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Effects of Salinity Stress on Growth and Biochemical Responses of Different Soybean Varieties

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Abstract

Soybean is a major source of plant protein and is also affected by the salt stress. In the present study, an attempt was made to assess the impact of salinity stress on different varieties of soybean viz. CO1, CO2, and CO (soy) 3 at different salt treatments (control, 50, 100, 150 and 200 mM). Increasing salt concentration resulted in a decrease in growth parameters, viz., shoot length, root length, fresh and dry weight of plants, and the number of leaves and branches per plant. CO (soy) 3 showed comparatively better tolerance variety and less damage compared to CO2 and CO1. CO (soy) 3 demonstrated the highest chlorophyll a (0.450 mg g⁻¹ FW), chlorophyll b (0.252 mg g⁻¹ FW) and total chlorophyll (0.702 mg g⁻¹ FW) even at 200 mM. Electrolyte leakage and malondialdehyde content increased with increasing salt stress in all varieties, indicating membrane damage and lipid peroxidation. These results demonstrate that CO (soy) 3 responded to salinity stress through enhanced osmolyte and antioxidant accumulation. EL (79%) and MDA content (43.40 nmol g⁻¹ FW) reached their highest level at 200 mM salt stress in the CO (soy) 3 variety. Proline content increased with salt stress, reaching its highest level at 200 mM (0.489 mg g⁻¹ fresh weight) in CO (soy) 3. Antioxidants, such as phenols (10.5 mg GAE g⁻¹ DW) and flavonoids (77.33 mg QE g⁻¹ DW) were also higher in CO (soy) 3 compared to CO2 and CO1.

Keywords

Salinity, Soybean, CO (soy) 3, MDA, Antioxidants, Proline

Introduction

Salinity stress is one of the main abiotic stresses that affects crop plants and reduces productivity globally. Approximately 1125 million hectares, or 6% of the world's land surface and 20% of irrigated land, are affected by this salinity [1]. Since soil salinity affects crop yields, salinization is a global problem that lowers productivity in areas with high salt concentrations [2]. It has become a major ecological concern on a worldwide scale. It has a direct effect on crop health, damages land structure, interferes with natural farming methods, and results in a low and irregular crop production [3]. We must put into practice efficient methods to improve saline soil management and reduce agricultural yield in order to alleviate the limitations that salt stress places on ecological growth and crop production [4]. High soil salt concentrations have an adverse effect on agricultural plant growth and yield losses because of the toxicity of the NaCl concentration and the decrease in soil moisture availability [5].

Plants under salt stress exhibit a wide range of reactions at different levels of their structural organization [6]. Additionally, salt stress can change how electrons are transported and how active photosystem II is, which oxidizes water molecules to create electrons [7]. High NaCl concentrations endanger a variety of physiological and biochemical processes, including protein synthesis, photosynthesis, water status, leaf area, lipid metabolism, and membrane integrity. Salt-related problems have been observed throughout the entire plant life cycle [8]. Plants respond to salt stress in a variety of ways at various levels of structure. Osmotic stress, ionic imbalance/toxicity, and oxidative stress are the three main limitations that salinity places on plants [6]. Osmotic stress affects cell expansion and growth by lowering the external water potential and reducing plants' ability to maintain their water content. Additionally, it causes stomata to close, which lowers the plant's capacity to absorb CO₂. Ionic stress is brought on by an overabundance of toxic salt ions, primarily Na⁺ and Cl⁻, which interfere with plants' regular metabolic processes. The build-up of toxic Na⁺ and Cl⁻ also causes a significant drop in cytosolic K⁺, which has a variety of effects on a cell's viability and metabolic activity [9]. Although Cl⁻ toxicity is not as severe as that of Na⁺, it can nonetheless seriously disrupt a variety of physiological and biochemical processes in susceptible species [10].

The domestication of soybeans (*Glycine max*) from wild soybeans (*Glycine soja*) occurred in China between 6000 and 9000 years ago [11]. It is today one of the most extensively grown and used food and economic crops in the world, providing more than two-thirds of the world's protein needs and a quarter of the edible oil market. Since

China is one of the world's largest consumers of soybeans and its import volume is increasing annually, it is imperative that it increase its domestic soybean output [12]. Soybeans (*Glycine max L.*), a member of the Fabaceae family, are one of the most important lowland cash crops in terms of both economic and nutritional value. It is a widely grown food and feed crop in Ethiopia for a variety of uses, including human consumption, animal feed, profit, and the production of fish and poultry meal. It is also a great intercrop, according to Alebel et al. [13] and Kokebie et al. [14]. Because of their nutritional worth and capacity to produce oil, soybeans are widely produced [15]. Despite their high degree of adaptability, soybean plants are vulnerable to salt and can lose as much as 40% of their production, photosynthesis, growth after outgrowth, and seed germination [16]. Ionic toxicity and osmotic stress in soybean plants are primarily caused by high salinity [17]. Previous studies have showed that NaCl adversely affects soybean growth and physiology; however, most studies have been conducted using soybean varieties other than CO1, CO2, and CO (soy)3. Comparative information on the salinity responses of these varieties remains limited. Furthermore, some studies have simultaneously evaluated growth performance, chlorophyll pigments, electrolyte leakage, malondialdehyde (MDA), proline accumulation, total phenol content, and flavonoid content under salt levels at the vegetative stage. Therefore, the present study investigated to provide a comprehensive assessment of the morphological, physiological and biochemical responses of soybean varieties CO1, CO2, and CO (soy) 3 to salinity stress and to identify attributes associated with salinity condition.

Results

Morphological parameters

Plant shoot length

The plant shoot length of different soybean varieties varied across different salt concentration i.e., (control, 50, 100, 150, and 200 mM). Shoot length drastically decreased with increasing salt concentration up to 200 mM (Figure 1). Out of the three varieties, CO (soy) 3 variety showed better shoot length response than CO1 and CO2 varieties i.e., (29 cm plant⁻¹, 18 cm plant⁻¹, and 21 cm plant⁻¹), respectively.

Plant root length

The root length of the different soybean varieties varied across different NaCl concentration i.e., (control, 50, 100, 150, and 200 mM). Figure 2 showed that the root length drastically decreased with increasing in salt concentration up to 200mM. Out of three varieties, the CO (soy) 3 variety showed better growth performance than the CO1 and CO2 varieties, i.e., (11 cm plant⁻¹, 5 cm plant⁻¹, and 7 cm plant⁻¹), respectively.

Number of leaves

The number of leaves in the different soybean varieties varied across different salt concentration i.e., (control, 50, 100, 150, and 200 mM). Figure 3 showed that the number of leaves drastically decreased with increasing salt concentration up to 200mM. Out of three varieties, CO (soy) 3 variety showed better growth response than the CO1 and CO2 varieties, i.e., 5 plant⁻¹, 2 plant⁻¹, and 3 plant⁻¹, respectively.

Number of branches

The number of branches in different soybean varieties varied across different salt concentration i.e. (Control, 50, 100, 150, and 200 mM). Figure 4 showed that the number of branches drastically decreased with increasing in salt concentration up to 200 mM. Out of three varieties, CO (soy) 3 variety showed better growth than CO1 and CO2 varieties, i.e., 3 plant⁻¹, 1 plant⁻¹, and 2 plant⁻¹, respectively.

Shoot fresh and dry weight

Shoot fresh and dry weight increased with plant growth, and the highest values were observed at 45 DAS. The different varieties of soybean varied across different salt concentration i.e., (control, 50, 100, 150, and 200 mM). Figure 5&6 showed shoot fresh and dry weight drastically decreased with increasing in salt concentration up to 200 mM out of three varieties, CO (soy) 3 variety showed better growth than CO1 and CO2 varieties, i.e., 3.03g plant⁻¹, 2.39 g plant⁻¹, and 2.52 g plant⁻¹ for shoot fresh weight and 0.76 g plant⁻¹, 0.52 g plant⁻¹, and 0.67 g plant⁻¹ for dry weight of shoot, respectively.

Root fresh and dry weight

The Root fresh and dry weight increased with the plant growth, and the highest values were observed at 45 DAS (Figures 7 & 8). The soybean varieties revealed variation under different salt concentrations (control, 50, 100, 150, and 200 mM). Therefore, the root fresh and dry weight decreased with increasing salinity, with the greatest reduction observed at 200 mM NaCl. Among the three varieties, CO (Soy) 3 variety was showed better results than CO 1 and CO 2. At 200 mM NaCl, root fresh weight was 0.62, 0.47, and 0.56 g plant⁻¹ in CO (Soy) 3, CO 1, and CO 2, respectively, while root dry weight was 0.15, 0.11, and 0.14 g plant⁻¹, respectively.

Physiological traits

Determination of chlorophyll pigments

Chlorophyll content (chl a, chl b, and total chl) was measured in all soybean varieties under different NaCl concentrations (Figures 9-11). Chlorophyll content decreased with increasing salinity in all varieties. Under control conditions, CO (Soy) 3 expressed higher chlorophyll content than CO 1 and CO 2. At 200 mM NaCl, CO (Soy) 3 was higher chl a, chl b, and total chl contents (0.450, 0.252, and 0.702 mg g⁻¹ FW, respectively) compared with CO 1 (0.413, 0.223, and 0.636 mg g⁻¹ FW, respectively) and CO 2 (0.375, 0.212, and 0.587 mg g⁻¹ FW, respectively). These results demonstrate that salinity stress significantly reduced chlorophyll content in soybean plants.

Electrolytic leakages

Electrolyte leakage increased with increasing NaCl concentration in different soybean varieties and was highest at 200 mM NaCl. As shown in Figure 12, all salinity treatments resulted greater electrolyte leakage than the control. At 200 mM NaCl, electrolyte leakage was 79% in CO (Soy) 3, 69.33% in CO 1, and 74% in CO 2, respectively.

Biochemical parameters

Determination of lipid peroxidation malondialdehyde (MDA)

Malondialdehyde (MDA), a marker of lipid peroxidation and membrane damage, was measured to assess the degree of membrane lipid peroxidation under salt stress. Figure 13 illustrates the effect of salinity on MDA content in the three soybean varieties. The results showed that the MDA content increased with increasing NaCl concentration in all soybean varieties. The highest MDA content was recorded at 200 mM NaCl, whereas the lowest values were observed in the control treatment. At 200 mM NaCl, CO (Soy) 3 recorded the highest MDA content (43.40 nmol g⁻¹ FW), followed by CO 2 (41.38 nmol g⁻¹ FW) and CO 1 (38.80 nmol g⁻¹ FW) varieties, respectively.

Determination of proline content

Osmolytes like Proline content increased with increasing NaCl concentration in all soybean varieties. Among the three varieties, CO (Soy) 3 recorded the highest proline content at 200 mM NaCl (0.489 mg g⁻¹ FW), followed by CO 2 (0.452 mg g⁻¹ FW) and CO 1 (0.392 mg g⁻¹ FW) (Figure 14). The enhanced accumulation of proline in CO (Soy) 3 indicates a better osmotic adjustment response under salt stress.

Determination of Total phenol content

Total phenolic content increased with increasing salinity in all soybean varieties. The highest phenolic content was observed at 200 mM NaCl (Figure 15). Among the varieties, CO (Soy) 3 exhibited the highest total phenolic content (10.5 mg GAE g⁻¹ DW), whereas CO 2 and CO 1 recorded 8.51 and 8.33 mg GAE g⁻¹ DW, respectively.

Determination of Total flavonoid content

Total flavonoid content increased with increasing NaCl concentration in all soybean varieties compared with the control treatment. As shown in Figure 16, all salinity treatments exhibited higher flavonoid content than the control. At 200 mM NaCl, CO (Soy) 3 recorded the highest flavonoid content (77.33 mg QE g⁻¹ DW), followed by CO 2 (67.00 mg QE g⁻¹ DW) and CO 1 (57.33 mg QE g⁻¹ DW).

Discussion

Increasing NaCl salinity decreased the growth parameter, via shoot and root length, on the 45th day of all varieties of soybeans. The similar findings coincided with those of other researchers, such as *Lactuca sativa* L. [18] *Glycine max* L. [19, 20]. According to Zorb et al. [21], crop plants' shoot and root lengths are negatively impacted by salinity. The shoot and root length of soybean cultivars were significantly decreased by 200 mM NaCl, according to our findings (Figure 1 & 2). The findings of Sivakumar and Priya (2017), who noted that salinity decreased black gram plant height, are similar to ours.

The reduction in biomass induced by salinity could be attributed to a lack of energy for growth, as the majority of the energy is consumed for salt transport and regeneration/repair of salt-damaged tissues. It could also be attributed to decreased water absorption due to osmotic pressure, as well as nutritional deficiency caused by ionic imbalance [22]. Loss of turgidity in cells reduces the efficacy of physio-biochemical processes, since enzymes generally act in the aqueous phase [23].

The photosynthetic efficiency of the soybean varieties gradually decreased, the chlorophyll content decreased with increasing concentration up to 200mM. This result matches those of other varieties, such as *Ocimum basilicum* L. [24], Rice [25], and Rose [26]. Wang et al. [27] found that grana degradation resulted in significant reductions in photosynthetic indices, chlorophyll amount, and chloroplast structural degradation. Betzen et al. [28] and Wani et al. [29] found that high salinity reduces PS II activity by destroying and disrupting the PS II reaction centres and oxygen-evolving complexes in wheat and mustard, respectively. Salinity reduced photosynthesis and stomatal conductance by damaging the ultrastructure and synthesis of chlorophyll [30].

The estimation of the oxidative parameters, including electrolytic leakage and MDA, in response to the stressor, e.g., salt, can help to determine the extent of the membrane damage and loss of integrity. In the current experiment, the values of oxidative parameters (EL and MDA) increased greatly with the salinity and higher values of these parameters were found at the highest concentration of NaCl. The instigating influence of the effect of Salinity on deterioration of the membrane can be the reason behind the increasing level of electrolytic leaking in salt-stressed plants. In CO (soy) 3, electrolytic leakage in leaf could be observed to a greater extent. This implies that salt induced oxidative stress that wrecked the membranes of the plant known as "CO (soy) 3" than it did in the other plants. Because of their great ion loss through leaf tissue and vulnerability to salty environments, this vegetation could have immature defence strategies.

Our results, EL and MDA concentrations were shown to rise with the salt concentrations are in agreement with the previous reports on lettuce and spinach [31], mung beans [32], water drop wort [33], rice [34], and *Punica granatum* L. [35]. The CO1 type compared with the other types possessed the lowest EL and MDA concentrations at all the salt concentrations. This results recorded that CO1 expressed lower membrane damage under salt stress compared with the other varieties, as reflected by its consistently lower EL and MDA levels was already reported by Momeni et al. [36] and Saxena and Partee [37] who found that salt-tolerant plants show lower EL and MDA levels than salt-susceptible plants. Although CO (soy) 3 exhibited significantly higher proline, phenol, and flavonoid contents, it also showed higher EL and MDA levels, indicating higher membrane damage under salt stress. Therefore, these antioxidant responses may represent a stress-response mechanism of superior salt tolerance.

Saline stressed plants can store up high concentrations of proline and other compatible osmolytes. In the present investigation, we found that salinity considerably raised the proline concentration in all soybean types as compared to the control treatment. Our findings are in line with previous studies that found proline content increased with salinity and showed a promising increase at the highest salt concentration [33]. Several agricultural crops have also been reported to increase the content of proline with increasing salt concentration in the crops such as alfalfa [38], lettuce [39], soybean [20], and wheat [40]. It has also been noted that proline levels are increased under salinity stress development in *Ocimum basilicum* [24].

We have reported proline accumulation significantly high in the case of "CO (soy)3" in all concentration of salt whereas in the case of CO1, the accumulation of proline was relatively low. The study suggest that plants can adapt to stresses such as salt through the synthesis of secondary metabolites such as phenolic compounds and flavonoids [41]. This was supported in our results which showed that the higher the salt levels, the higher was the phenol and flavonoid contents of all varieties of soybean. We demonstrate that at all the NaCl concentration levels,

CO (soy) 3 accumulated better phenol and flavonoid contents than the other varieties. This manifest activation of antioxidant defense mechanisms in response to salinity stress. However, antioxidant accumulation alone should not be considered adequate evidence of better salt tolerance without support from membrane stability and growth-related attributes. These findings observed that antioxidant accumulation is an important component of the stress response under salt stress; however, it should be explicate together with growth and membrane stability traits while evaluating salt tolerance.

The findings obtained in the present study demonstrated significant variation among soybean varieties in their responses to salt stress. The soybean varieties showed physiological and biochemical responses when exposed to salinity stress. Compared with the other varieties, CO (soy) 3 variety retained higher chlorophyll pigments and showed better accumulation of proline, phenolic compounds, and flavonoids under saline conditions. Nevertheless, this varieties also exhibited elevated EL and MDA contents, suggesting a higher extent of membrane damage under salt stress. However, CO (soy)3 variety also showed higher EL and MDA values, indicating increased membrane damage under high salt conditions. These results recorded that CO (soy) 3 responded to salinity stress through enhanced osmolyte and antioxidant accumulation. However, the significantly increase in EL and MDA indicates that these responses were not sufficient to completely protect membrane damage under salt conditions. Therefore, the evaluation of salt tolerance should be based on the integrated response of growth, photosynthetic pigments, membrane stability, and osmolyte accumulation rather than on a single parameter alone. depends on the parameters evaluated in the present study, no single variety could be conclusively identified as the most salt tolerant because different traits showed contrasting responses under salinity stress Further studies incorporating ion homeostasis (Na^+/K^+ balance), and plant water level are required to validate varietal differences in salt tolerance.

Methods

Soil preparation

The farmyard Manure and soil were gathered at Annamalai University's Botanical Garden, in the Department of Botany. A portable pH meter revealed that the soil had a sandy loam texture and a pH of 7.8. Using an EC meter in the lab, the soil sample's electrical conductivity (EC) measured 0.86 dS m^{-1} . After that, the soil composite was put inside a polythene bag that was the same size, 25 cm in height and 13 cm in breadth.

Plant material and experimental design

Three types of soybean seeds (CO1, CO2, and CO (soy) 3) were acquired from the Coimbatore Agriculture University. The seeds underwent several rinses with distilled water after being surface sterilized for 15 minutes with 0.2% mercury chloride (HgCl_2). The experimental was conducted at the Botanical Garden, Department of Botany, Annamalai University. The experiment was arranged in three soybean varieties i.e., (CO1, CO2, and CO (soy) 3), five salinity treatments (control, 50, 100, 150, and 200 mM NaCl), and three replicates. Each replicate consisted of one independent grow bag and was considered the experimental unit.

Salt treatment and growth conditions

Each variety's ten consistent, healthy seeds were planted in a grow bag with a 1:2:1 mixture of sand, red soil, and farmyard manure, and they were watered every two days with tap water. Germination resulted in consistently 5 seedlings per grow bag. Thinning was performed, and all emerged seedlings were maintained throughout the experimental duration. Plants were treated with different NaCl concentrations (control, 50, 100, 150, and 200 mM NaCl) two weeks after sowing. Salt treatments were applied four times at weekly intervals using 250 mL of the respective NaCl solution per each grow bag. Control plants received an equal volume of tap water. All morphological and biochemical parameters were measured using samples collected from the three replicates, and the resulting data were used for statistical analysis. Morphological and biochemical observations were recorded on the 45th day after sowing (DAS).

Morphological traits

Morphological traits were measured on the forty-fifth day, including the length of the plant's root and shoot, the number of leaves and branches, and the fresh and dry weight of the root and shoot. The length of the root and shoot was measured with a ruler and noted in millimetres. The dry weight of the shoot and root was measured using a computerized electrical scale after the plant tissues were dried for 24 hours at 80°C in a hot air oven.

Measurement of physiological parameters

Determination of chlorophyll pigments

The photosynthetic pigment content of the leaves, including the amounts of chlorophyll a, chlorophyll b, and total chlorophyll, was ascertained using the procedure described by Arnon [42]. Ten millilitres of 80% acetone were used to extract 0.5 grams of fresh leaves after they had been weighed. A UV-VIS spectrophotometer was used to detect the absorbance (A) at 645 and 663 nm following centrifugation. Lastly, the following formula (mg g^{-1} FW) was used to assess the concentration of chlorophyll pigment.

$$\text{Chlorophyll a} = (0.0127) \times (O.D. 663) - (0.00269) \times (O.D. 645)$$

$$\text{Chlorophyll b} = (0.0229) \times (O.D. 645) - (0.00468) \times (O.D. 663)$$

Electrolyte leakage

The Dionisio-Sese and Tobita [43] approach was used with slight modifications to quantify the electrolyte leakage (EL) of leaves, a measure of the degree of membrane damage. Three randomly chosen leaf samples from each plant were punctured with a 1 cm stainless steel cork borer to create leaf disks. A test tube filled with these leaf disks (two for each leaf) was rapidly filled with ten milliliters of distilled water, and the test tube was agitated for five hours. A digital conductivity meter was used to measure the first electrical conductivity (EC1) following five hours of incubation. The leaf disk-containing solution was immersed in a water bath that had been heated to 100°C for 30 minutes. After that, it was let to cool for ten minutes in an ice box. Lastly, the electrical conductivity of this solution (EC2) was measured in order to determine the EL of the leaf leachates:

$$\text{Electrolyte leakage (\%)} = \frac{EC_1}{EC_2} \times 100$$

Determination of lipid peroxidation malondialdehyde (MDA)

The membrane peroxidation of lipids was detected by measuring malondialdehyde (MDA), the breakdown product of the polyunsaturated fatty acid (PUFA) component of the membrane under stress. MDA, the by-product of lipid peroxidation, was measured using the thiobarbituric acid (TBA) method of Heath and Packer [44], with a few minor adjustments. From each plants, three fresh leaf samples were taken, then using a mortar and pestle, 0.5 g of fresh leaf tissues composites were extracted using 10 mL of a 0.1% (w/v) trichloroacetic acid (TCA) solution. After centrifuging the mixture for 30 minutes at 8,000 rpm, one millilitre of the supernatant was combined with four milliliters of 20% TCA that contained 0.5% TBA. After 30 minutes of heating the mixture to 100°C in a water bath, it was cooled for 5 minutes in an ice bath. Lastly, the specific and non-specific absorbance values were measured at 532 nm and 600 nm, respectively, using 20% TCA and 0.5% TBA as a blank. The MDA concentration was calculated using Lambert's extinction coefficient and expressed as nmol g⁻¹ FW.

Measurement of Biochemical parameters

Determination of proline content

Bates et al. [45] developed the acid-ninhydrin method; it was used to determine the proline concentration of leaves. D-proline was applied as a reference. Five millilitres of 3% 5-sulfosalicylic acid were added to each replicate after around 250 mg of fresh leaf tissues composite had been extracted using a mortar and pestle. The mixture was then centrifuged for 30 minutes at 8,000 rpm. After adding one millilitre of acid ninhydrin solution, one millilitre of glacial acetic acid, and one millilitre of supernatant, the mixture was incubated in a water bath at 100°C for an hour. The absorbance was measured at 520 nm using toluene as a blank after the mixture was vortexed with two millilitres of toluene for five minutes. Using the proline standard curve, the proline content was determined and expressed in milligrams per gram of fresh weight. The results were calculated using the formula.

$$\text{Proline} = \frac{O.D}{0.1} \times \frac{6.15 \times 4 \times 2}{1000} \times 100$$

Determination of total phenol content

The Folin-Ciocalteu (FC) method was used to calculate the total phenol content (TPC) [46]. Using a computerized analytical precision balance, 250 milligrams of dried leaf composite were extracted using 10 millilitres of distilled water. One millilitre of the extracts, five millilitres of distilled water, and one millilitre of FC reagent were then added to a 10-millilitre volumetric flask. The mixture was allowed to stand at room temperature for 10 minutes. The reaction mixture was incubated after 1 millilitre of 7.5% Na₂CO₃ was added to the reaction mixture after an hour. Finally, the absorbance of this blue complex at 750 nm was determined with the help of the blank that contained a

mixture of 7.5% Na₂CO₃ and diluted FC reagent. The calibration curve of the gallic acid was used to approximate the TPC, which was in turn converted to mg GAE g⁻¹ DW.

Determination of total flavonoid content

The flavonoid content of the leaves was determined using a slightly modified aluminium chloride colorimetric method by Chia-Chi et al. [47]. The sample of 0.3 g of dry leaf was used and 10 mL of 70 percent ethanol was added. Based on this extract, 1 mL of this extract was added to a 10 mL volumetric flask holding 300 µL of 10% AlCl₃ and 300 µL of 5% NaNO₂. The mixture was left to incubate for 6 minutes and 1 M NaOH was added at the end of the incubation period and the solution was again left to incubate for 30 minutes at room temperature. The resulting yellow-brown complex was measured for absorbance at 510 nm using a UV–visible spectrophotometer. The total flavonoid content was calculated using a quercetin standard curve, and the results were expressed as mg QE g⁻¹ DW.

Statistical analysis

The experimental data were analysed using two-way ANOVA to assess the effects of variety, salinity, and their interaction. When significant differences were detected, Tukey's multiple comparison test was used for post-hoc analysis. Statistical analyses were performed using GraphPad Prism version 8. Differences were considered statistically significant at P < 0.05, the data are showed as the mean ± standard deviation (SD).

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Author's Contribution

RM collected samples, performed experimental part, analysed data and wrote the original draft, DD and DM analysed and wrote original draft. AV conceived and supervised the study. All the authors have read and approved the final version of the manuscript.

Clinical Trial Number

Not applicable

Declaration

Ethical approval and Consent to participate. Soybean (*Glycine max* (L.) Merr.) seeds used in this study were obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore, India, and were cultivated under experimental conditions. The collection and use of plant materials complied with all applicable institutional, local and national guidelines. No specific permits or licences were required for the use of this cultivated plant material.

Consent to publish

Not applicable.

Conflicts of interest

The authors state that none of the work described in this study could have been influenced by any known competing financial interests or personal relationships.

Data availability statements

Data will be made available on request.

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AI declaration:

We have used AI tools (Google Gemini Pro) for language improvement and polishing.

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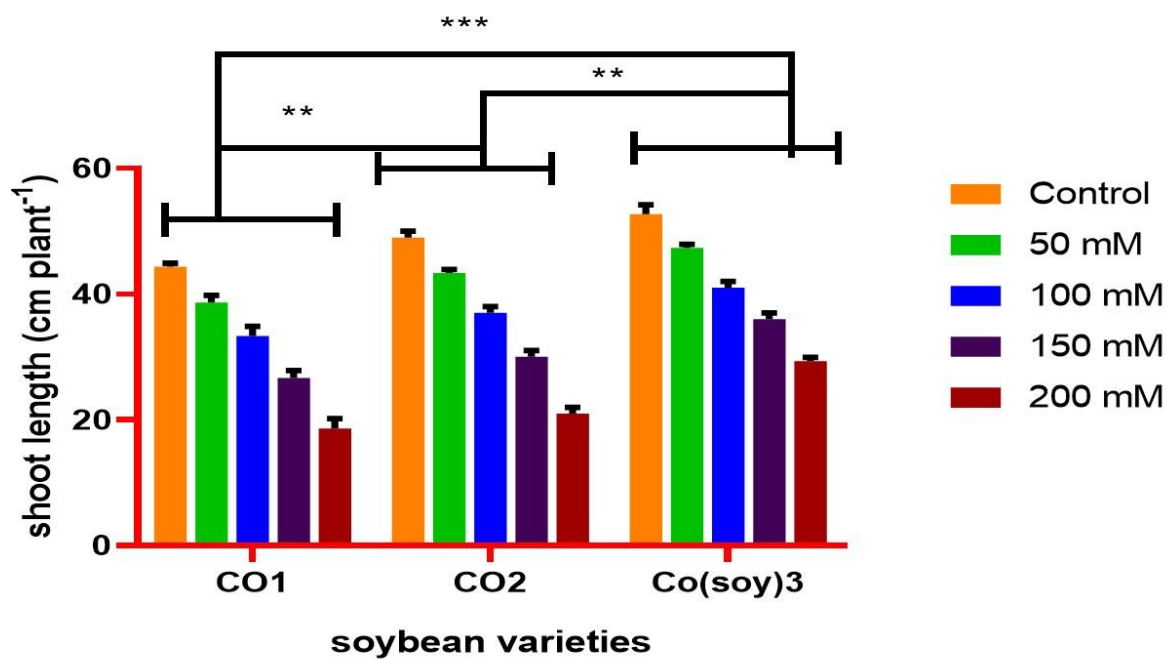


Figure 1. Effect of salinity on shoot length at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (**P < 0.01, ***P < 0.001).

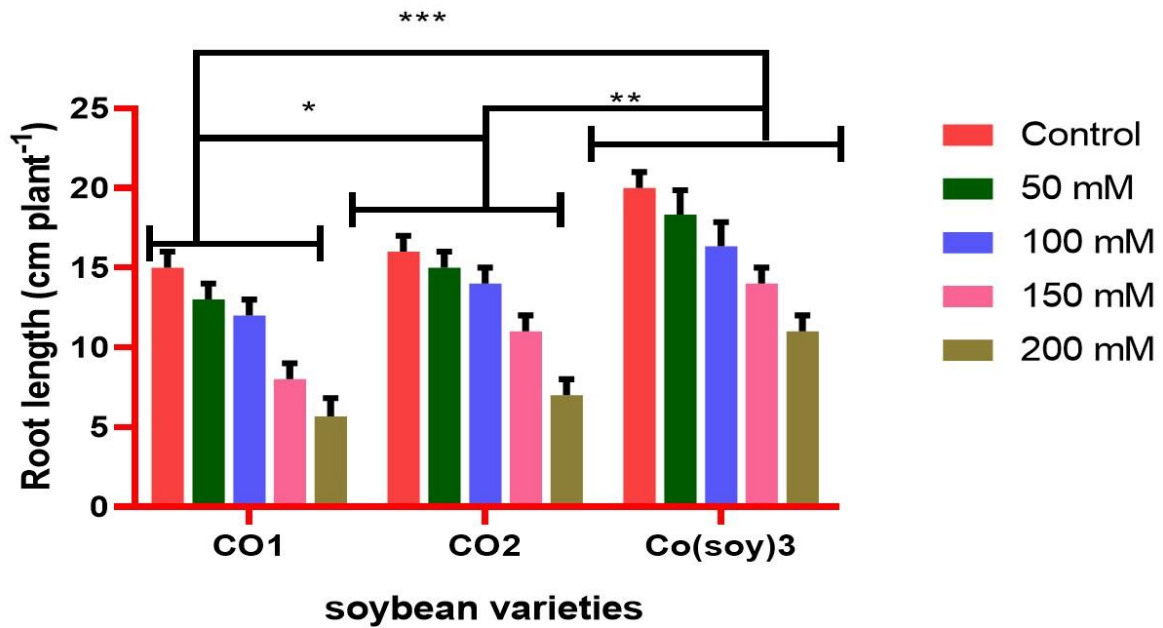


Figure 2. Effect of salinity on root length at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (*P < 0.05, **P < 0.01, ***P < 0.001).

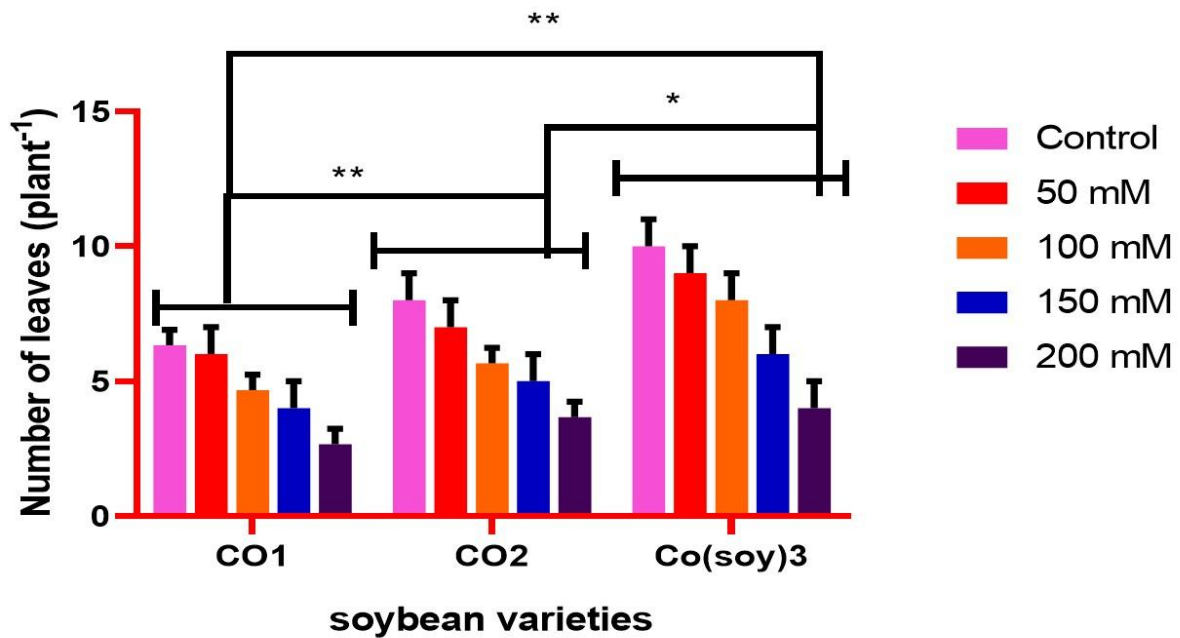


Figure 3. Effect of salinity on number of leaves at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (* $P < 0.05$, ** $P < 0.01$).

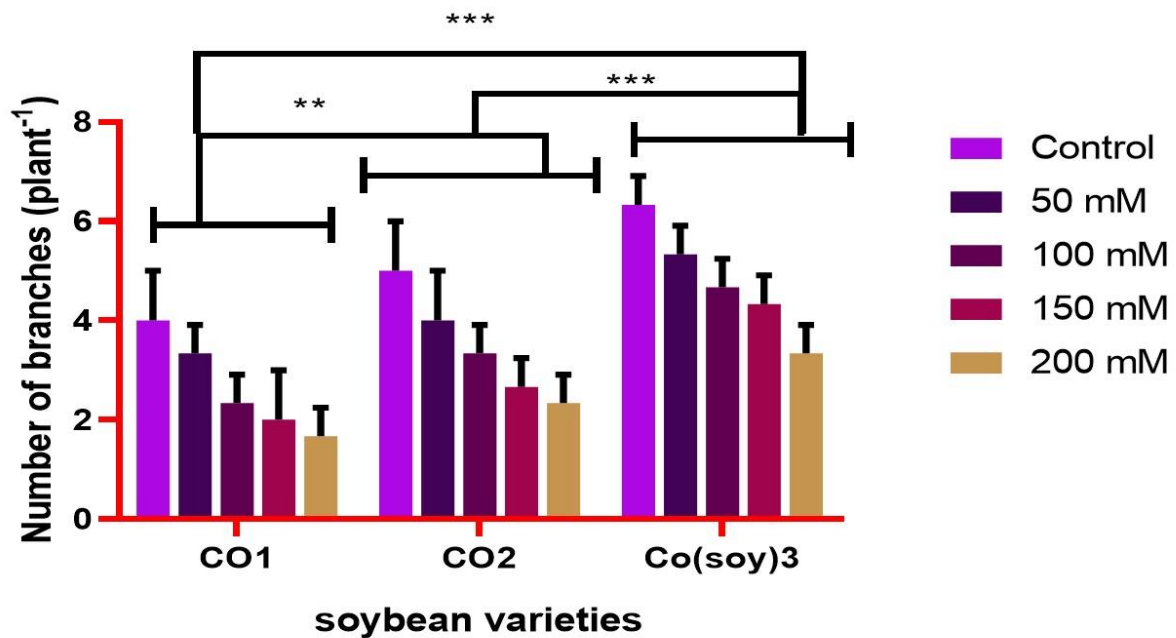


Figure 4. Effect of salinity on number of branches at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (**P < 0.01, ***P < 0.001).

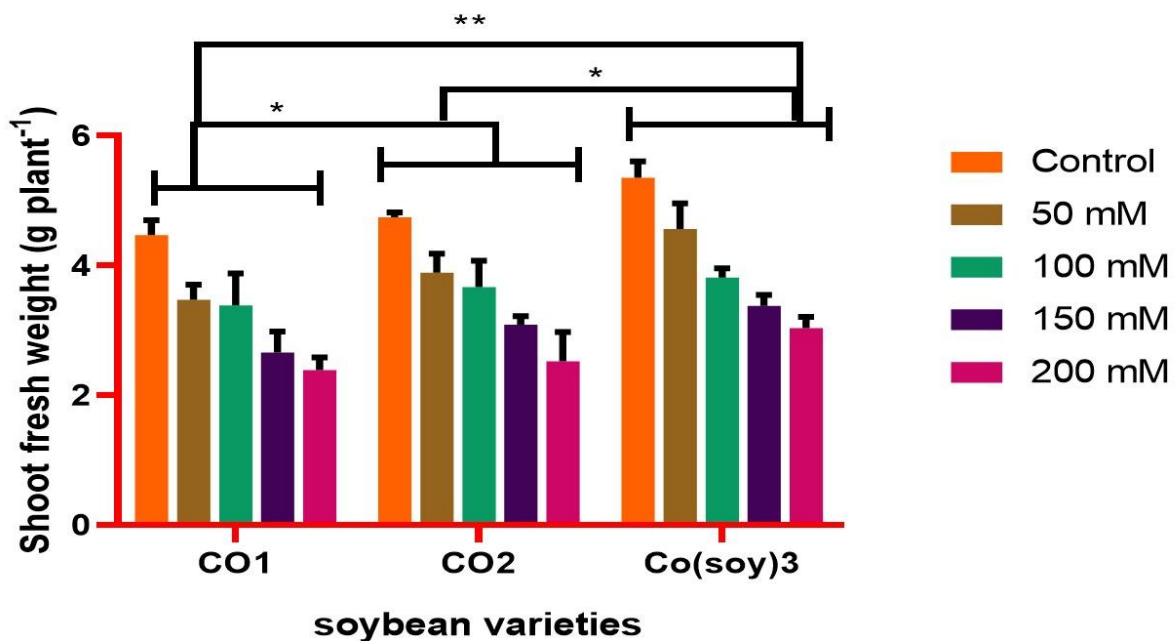


Figure 5. Effect of salinity on shoot fresh weight at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (*P < 0.05, **P < 0.01).

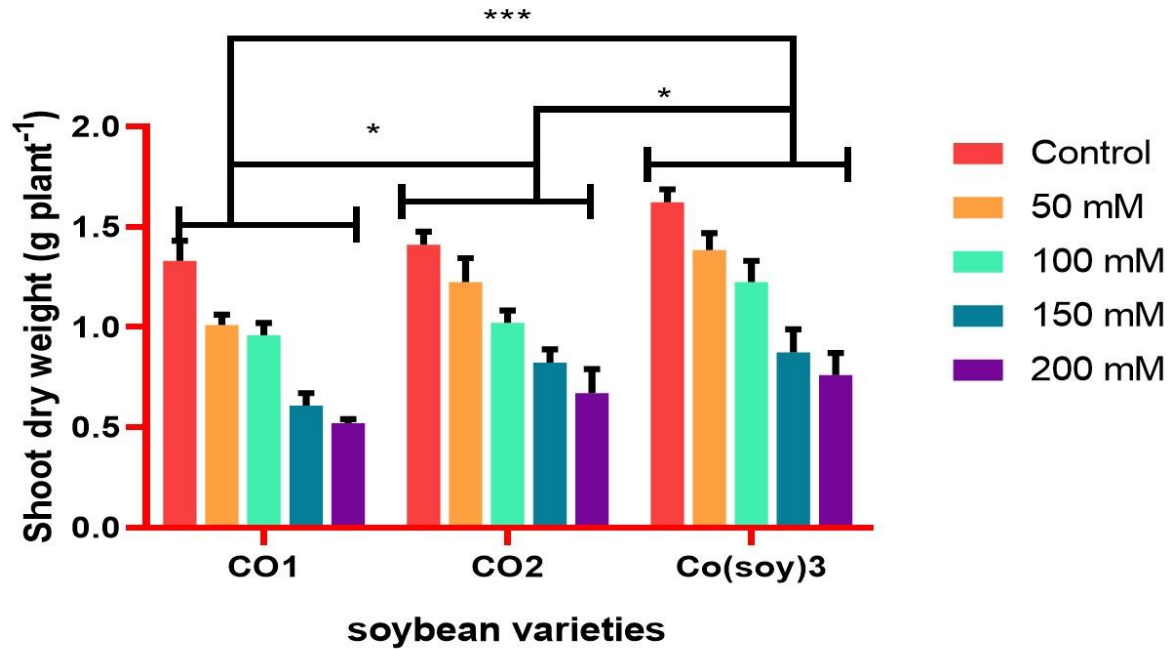


Figure 6. Effect of salinity on shoot dry weight at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (*P < 0.05, ***P < 0.001).

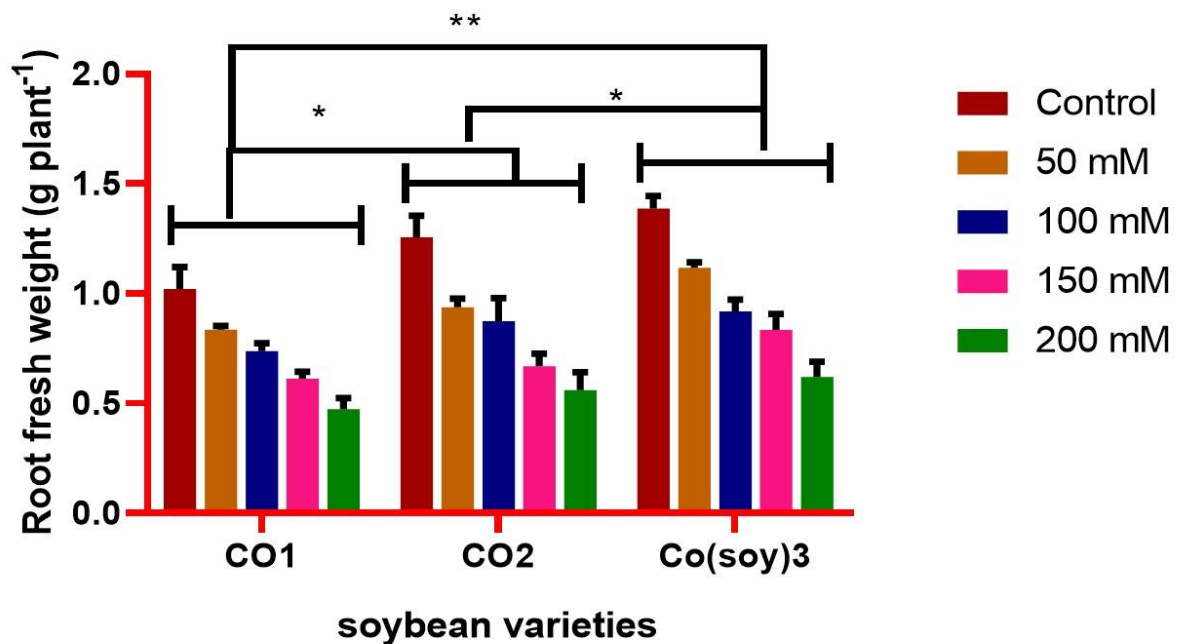


Figure 7. Effect of salinity on root fresh weight at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (*P < 0.05, **P < 0.01).

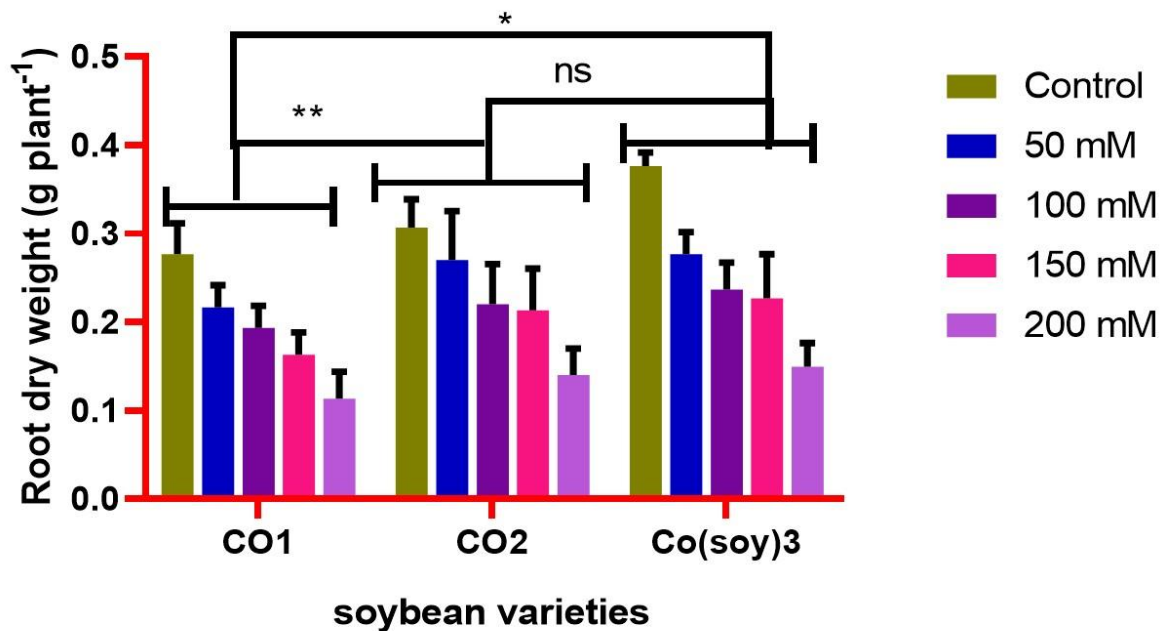


Figure 8. Effect of salinity on root dry weight at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (* $P < 0.05$, ** $P < 0.01$), and ns indicates no significant difference ($P > 0.05$).

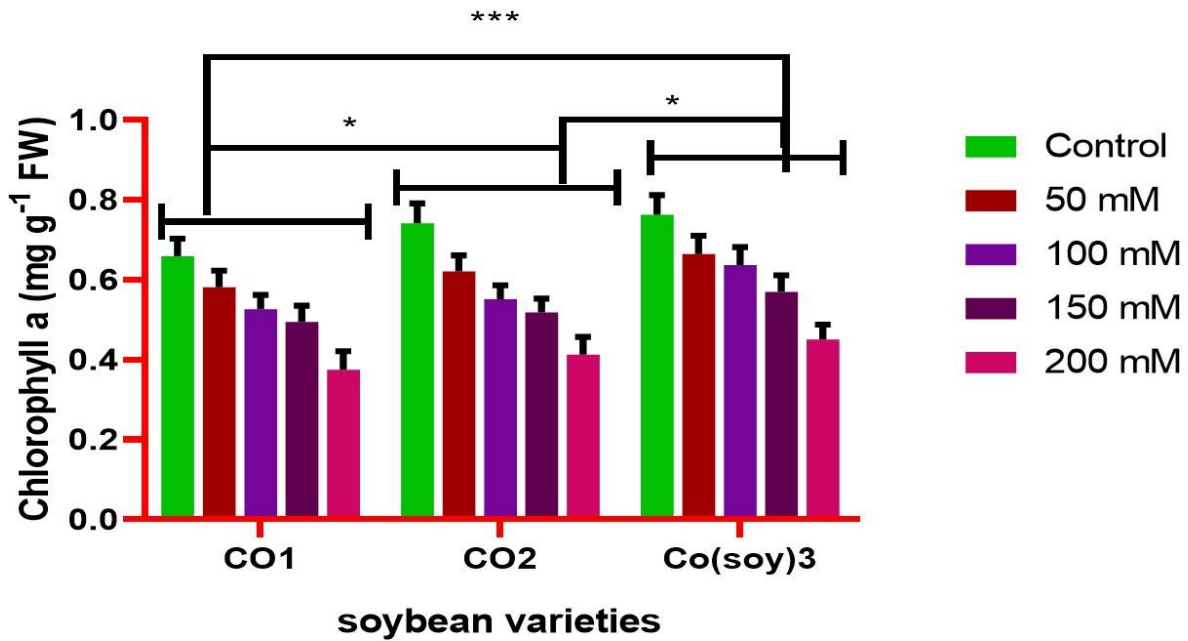


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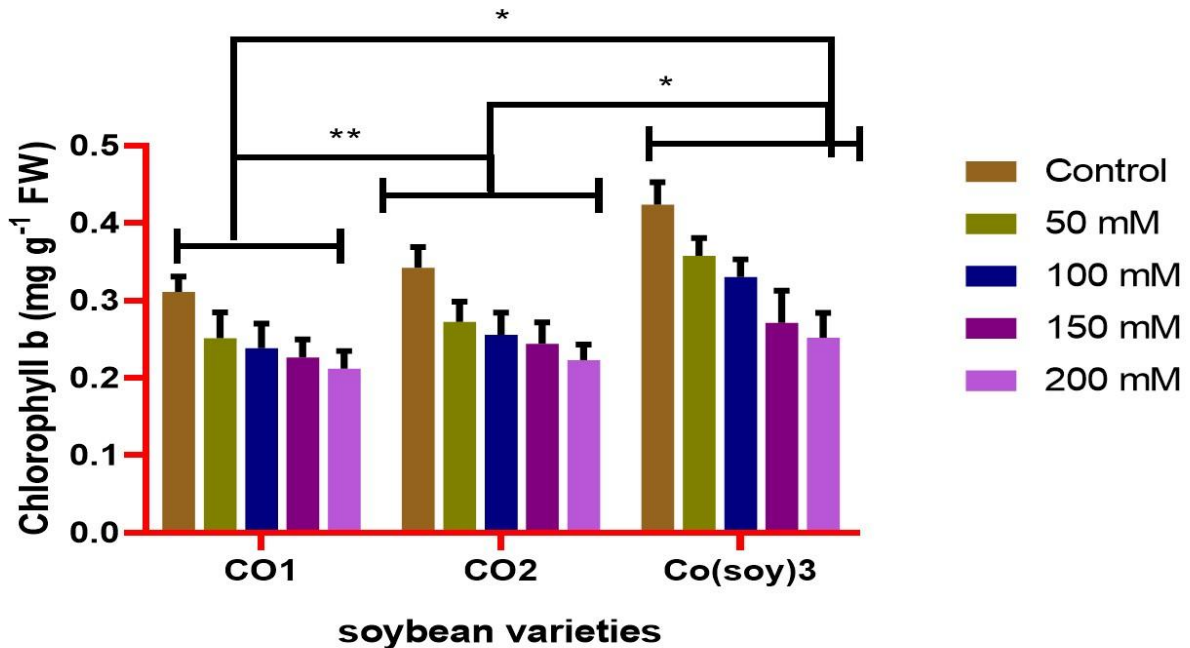


Figure 10. Effect of salinity on chlorophyll b at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (*P < 0.05, **P < 0.01).

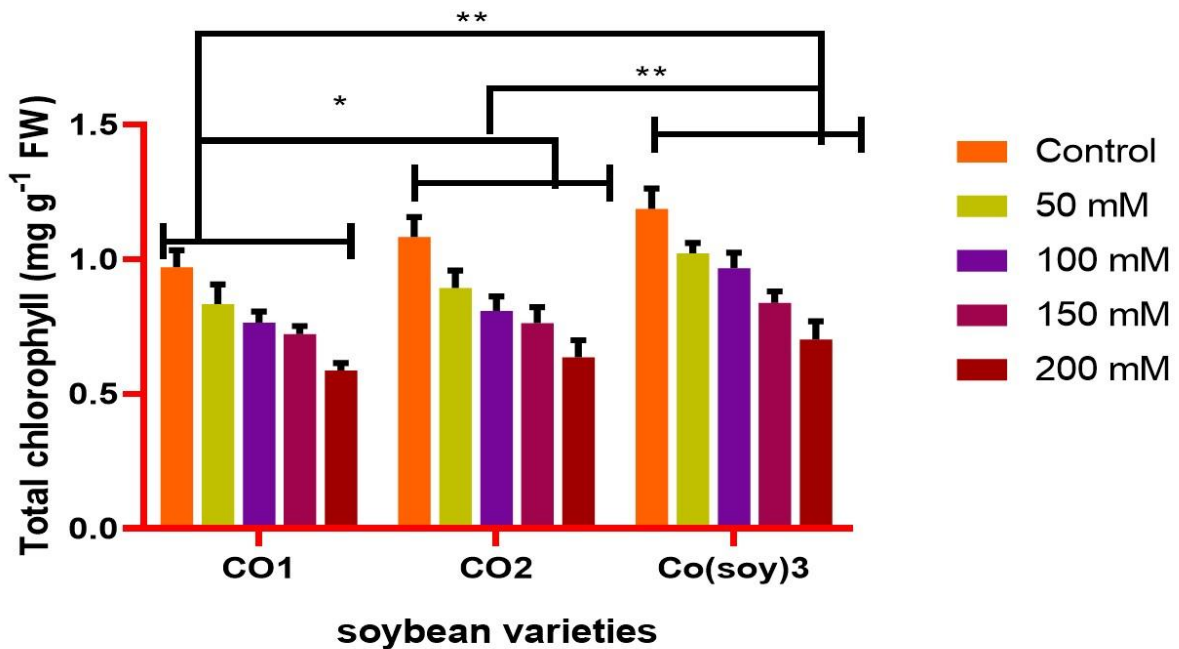


Figure 11. Effect of salinity on total chlorophyll at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (*P < 0.05, **P < 0.01).

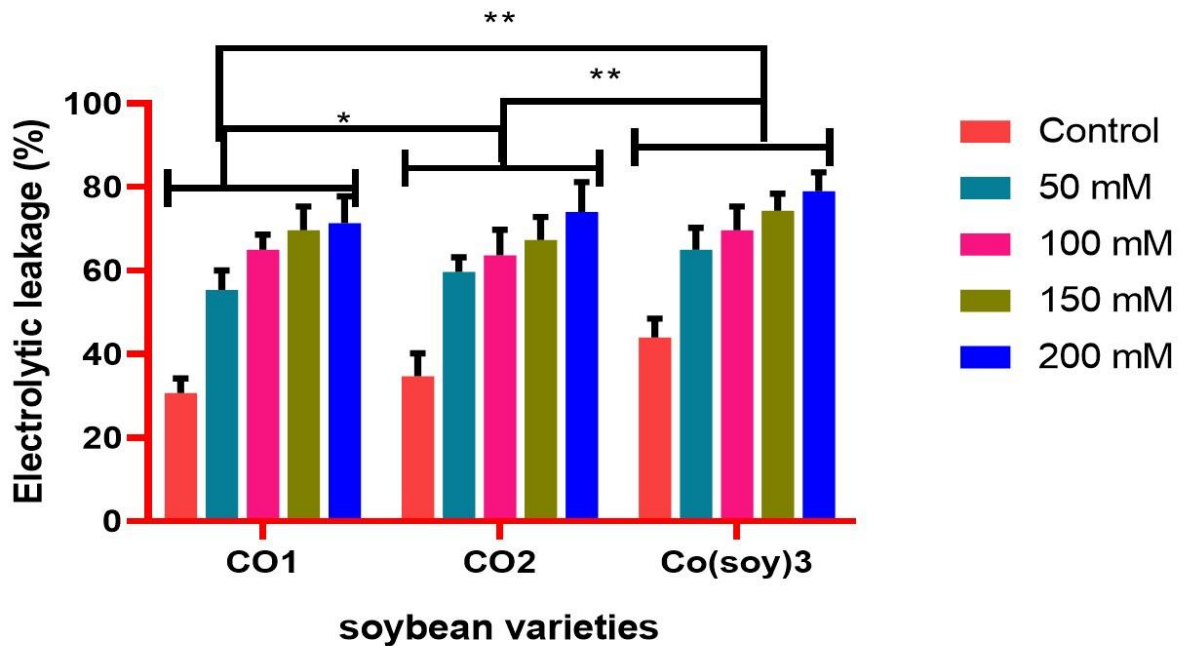


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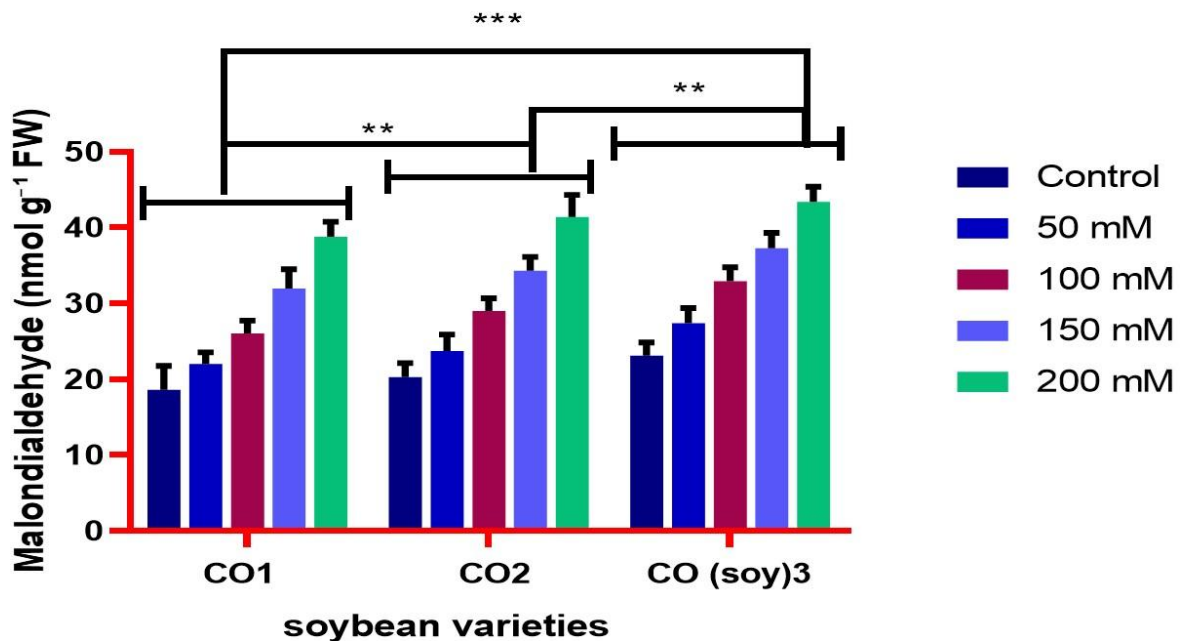
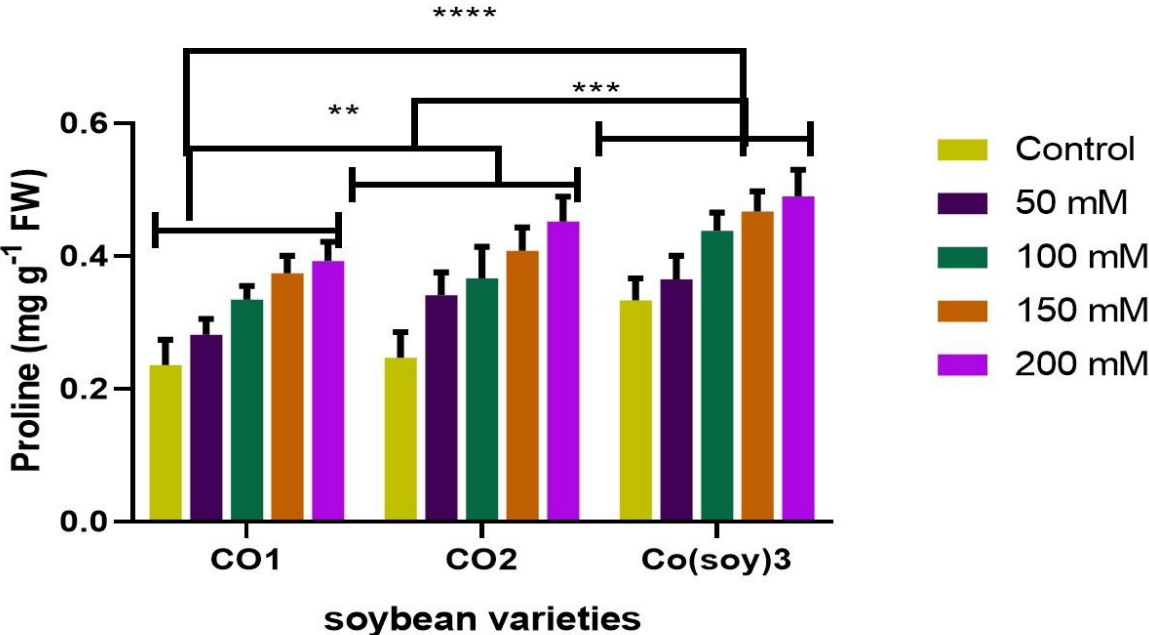
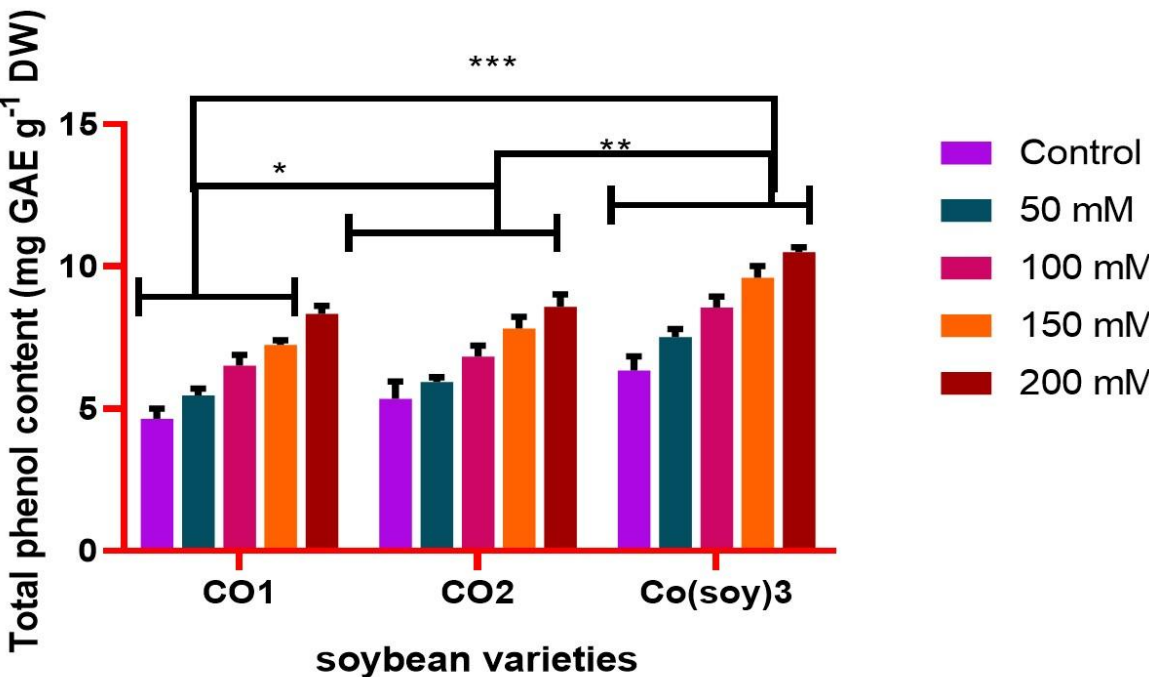


Figure 13. Effect of salinity on MDA at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (** $P < 0.01$, *** $P < 0.001$).



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551 **Figure 14.** Effect of salinity on proline content at different soybean varieties at 45 DAS. Each
552 value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences
553 among soybean varieties according to Tukey's multiple comparison test (**P < 0.01, ***P < 0.001,
554 ****P < 0.0001).



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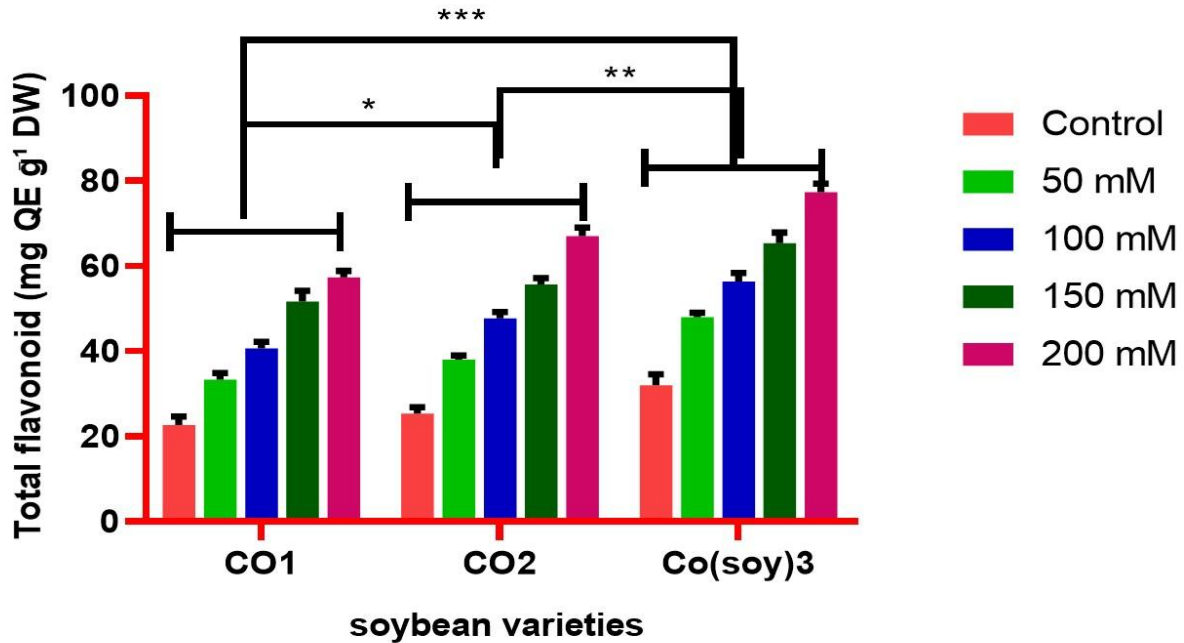


Figure 16. Effect of salinity on Total flavonoid content at different soybean varieties at 45 DAS. Each value are expressed as the mean $\pm$ SD (n=3). Horizontal brackets indicate significant differences among soybean varieties according to Tukey's multiple comparison test (*P < 0.05, **P < 0.01, ***P < 0.001).

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