation of Na$^+$ ion in the cell, which damages the chlorophyll structure by replacing the Mg^{2+} ion present in chlorophyll structure by Na$^+$ ion. This phenomenon results in the weakening of the pigment-protein-lipid complex. The activation of chlorophyllase enzyme activity is also attributed to chlorophyll reduction under salt stress (Nahar *et al.*, 2016). Foliar application of spermidine enhances the chlorophyll molecules. Spermidine acts as an antioxidant, preventing the cell from oxidative damage through ROS. It alters the membrane's stability and permeability and damages chloroplast structure. The results show an increase in total chlorophyll in the leaves of chickpeas by spermidine (Hu *et al.*, 2016). Similar results were obtained in the case of Amin *et al*. (2013) in chickpea and Al-Mushhin (2022) in *Vigna angularis*. The present study showed a positive correlation between salt stress and pigment molecules (Flavonoid and anthocyanin). Salt stress significantly increased the flavonoid and anthocyanin content in all chickpea genotypes (Fig. 4C-D).

Flavonoid represents the main and most complex subgroup of polyphenols with a wide array of biological function. It is the most widely distributed secondary metabolite and plays role as signaling molecule, antioxidant and free radical scavengers. On the other hand, anthocyanin is color water soluble pigments belonging to the phenolic group (Borghesi *et al.,* 2011). They harvest the light energy for photosynthesis. These are non-enzymatic antioxidants that counter oxidative stress and detoxify the plant from ROS under salt stress. They accumulate in the plant tissue and inhibit lipid peroxidation (Zhou *et al.,* 2018). Spermidine application further increases flavonoid and anthocyanin content accumulation under salt stress. They act as the most crucial antioxidants for scavenging. Flavonoid inhibits the enzyme lipoxygenase, which converts the polyunsaturated fatty acid to oxygen-containing derivatives (Eryilmaz, 2006). The results are consistent with the finding of Dadasoglu *et al.* (2022) in chickpeas, Taibi *et al.* (2016) in *Phaseolus vulgaris*, Genzel *et al.* (2021) in capsicum, Kiani *et al.* (2021) in wheat, Jeon *et al.* (2020) in *Sorghum bicolor*. Nitrogen Balance Index represents the nitrogen nutritional status of the plant. In the present work salinity stress significantly reduces the NBI of chickpea genotypes (Fig. 4B). Nitrogen uptake and transport are sensitive to salinity. Under salt stress, the Cl^- ions have antagonistic effect on N_2. So, it reduced the N_2 uptake, leading to nitrogen deficiency (Ashraf *et al.,* 2018). NBI is the ratio of chlorophyll and flavonoid. So, under salt stress, nitrogen deficiency reduced the chlorophyll synthesis and increased the flavonoid content. Also the nitrogen uptake depends upon the mobility of NH_4 and NO_3, which initially dissolve in water and are then absorbed by the plant. Due to osmotic stress, the water uptake is reduced under salt stress and so N_2 uptake (Mansour *et al.,* 2000).

Nitrogen is an important nutrient that influences plant growth because it is the necessary component of various biomolecules such as amino acids, proteins, polyamines, etc., involved in various metabolic activities. Spermidine application enhances the NBI under salt stress in chickpea genotypes because spermidine is a nitrogen-containing compound and exogenous application of spermidine enhances its endogenous level, which then accumulates in plant cells and provides protein and nucleic acids, which in turn control many cellular activities in plants that depend on nitrogen. The result is in agreement with the finding of Fan *et al.* (2022) in wheat, Hasan and Miyake (2017) in *Zea mays*. Salinity also reduced the seed quality of chickpea crops (Fig. 5). It enhances ash and chloride ion content and decreases seed fiber and moisture content. The low moisture content in seed (Fig. 5A) might be due to less water uptake under stress conditions and, ultimately, less moisture content (Sarker *et al.,* 2018). Cl^- ion accumulation leads to increased seed ash content and decreased fiber content (Fig. 5B, C, and D). The Cl^- ion accumulation in seed might decrease the water uptake, inhibiting cell wall development and cell elongation, which is crucial for fiber content (Ali *et al.,* 2017). It also inhibits the uptake of essential nutrients such as calcium and potassium, leading to a decrease in fiber content of the seed. In the present study, the spermidine application reduced the Cl^- ions accumulation in the seed by reducing the osmotic and oxidative stress in chickpeas under salt stress, improving the seed quality and enhancing the moisture and fiber content in all chickpea genotypes under salt stress.

Conclusion

Chickpea is an important crop agronomically and ecologically, but being a salt-sensitive crop, its seed quality is greatly affected by salt stress. Salinity stress induced osmotic stress in the crop, reducing plant growth and development. It reduced the plant height and root length, ultimately reducing water and essential nutrient uptake. In the leaf, it inhibited chlorophyll synthesis, reducing photosynthesis and development. It also inhibited nitrogen uptake and accumulation, possibly because of Cl^{-1} ion accumulation in leaf. In response to the salt stress, chickpea genotypes show increased flavonoid content with high antioxidant properties. The exogenous application of spermidine enhanced the endogenous spermidine levels, which involve various protective responses to ameliorate the salt stress effects. It helps to mitigate salt stress damage on plant height, biomass, chlorophyll, nitrogen balance index, flavonoid, and anthocyanin content. Further, the application of spermidine improved the seed quality by maintaining more seed fiber and moisture content, thereby decreasing the ash and Cl^- ions under salt stress. Spermidine application of 1.0 mM enhanced tolerance against salt stress, especially in salt-sensitive chickpea genotypes. Among all the genotypes studied in the present study, HC 5 performed poor than the other chickpea genotypes under high salinity and not to be used in the highly salt-affected area for further breeding programs.

Conflict of interest
The authors declare that they have no conflict of interest.

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