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Mitigating salinity stress and improving cotton productivity through integrative use of gypsum and compost amendments with exogenous proline

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ABSTRACT

Abiotic constraints, such as salinity, significantly damage crop yields worldwide. Cotton, though moderately salt-tolerant, suffers from reduced growth and yield under saline-sodic soil conditions. Effective integrated mitigation strategies are crucial to address this challenge. Our study, conducted in Lodhran, Punjab, Pakistan, investigated six treatments using an integrated strategy such as gypsum, compost and exogenous proline combining effect in improving cotton productivity under salinity stress. We assessed plant growth, agronomic traits, physio-chemical parameters, cotton yield, and soil characteristics. Our experimental results showed that the combined application of amendments such as T5: gypsum + proline and T6: compost + proline gave better results as compared to individual treatments (T2, T3 & T4) over the control (T1). A significant improvement was observed in plant length and dry weights of shoot and root by 64 %, 81 % and 47 %, respectively under the effect of T5, also increased cotton yield up to 2 folds (888 kg) over control (324 kg ha^{-1}). Likewise, significant improvement in the plant physio-chemical parameters was recorded such as high activities of antioxidant enzymes and the maximum accumulation of malondialdehyde (MDA) content by 60–71 %, as well as reduction in the oxidative burst by 55–65 % after the integrated treatments (T5 & T6) as compared to salt-stressed plants (control). Likewise, contents of nutrients are improved in plants viz., N: 70; P: 61 %; K: 33 % and Mg: 86 % under the positive effect of gypsum + proline over control. Results of soil analysis showed that the soil was moderately saline-sodic. Furthermore, soil analysis revealed that there was a significant improvement in NPK, S and Mg content in the soil after treated salt-stressed soil with gypsum and compost (T2, T3, T5 and T6) while a significant reduction was observed in Ca (17 %) and Na content (28 %), as well as EC (dSm^{-1}) was decreased by 38 % and SAR (mmol/L)1/2 by 27 % under the effect of gypsum + proline over the control treatment. The outcomes of the current study reveal that the reclamation potential of gypsum and compost applied individually or together with exogenous proline improved plant growth, yield and plant defense system under salinity stress.

1. Introduction

Land degradation is considered a global challenge influencing all areas of human prosperity, reducing crop productivity with huge economic loss and putting the livelihood of farmers and food security at risk (AbdelRahman, 2023). The major causes of soil degradation include soil salinity, water logging, poor soil fertility, loss of soil cover, soil erosion, and depletion of essential minerals and organic matter contents (Cárceles Rodríguez et al., 2022).

Salt-stressed soils exhibited 1 billion hectares of land around the globe which is equivalent to 8.8 % of the planet (Ivushkin et al., 2019). Salinity is one of the major constraints responsible for land degradation,

Abbreviations: SOD, superoxide dismutase; CAT, Catalase; APX, ascorbate peroxidase; DHA, docosahexaenoic acid; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; O_2•−, superoxide.

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In Pakistan, 6.67 million hectares of agricultural land out of the total cultivated land area (20.36 mha) is salt-affected (Ilyas et al., 2020) and more than 70 % of the tube wells are responsible for pumping out brackish water in saline areas (Qureshi, 2020). High concentration of salts in the soil deteriorates soil fertility and its productivity leading to a drastic reduction in crop yield that is alarming for the national economy (Akhter et al., 2021; Ahanger et al., 2020; Ahmad et al., 2016).

Cotton (*Gossypium hirsutum* L.) often referred to as "white gold," is the primary cash crop of Pakistan that is being cultivated in arid and semi-arid regions. It holds significant importance in the agricultural sector and contributes to the livelihood improvement of farmers, particularly in the provinces of Punjab and Sindh (Kolachi et al., 2021). Salinity is a brutal stress for cotton crops, it can stand with a salinity threshold level of 7.7 dS m^{-1} at the seedling stage but high salinity levels severely affect crop growth, yield and fiber quality (Sharif et al., 2019). Salt-affected soil interferes with the ionic influx in the roots and disrupts their translocation toward the shoot causing severe limitations in the growth and plant physiological parameters (Nawaz et al., 2010).

To combat salt-stressed soil conditions, soil amendments should be used to improve soil health and fertility because nutrient imbalances are always been a problem in saline-sodic areas (Ramzani et al., 2017). Among the different forms of soil amendments, compost application is considered a sustainable approach due to its favorable long-lasting effect on soil health which improves soil fertility, organic matter, physical properties and water-holding capacity of the soil (Jan et al., 2020). Besides, some studies also showed that proline is an excellent enzyme that serves as an essential component in plants, playing a highly significant role when they are exposed to various stress conditions (Hayat et al., 2012). It was studied that when plants are grown under salt stress and supplied with the exogenous proline, plants enhance tolerance against stress by improving seed germination, photosynthesis, gas exchange, plant growth and biomass (El Moukhtari et al., 2020). Additionally, proline application also improves the antioxidant defense system of stressed plants and protects them from oxidative bursts and ROS production (Raza et al., 2023). Henceforth, beneficial outcomes of proline in plants can be further enhanced by utilizing it in combination with various types of soil amendments (i.e., gypsum and compost).

Keeping in view the importance of soil amendments and exogenous application of proline, The current study places a special emphasis on a sustainable plan by using an integrated approach of soil amendments gypsum and compost also with the exogenous proline on cotton crops growing in salt-stressed land. Therefore, the proposed hypothesis suggests that proline may enhance the activities of antioxidant enzymes. This enhancement could potentially reduce the detrimental effects of salinity on cotton plants by initiating various metabolic processes that support plant growth.

2. Experimental methodology

2.1. Study site and experimental design

The current field trial on cotton production was conducted in Sham Kuliyar Lodhran (29° 31′ 59.99″ N, 71° 37′ 59.99″ E) south Punjab, Pakistan (Fig. 1). Soil of the selected area was assessed before setting up the field trial, which was moderately saline-sodic having 4.25 dSm^{-1} EC and 16.6 $(mmol\ L^{-1})^{1/2}$ SAR. The amount of organic matter was 0.6 % with 8.6 soil pH while sodium (Na) and potassium (K) were 2.2 g kg^{-1} and 123 mg kg^{-1}, respectively. Among soil amendments, gypsum (1 % gypsum w/w) and compost (10 t/ha) were used to remediate soil and exogenous proline applications (3 foliar sprays at different intervals) were used to improve the plant defense system under stress. This experiment was piloted by WWF-Pakistan under the Better Cotton project. The experimental treatment design includes six different treatments (Table 1). Each treatment was replicated thrice and set in a randomized complete block design (RCBD).

Gypsum was applied after soil analysis and the gypsum rate was calculated by using the Schoonover method while compost was applied at the rate of 1 % (w/w). Only 3 sprays of proline were done during the crop cycle such as 1st: after 25 days of seed germination, 2nd: after 45 days of seed germination and 3rd: after 65 days of seed germination Each experimental treatment plot size was 4 × 4 m^2 and land preparation practices were done as per the requirement for cotton cultivation. Moreover, good-quality irrigation was applied throughout the crop period.

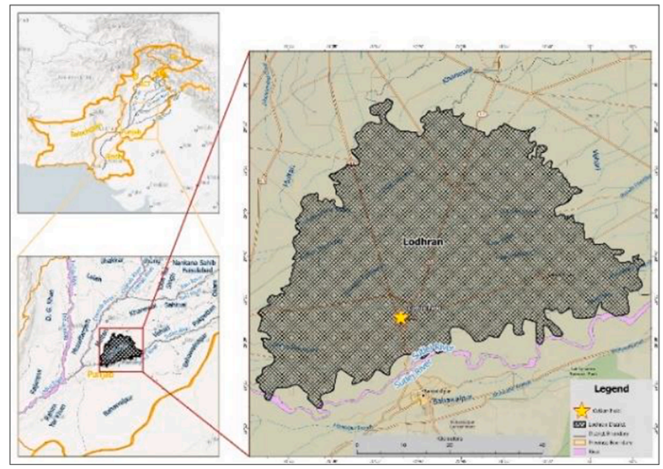

Fig. 1. GIS Map of the experimental site in Pakistan.

Table 1
Experiment treatment design in the cotton growing field.

Treatments	Description
T1	Salt-stressed soil without amendments is considered a control
T2	Gypsum (1 % w/w) is amended in salt-stressed soil as a sole treatment
T3	Compost (10 t/ha) is amended in salt-stressed soil as a sole treatment
T4	Exogenous proline application at three different intervals (3X)
T5	Gypsum soil amendment along with proline foliar application (3X)
T6	Compost soil amendment along with proline foliar application (3X)

2.2. Studied parameters

To evaluate the significant difference among the treatments (T1-T6) in salt-affected soil, the data were collected for the following studied parameters as chemical parameters of soil and plants.

Plant physiological assays such as antioxidants and ROS assays were done after 45 days of plant germination. While the data related to growth and yield perameters have gathered right after harvest (post-harvest).

2.3. Agronomic attributes

The data relating to growth and agronomical parameters included plant length (cm), fresh and dry weight (g) of shoot and root, relative water content (RWC), electrolyte leakage (EL), number of bolls per plant (n) and cotton yield (kg ha^{-1}) were recorded after cotton harvest (Awan et al., 2022; Awan et al., 2023).

2.4. Chemical analysis of soil and plant

Analysis related to the chemical parameters of soil and plants included the content of nitrogen (N), phosphorus (P), potassium (K), sodium (Na), calcium (Ca), and magnesium (Mg) were assessed in plants (g kg^{-1}) and soil (mg kg^{-1}). Furthermore, soil electrical conductivity (%) and sodium absorption ratio (SAR) were computed.

2.5. Physio-chemical analysis of plant

Cotton leaves were sampled after 45 days of plant germination from each treatment for the assessment of physiological and biochemical changes in the cotton plants grown under salt stress. These assays include chlorophyll content (*a* and *b*), for this cotton leaves (0.5 g) were homogenized in 80 % ethanol and centrifuged at 14000 rpm for 10 min to collect the clear extract. The absorbance for chlorophyll *a* (645 nm) and chlorophyll *b* (663 nm) were taken and calculated according to the formula given by Hartmut and Alan (1983).

The defense-related enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), docosahexaenoic acid (DHA) were estimated from fresh cotton leaf sample (0.5 g) and thoroughly homogenized in a potassium phosphate buffer (50 mM, 4 mL, neutral pH) having 100 mM KCl, 5 mM β-mercaptoethanol and ascorbic acid [(AsA), 1 mM, 10 % (w/v)]. The prepared mixture was centrifuged at 14000 rpm for 10 min and the supernatant was attained. Afterward, CAT, SOD, APX, and DHA activities were analyzed and the constituents of ROS i.e., malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide (O_2•−) (i.e., MDA, H_2O_2 and O_2•−) were also assessed in a separated clear supernatant by adopting various standard procedures (Rasool et al., 2021; Awan et al., 2022).

2.6. Data analysis

The recorded data were subjected to the analysis of variance (ANOVA) and means between treatments were compared by using the least significant difference (LSD) test at a level of $p \leq 0.05$ using the software Statistix v10.

3. Results

Our results showed that amongst all treatments, the combined application of amendments such as T5 [Gypsum (1 %) + Proline (3S)] and T6 [Compost (10 t/ha) + proline (3S)] exhibited better results of agronomic, physio-chemical and yield attributes as compared to the individual/sole treatment viz., T2 and T3, and the treatments with soil amendments were considerably different from the control treatment (T1: plants without any amendment).

3.1. Assessment of agronomical parameters

The plant height significantly increased by 64 % and 50 % in T5 and T6, respectively as compared to the control (Fig. 2). Likewise, shoot fresh and dry weight significantly improved by 49 and 81 %, respectively under the combined effect of gypsum (1 %) + proline (3S) in T5 followed by T6 that showed 39 % and 67 % improvement, in shoot fresh and dry weights, respectively as compared to the control plants. Similarly, root fresh and dry weight also significantly increased by 66 % and 47 %, respectively in T5 over the control followed by T6 which showed 51 % and 33 %. Furthermore, the number of branches of cotton plants also significantly increased by 70 % and 85 % under the combined effect of compost + proline (T6) and gypsum + proline (T5), respectively over the control plants.

3.2. Cotton yield

Cotton yield was assessed in all treatments which depicted the same trends as agronomic traits. The results exhibited a significant augmentation in the cotton yield when the plants were treated with individual and combined applications of gypsum and compost with proline. Our results revealed that T5 [Gypsum (1 %) + Proline (3S)] showed a pronounced effect with a significant improvement in cotton yield by 2 folds (2X) with the maximum recorded yield 888 kg ha^{-1} followed by T6 [Compost (10 t/ha) + Proline (3S)] with 783 kg ha^{-1} cotton yield as compared to T1 (324 kg ha^{-1}) (Fig. 3).

3.3. Physio-chemical analysis of plants

Generally, the application of amendments such as Gypsum (1 %) and

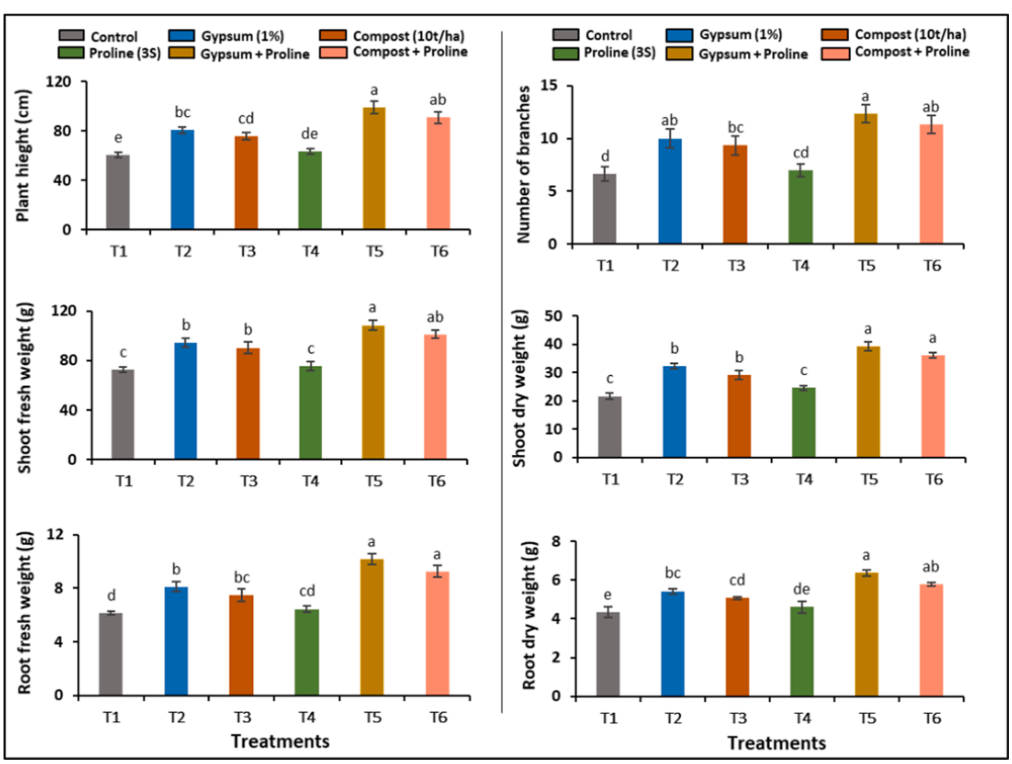

Fig. 2. Effect of individual and combined applications of soil amendments and proline spray on the agronomic parameters of cotton plants (height, number of branches, fresh and dry weights of shoot and root) gown in the salt-affected soil.

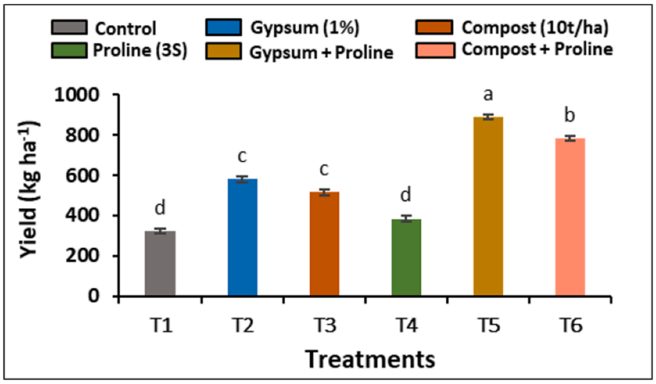

Fig. 3. Effect of individual and combined applications of soil amendments and proline spray on the cotton yield (kg ha^{-1}) gown in the salt-affected soil.

Compost (10 t/ha) in combination with Proline (3S) as T5 and T6 exhibited a significant (at $p \leq 0.05$) improvement in physio-chemical parameters of cotton plants have grown under salt stress field. Photosynthetic pigments such as chlorophyll *a* and b significantly improved under the integrated effect of gypsum + proline (T5) and compost + proline (T6). Results revealed that chlorophyll *a* and *b* were significantly improved by 44 % and 36 % in T5 and pronouncedly improved by 65 % and 57 % in T6 as compared to a control T1 (Fig. 4). The results related to plant biochemical parameters such as defense-related antioxidant enzymes exhibited the significant (at $p < 0.05$) improvement after the integrated treatments (T5 & T6) in salt affected soil (Fig. 4). The antioxidant enzymes activity was remarkably increased viz., SOD high by 4 times, APX and CAT increased by twofold and DHA by 71 % under the effect of treatment 6 in saline soil as compared to the control plants in T1. Results clearly showed that T6 was more prominent for the significant reduction of ROS over the control (T1). A remarkable reduction was noticed in the generation rate of MDA by 60–71 % and reactive oxygen species viz., H_2O_2 and $O_2^{\bullet-}$ by 65 % and 58 %, respectively in the combined treatments (T5 & T6) as related to the control (Fig. 4).

Likewise, current results revealed that the RWC of the plants significantly amplified by 21–25 % when plants were treated with combined applications of Gypsum (1 %) + Proline (3S) in T5 and Compost (10 t/ha) + Proline (3S) in T6 as compared to the control plant. Whereas the electrolyte leakage was significantly reduced by 28–34 % in the same combined treatments (T5 and T6) as compared to the control plants under the salt-affected soil (Fig. 5).

3.4. Chemical analysis of plants

Chemical analysis of the shoots of cotton plants showed that primary macronutrients such as nitrogen, phosphorus and potassium (NPK) content (g kg^{-1}) were significantly increased by 70 %, 61 % and 33 %, respectively in the treatment T5 combined application of Gypsum (1 %) + Proline (3S) in saline soil as compared to the control plants in T1. Likewise, magnesium content in the plants was also significantly augmented by 86 % followed by 70 % in T5 and T6, respectively. While calcium and sodium content in the shoots was significantly reduced ($p \leq 0.05$) by 40–50 % in both combined treatments (T5 and T6) over the control treatment (T1) (Fig. 6).

3.5. Chemical analysis of soil

Chemical analysis of soil samples showed that the availability of nitrogen and phosphorus content (mg kg^{-1}) were significantly increased in the treatments with the individual application of compost in treatment T3 by 10–15 % and also the combined treatments (T5 and T6) showed 10–19 % increase in nitrogen and phosphorous content over the soil sampled from the control treatment (Fig. 6). While, potassium content (mg kg^{-1}) was significantly increased in combined treatments of T5 and T6 by 18 % and 12 %, respectively as well as in T2 (individual application of gypsum) by 10 % as compared to the control. The soil analysis results displayed that macronutrients such as sodium (Na) and calcium (Ca) content were significantly reduced under the individual and combined effect of gypsum by 17 % and 28 % as compared to the

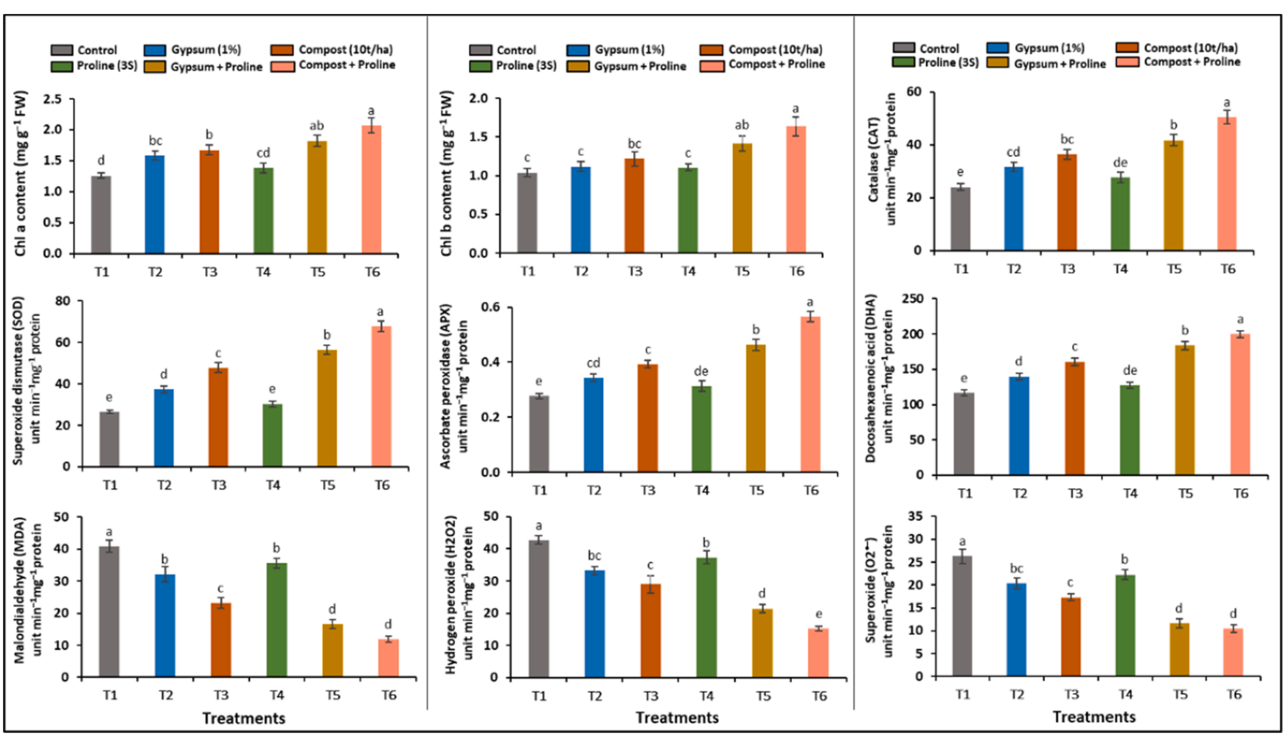

Fig. 4. Effect of individual and combined applications of soil amendments and proline spray on the physio-biochemical attributes of cotton plants gown in the salt-affected soil.

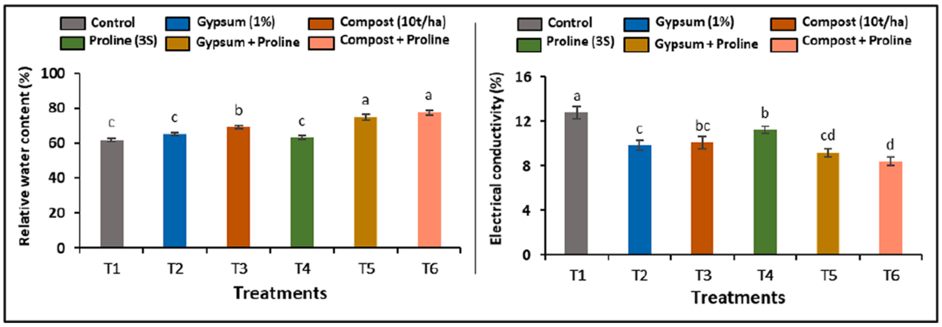

Fig. 5. Effect of individual and combined applications of soil amendments and proline spray on the relative water content and electrolyte leakage of the cotton plants gown in the salt-affected soil.

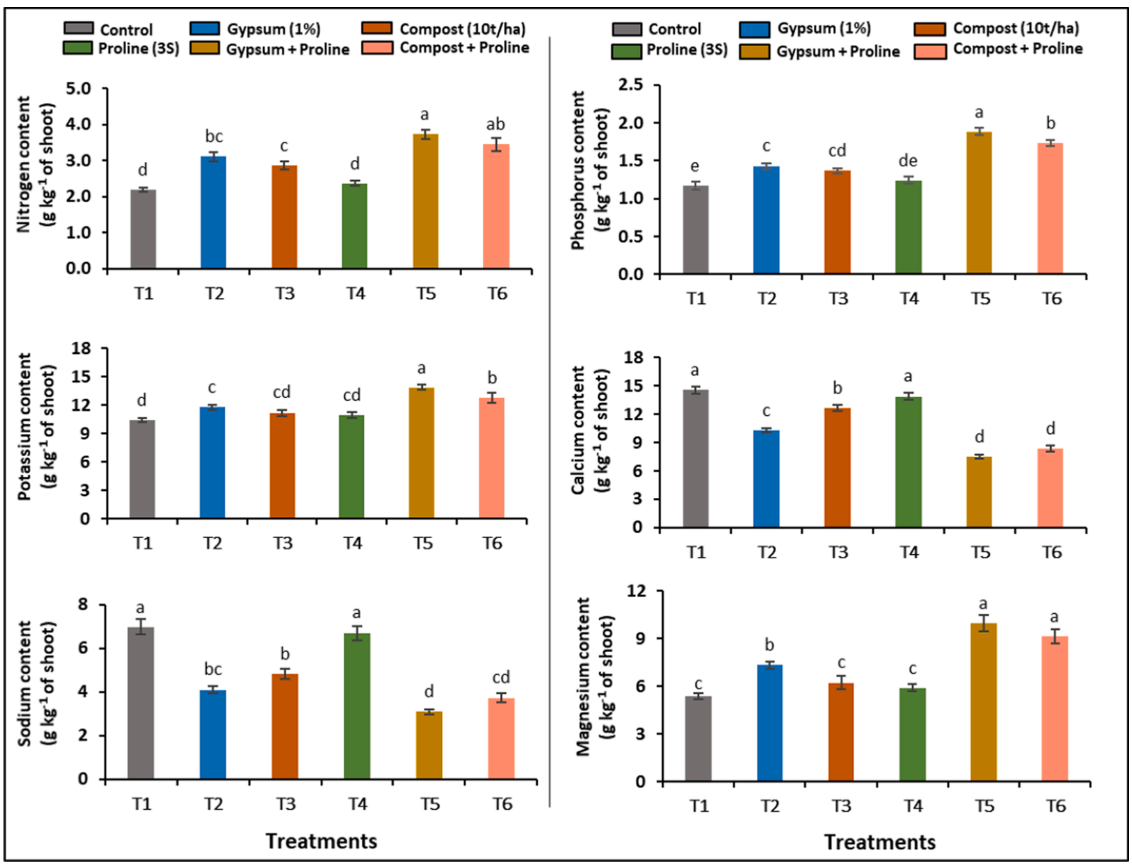

Fig. 6. Effect of individual and combined applications of soil amendments and proline spray on the chemical properties of the plants such as nitrogen, phosphorus, potassium calcium, sodium and magnesium content (g kg^{-1}).

control treatment. In contrast, magnesium (Mg) and sulfur (S) contents were significantly increased in the soil after the individual and combined applications of gypsum (T2 and T5) by 12–15 % as compared to the control treatment (T1). Moreover, the analysis related to soil sodium adsorption ratio and electrical conductivity (%) estimated a significant reduction of 25 % and 37 %, respectively after the single and combined application of gypsum and proline (T2 and T5) as compared to the soil in T1 (Fig. 7).

4. Discussion

Previous studies showed that several approaches can be integrated to minimize the negative effects of salinity and for the reclamation of salt-stress land (Ramzani et al., 2016; Jan et al., 2020; Nehela et al., 2021). Our results showed that salinity drastically reduced plant growth and yield of cotton in non-treated salt-stressed soil (control treatment: T1). The same results were reported previously with the reduction in biomass and growth of various economically important crops (cotton, wheat, maize, rice, and tomato) under the adverse of salinity (Gharsallah et al., 2016; Ramzani et al., 2016). Likewise, Munawar et al. (2021) and (Ahanger et al., 2020) also studied plant growth and plant development under salinity stress compromising plant height, fresh and dry weights, root-to-shoot ratio, physiological parameters and crop yield. Because salt imbalances such as sodium (Na^+), chloride (Cl^-), nitrate (NO^{3-}), calcium (Ca^{2+}), potassium (K^+), K^+/Na^+ and $Ca^{2+/}Na^+$ ratios in salt-affected soil ultimately reduce plant growth (Zhang et al., 2014). Our results indicated that the growth and yield of cotton showed significant improvement under the combined treatments of gypsum and compost with proline (T5 and T6) but the best findings were achieved with the application of gypsum + proline (T5) as compared to the control

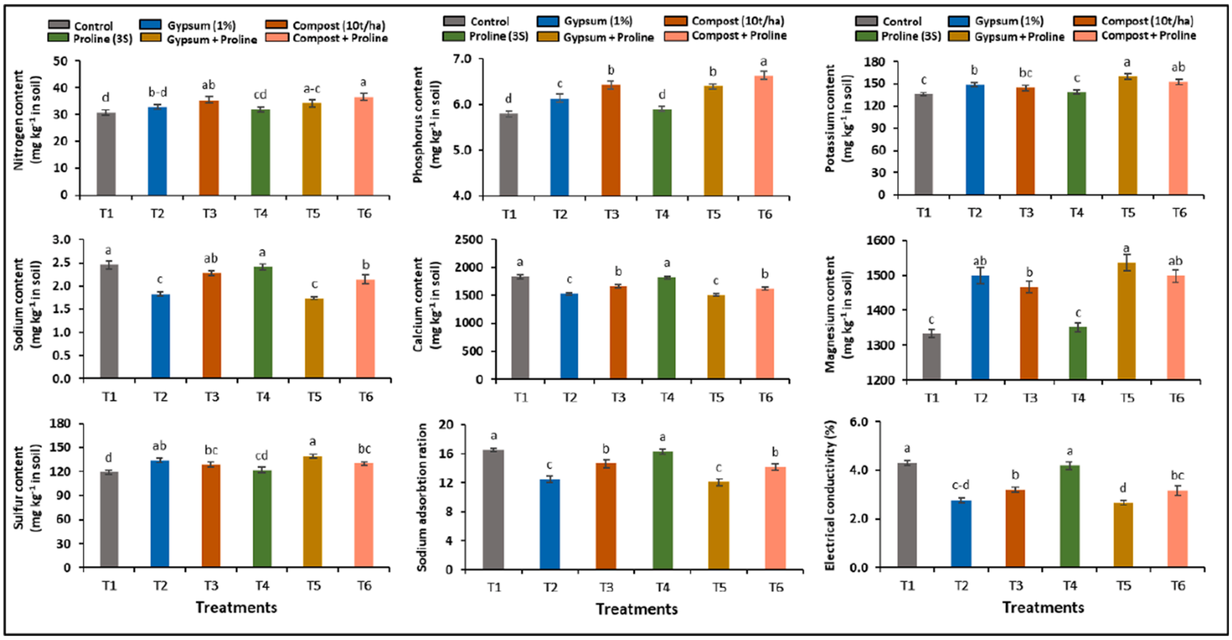

Fig. 7. Effect of individual and combined applications of soil amendments on the chemical properties of the soil such as nitrogen, phosphorus, potassium calcium, sodium, magnesium and sulfur content (mg kg^{-1}).

treatment. The application of gypsum is attributed to the increased production of essential substances like phytohormones, amino acids, glutathione, and osmoprotectants. Additionally, the use of gypsum leads to increased crop yield and improved nitrogen use efficiency across various crops (Bello et al., 2021a). Likewise, compost application enhances soil nutrient levels and organic matter and also increases the water-holding capacity and water infiltration rate of saline-sodic soils, while in plants, resulting in increased plant growth, and improved yield (Kolachi et al., 2021). Like soil amendments, the application of exogenous proline has been found to enhance plant growth and productivity by meditating plant tolerance under salt stress. Therefore, proline mitigates salinity stress by regulating nutrient balance and enzymatic activities and enhancing plant tolerance (Hamani et al., 2021).

Besides plant growth, cotton yield was significantly improved under the combined treatments of gypsum and compost with proline (T5 and T6). Hence, the main reason behind the better growth and yield may be soil reclamation strategy with the soil amendments (i.e., gypsum and compost) improves the physio-chemical properties of saline soil where the supply of more nutrients to plants was ensured (Ramzani et al., 2017; Jan et al., 2020; Kolachi et al., 2021).

Salinity stress interferes with the plant photosynthesis process, leading to reduced efficiency in converting sunlight into energy. Additionally, it disrupts ionic balance within the plant, causing an accumulation of harmful ions like sodium and chloride (Zhu et al., 2013). Moreover, salt stress induces oxidative injury to enzymes and other biomolecules, creating a harmful environment within the plant cells as a result of reactive oxygen species (ROS) generated in plant cells by the leakage of electrons into O_2 (Huang et al., 2019). Kesawat et al. (2023) reported that under salt-stressed conditions, reactive oxygen species were acting as signaling molecules, triggering signal transduction pathways and reducing CO_2 fixation due to low stomatal conductance. Furthermore, reactive oxygen species [viz., MDA, H_2O_2, and $O_2^{\bullet -}$] inflict irreversible cellular damage due to their potent oxidative properties, leading to alteration in plant morphology under stress (Frederickson Matika and Loake, 2014). Our results displayed that the activities of the defensive enzymes (SOD, CAT, APX and DHA) were elevated in the integrated treatments such as T5 and T6. Previous investigations showed that antioxidants play a crucial role in alleviating salinity stress by neutralizing harmful reactive oxygen species (ROS). By scavenging ROS, antioxidants protect the photosynthetic machinery from oxidative damage. This leads to improved photosynthesis, which is essential for maintaining plant growth and mitigating the negative impacts of salinity stress on crop productivity (Chawla et al., 2013). The increases in CAT, APX and DHA activities are adaptive traits in plants that help them overcome salt damage by reducing the toxic levels of hydrogen peroxide (H_2O_2), a reactive oxygen species that accumulates under salt stress conditions. The increase of these defensive enzymes was relative to the salt stress soil in control showed enhanced defense against ROS accumulation (Ramzani et al., 2017) because of the lower salinity stress in the integrated treatments.

Moreover, a significant enhancement in N, P and K content was estimated in plants grown in salt-stressed soil under the combined effects of gypsum and compost (T5 & T6). Ganjegunte et al. (2014) and Stavi et al. (2021) studied that salinity reduces nutrient concentration (especially NPK) in plants by creating an osmotic effect and causing deficiencies/toxicity issues in plants under salt-stressed conditions. Likewise, soil chemical analyses also showed that NPK contents were significantly high under the individual and integrated treatment of compost (T3 and T6) which was reported by Lawrence and Melgar (2023) that the use of compost increased the contents of organic matter (OM) and nitrogen in the soil which may improve the nutrients and water status in the salt-stressed soil. Post-harvest soil analysis indicated that compost treatment significantly reduced the electrical conductivity (EC), Ca, Na and sodium adsorption ratio (SAR) than that of the control soils (un-treated) and saturated the exchange complex with Ca^{2+} thus enabling better plant growth in salt-affected soils as studied by Chaganti et al. (2015). Results of soil analysis were in line with the previous studies which reported that organic matter (compost) in saline soils can accelerate Na^+ leaching, and decrease the ESP, EC, and Cl^- toxicity but it works slowly and less effectively as compared to gypsum treatment (Tejada et al., 2006; Bello et al., 2021b). Moreover, Abdel-Fattah et al. (2015) and Suleiman et al. (2021) also described that soil treatment with gypsum improves ionic balance more efficiently in sodic saline soil as gypsum will normally dissociate easily and remediate saline-sodic soil by lower the soil pH. Furthermore, gypsum treatment reclaims salinity leaching basic cations especially by replacing Na with Ca, and improving soil structure by flocculation and maintaining nutrient balance (Capaldi et al., 2015; Ahmed et al., 2016).

5. Conclusion

The research findings concluded that the integrated application of gypsum, compost and foliar application of proline sprays (applied during vegetative, flowering and fruit formation stages) is the most effective practice for enhancing cotton productivity in saline soils and remediate salt-affected areas. Among the six treatments, T5: gypsum + proline significantly improved all morphological and chemical attributes of the cotton plants (including growth, plant physiology, plant chemistry and crop yield) as compared to the control. Additionally, a notable reduction in reactive oxygen species (ROS) was observed, along with a maximum increase in the activity of antioxidant enzymes in cotton plants grown in salt-stressed soil treated with the integrated application of gypsum and compost with proline (T5 and T6). This integrated approach is eco-friendly, cost-effective and easily adoptable by farmers, who can recoup their investment within a single cotton growing season. By raising awareness and promoting this integrated strategy, cotton farmers can effectively manage salinity stress and improve crop productivity in affected regions.

CRediT authorship contribution statement

Zoia Arshad Awan: Writing – review & editing, Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Pia Muhammad Adnan Ramzani:** Writing – original draft, Visualization, Supervision, Data curation, Conceptualization. **Liaqat Ali Khan:** Supervision, Project administration, Conceptualization. **Asad Imran:** Writing – review & editing, Project administration, Conceptualization. **Sheza Ayaz Khilji:** Writing – review & editing, Conceptualization. **Abdel-Rhman Z. Gaafar:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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