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Impact of boron on *Glycine max* L. to mitigate salt stress by modulating the morpho-physiological and biochemical responses

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Abstract

Background Boron (B) is an essential micronutrient in higher plants, contributing to various physiological processes. However, its protective mechanism in mitigating salt stress remained less understood. This study investigates that exogenous boron (0, 1, 2 kg ha^{-1}) can help alleviate salt stress (0, 60, 120 mM NaCl) in two soybean cultivars AARI-2021 (V1) and Ajmeri (V2). It examines B role in reactive oxygen species (ROS), secondary metabolites, and antioxidant defense systems in mitigating salt stress.

Results Salt stress negatively impacted morph-physiological and biochemical attributes. Boron supplementation (2 kg ha^{-1}) reduced oxidative stress indicators, such as malondialdehyde (by 18% in V1 and by 21% in V2) and hydrogen peroxide (by 30% in V1 and by 38% in V2). Moreover, foliar application of boron (2 kg ha^{-1}) increased the catalase (CAT) (58% in V1 and 57% in V2), superoxide dismutase (SOD) (7% in V1 and 10% in V2), and peroxidase (POD) (42% in V1 and 32% in V2) activities under salt stress. Salt stress also led to an increase in Na^+ and a decrease in K^+ and Ca^{2+}. However, boron supplementation enhanced K^+ and Ca^{2+} in salt-stressed plants. Furthermore, boron application (2 kg ha^{-1}) increased the activity of secondary metabolites, total phenols content (TPC) (by 52% in V1 and by 59% in V2), total flavonoid content (TFC) (by 27% in V1 and by 21% in V2), and anthocyanins (ANTs) (by 33% in V1 and by 25% in V2) under salt stress.

Conclusion This study suggests that B can reduce salinity-induced oxidative damage in soybean plants by modifying antioxidant defense and secondary metabolites and preserving ion homeostasis.

Introduction

Soybean (*Glycine max* L.) is an essential oilseed crop in the *Fabaceae* family, native to East Asia. Soybean is a significant legume, accounting for 25% of global edible oil production. As early as 7000 BC, it originated in central China for the first time [1]. Soybean is a short-day plant that requires warm conditions for optimal growth. It can be grown all year in most tropical regions. Total global production for the 2023/2024 year is estimated to be between 378.1 and 398.2 metric tonnes, representing a 5.3% increase over the previous year. In 2020, soybeans production was 353,463,735 metric tonnes and in 2019 it was 336,329,392 metric tonnes. Brazil is the world's largest soybean producer, accounting for 34% of the world's production [2]. During 2022 (July-March), 2.843 million tonnes of edible oil was imported by Pakistan. The total import bill was Rs 664.682 billion. During this period,

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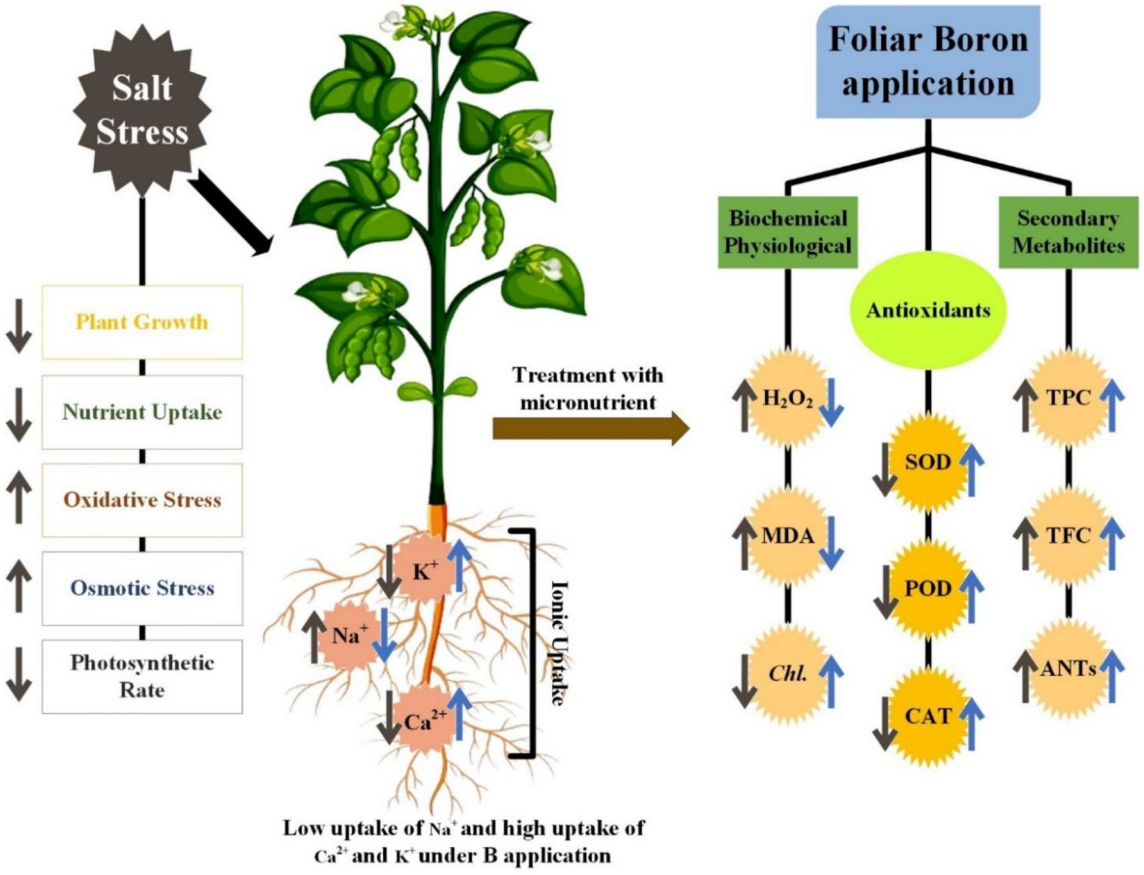
Graphical abstract



Keywords Antioxidant enzymes, Ion homeostasis, Reactive oxygen species, Secondary metabolites, Soybean

local edible oil production was provisionally estimated at 0.463 million tonnes [3]. Soybeans are processed into different soy products: soy meal, soy milk, soy flour, cottage cheese, and fermented products. Economically, soybean is the world's most significant bean that serves as a high protein ingredient for food, including vegetarian foods [4]. Soybean is an upright branching plant and can achieve 6.5 feet in height. It contains 35–50% proteins and 18–24% oils, depending on the variety and growth conditions. It includes one to four seeds per pod that can be yellow, brown, black, or maybe bicolored. They are spherical, oval, flattened, and elongated in shape [5]. Soybeans consist of a taproot system usually found in the top 30–60 cm of the soil and a well-developed stem with 0–6 lateral branches that grow to a height of 20 cm. Soybean leaves consist of three oval leaflets that extend to a length of 3–10 cm [6].

Soybean is a salt-sensitive crop. Salt stress negatively impacts soybean yields, involving changes in plant morphology, physiology, and metabolism by inhibiting seed germination [7]. High concentrations of salts affect soybeans in different ways. It prolongs root development, shoot length, and seed germination [8]. Increased salinity can cause a 40% reduction in soybean growth. Root nodule formation is a unique characteristic of legumes. Salinity reduces the efficiency of nitrogen fixation, which causes a decrease in biomass and the number of root nodules [9]. In response to high concentrations of reactive oxygen species (ROS), generation and salt stress result in oxidative stress. ROS production subsequently creates worse conditions for plants, reducing crop yield. Salt stress decreases plant growth by impairing water uptake and nutrient assimilation, which in turn can inhibit photosynthesis resulting into retarded growth. It could also lead to ion toxicity, damage metabolic processes and ultimately reduce the yield [10, 11]. Approximately 23% of all farmed land globally is impacted by salt, while another 37% is sodic. Salt harms around 20 million hectares of land, which is unusable for agricultural production [12]. This issue is much more acute in those

regions because there is a lack of water in Asia and Africa. Salt stress affects 20% of the farmed land and 50% of the irrigated areas [13].

Boron is essential to the growth of soybeans because it is required to form cell walls during expansion and for the healthy development of root nodules, which are responsible for nitrogen fixation [14]. Soybeans require a constant boron supply, especially during flowering and seed development. Foliar sprays are essential for those plants in which Boron is immobile, like soybean, considered one of the B-immobile plant species [15]. Because boron is essential for flower development and seed formation, reducing boron availability during this critical time may result in decreased yields. Under severe B deficiency, meristematic growing regions frequently die and exhibit stunted growth [16]. Other frequent side effects include stunted root development, inability of blossoms to produce seeds, and fruit abortion. Low B levels may also harm pollination and seed germination, even without visible leaf symptoms [17, 18]. In most plant species, B exhibits very weak phloem mobility. Therefore, in sufficient amounts, B in leaf tissue cannot be conveyed to reproductive organs (e.g., stem ends, blooms, flowers, seeds, etc.). Due to its low mobility, soluble B must be present in soil solution throughout all stages of plant development, but especially during reproductive growth (e.g., during seed setting) for optimal plant nutrition [16, 19]. It can be hypothesized that B supplementation will regulate physiological processes, strengthen cytosolic organs, boost antioxidant defenses, and protect plants from stress. The main objective of this study is to explore the role of boron on secondary metabolites and antioxidant activities in modulating the morpho-physiological and biochemical responses of soybean (*Glycine max* L.) under salt stress. Additionally, we also examined the effect of supplemental B on antioxidants, secondary metabolites, ions uptake, and osmotic regulation.

Materials and methods

An experiment was carried out to investigate the impact of boron on morpho-physiological, biochemical, secondary metabolites, and enzymatic antioxidant activities of soybean varieties (AARI-2021 and Ajmeri) under salt stress. A pot experiment was conducted in winter season (2022–2023) at the University of Agriculture Faisalabad Community College PARS, Pakistan. Plastic pots (30 cm depth and 24 cm diameter) containing 8 kg of dry river sand were used to sow soybean seeds. Seeds were collected from the Department of Oilseeds at Ayub Agricultural Research Institute (AARI), Faisalabad. Seeds were soaked in water for 24 h before sowing to boost germination. In a Completely Randomized Design, 10 soybean seeds were sown in each pot at similar depth and spacing with three replicates (Fig. 1).

Immediately after germination (3–4 weeks), the plants were subjected to a mild stress of 60 mM NaCl and a severe stress of 120 mM NaCl. A separate set of control plants was grown without exposure to NaCl. Boron treatment (foliar application of 1, 2 kg ha^{-1} B) was administered to the control and stressed plants after one week of salt stress. Soil moisture was maintained using weekly watering and 250 mL full-strength Hoagland's nutrient solution was supplied to each pot until the experiment's completion (Fig. 2).

The pots were placed in a controlled environment to provide optimal temperature and sufficient light conditions. The presence of pests and diseases was consistently monitored, with manual removal of any detected

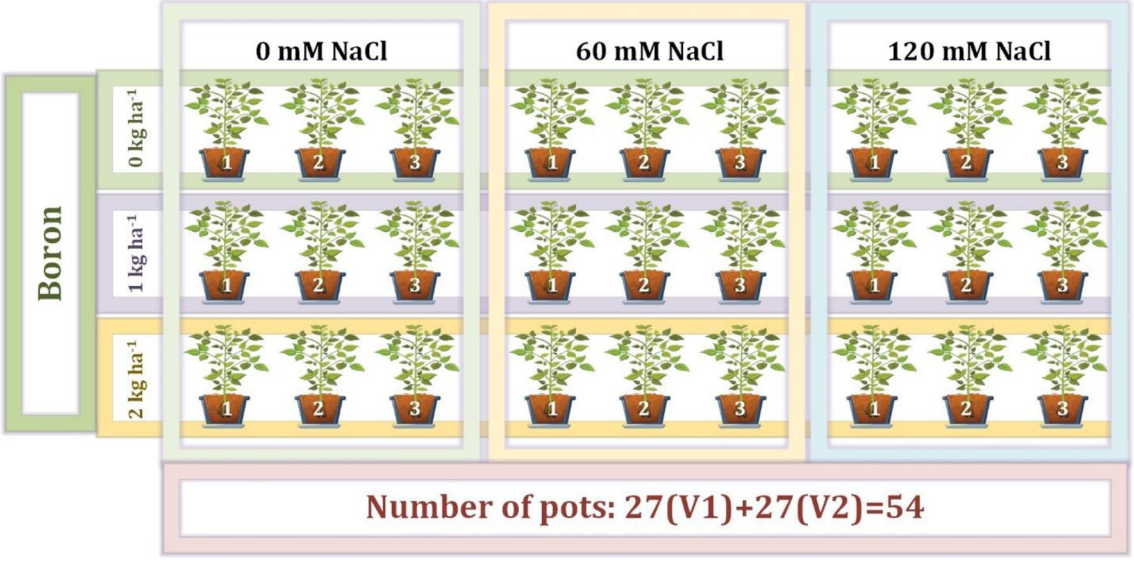


Fig. 1 Diagrammatic illustration of experimental design

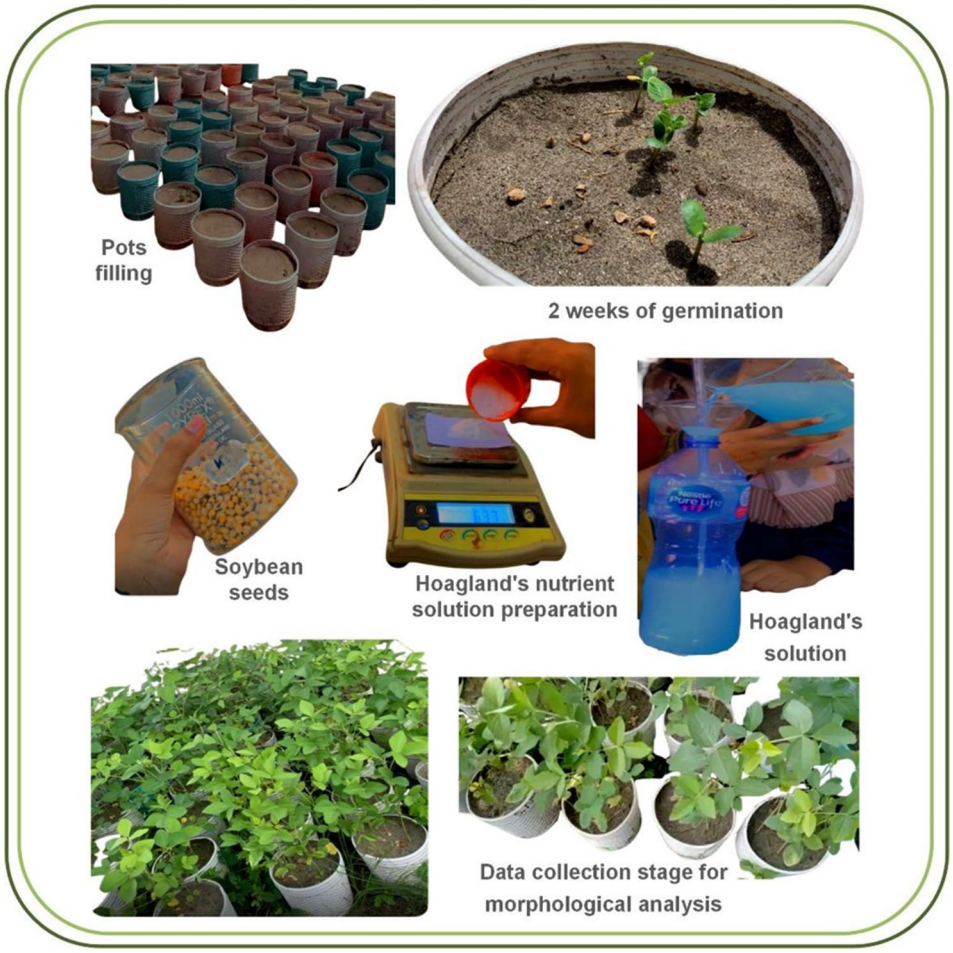


Fig. 2 Overview of germination and morphological data collection

pests. After 6 weeks of exposure to stress and boron, the relevant morpho-physiological, biochemical, secondary metabolites, and enzymatic characteristics were determined.

Morphological parameters

The number of leaves and branches plant^{-1} was calculated from five sample plants uprooted from each replication. Five plant samples were chosen from each replication, shoots were separated from roots, and their length (cm) was assessed using a measuring scale. Meanwhile, after weighing the shoot and root fresh weight (FW), plants were dried in an oven at 85°C for 48 h to determine the dry weight (DW) of plant^{-1}.

Determination of physiological parameters

Photosynthetic pigments were determined using Lichtenthaler and Buschmann [20] method. Fresh young broad bean leaves (0.5 g) were homogenized in 5 ml of 80% acetone to measure chlorophyll *a*, chlorophyll *b*, and carotenoids. Supernatant absorbance was measured by using spectrophotometer (InnoTech, Inno-UV2000, China) at three wavelengths 480 nm, 645 nm, and 663 nm for carotenoids, chlorophyll *b*, chlorophyll *a* respectively. After determination, the pigment concentration was measured in mg/g of leaf fresh weight.

Estimation of ionic content

Dried soybean root and shoot samples were ground finely, and then 0.2 g weighed out before being carbonized on an electric stove. For digestion, the technique of Wolf [21] was used, the samples were mixed with 0.1 M HCl, filtered, and ashed in a muffle furnace at 500 °C for four hours. Flame photometry (Jenway, PFP-7, Staffordshire, UK) was used to measure ions (Na^+, K^+, Ca^{2+}) of the filtrate.

Determination of antioxidant enzymatic activities

Peroxidase (POD) and catalase (CAT)

Catalase enzyme activity was measured using the technique of Velikova, et al. [22]. 1 mM of phenylmethylsulfonyl fluoride was mixed with ice-cold sodium phosphate buffer (5 mL) with a pH of 7.5. This mixture was then homogenized with 0.5 g of frozen plant material. The

extract was centrifuged at 12,500 g for 20 min at 4 °C. The supernatant, which consisted of potassium phosphate buffer (2.6 mL), hydrogen peroxide (400 µL), and enzyme extract (40 µL), was used to perform the assays. The absorbance at 240 nm of the H_2O_2 breakdown was suppressed using a spectrophotometer (Peak Instruments, C7200S, USA). According to Hemeda and Klein [23], Peroxidase activity was measured. Guaiacol oxidation was detected at 470 nm-spectrophotometry. 30% H_2O_2, methoxyphenol, and sodium phosphate buffer (pH 6.0) make up the reaction buffer. To determine activity, a 6.58 mM^{-1} cm^{-1} extinction coefficient was used.

Superoxide dismutase (SOD)

Using the technique of Beauchamp and Fridovich [24], the SOD activity was carried out. By using spectrophotometry (Peak Instruments, C7200S, USA) at 560 nm, superoxide dismutase (SOD) was needed to inhibit NBT photochemical degradation by 50%. A 3 cm^3 reaction mixture included 0.0033 mM riboflavin, 0.66 mM EDTA, 10 mM L-methionine, and 50 mM Na phosphate buffer at pH 7.8. With PPFD of about 300 µmol $m^{-2}s^{-1}$, reactions lasted 10 min at 25 °C.

Determination of MDA and H2O2 content

By following the method of Heath and Packer [25], At 532 and 600 nm, spectrophotometer (Peak Instruments, C7200S, USA) was used to measure MDA concentration. Data was collected using thiobarbituric acid reactive compounds (TBARS). The data was presented as nmol g^{-1} FW. According to Yang, et al. [26], leaf samples of 0.5 g were homogenized and centrifuged at 11,500×g using trichloroacetic acid (TCA). Potassium iodide and K-P buffer at pH 7.0 were added to the supernatant. The supernatant's optical absorbance at 390 nm was used to determine hydrogen peroxide content using spectrophotometer (Peak Instruments, C7200S, USA).

Estimation of secondary metabolites content

Total phenols content (TPC)

According to Dihazi, et al. [27], Folins-Ciocalteu-based tannic acid equivalent (TAE) was used in determining phenolic component content. 50 ml of 80% cold methanol (volume/volume) was mixed with 1 gram of fresh leaves for extraction three times at a temperature of 90 degrees Celsius. Following the filtration of the extract, methanol was used to dilute it to a previously established level. After carefully combining the extract, it was stored in the dark for 60 min. After that, absorbance was measured at 750 nm by using spectrophotometer (Peak Instruments, C7200S, USA).

Total flavonoid content (TFC)

Flavonoids were measured using the method of Sultana, et al. [28]. Mixing 0.5 g fresh leaves with 10 mL 80% aqueous methanol before filtering. After 5 min, extract (1 mL), distilled water (4 mL), 5% $NaNO_2$ (0.3 mL), and 10% $AlCl_3$ (0.3 mL) were added and incubated for 6 min before adding NaOH (2 mL) and H_2O (10 mL). Quercetin-serious solutions identified flavonoids at 430 nm by using spectrophotometer (Peak Instruments, C7200S, USA).

Anthocyanin (ANTs) contents

Anthocyanin was measured using the method of Mancinelli [29]. Fresh plant samples were crushed in 10 mL of acidified methanol (1:99 HCl). Homogenate was centrifuged at 18,000 g for 30 min at 4 °C. The supernatant was incubated at 5 °C in the dark for 24 h after filtering using Whatman's filter paper No.1. At 550 nm, the spectrophotometer (Peak Instruments, C7200S, USA) detected anthocyanin.

Statistical analysis

Analysis of variance of data for all the parameters was calculated under completely randomized design (CRD) with 3 replications and mean values by using CoStat-CoHort Software and Statistix 10 following Snedecor [30] methodology. Graphical illustrations and diagrams were created using Sigma Plot and Microsoft Visio, while R was utilized for generating heatmaps, conducting PCA, and analyzing correlations.

Results

Morphological parameters

Upon being subjected to mild and severe salt stress (60 mM and 120 mM NaCl stress), the reduction in number of leaves was reduced by 25% and 38%, respectively, in V1 and reduced by 21% and 45% in V2, as compared to 0 mM NaCl. However, supplementation of 1 and 2 kg ha^{-1} B increased the number of leaves by 26% and 58% in V1 and 18% and 31% in V2. Both stress levels reduced the number of leaves, and adding B substantially impacted the number of leaves under salt stress (Fig. 3A; Table 1). In V1, the number of branches decreased by 7% and 33% when subjected to a stress of 60 and 120 mM NaCl, respectively. In V2, the number of branches reduced by 15% and 38%, respectively, as compared to plants that were not treated. Boron supplementation increased the number of branches by 34% and 67% at 1 and 2 kg ha^{-1} B, respectively, in V1 and 30% and 57% in V2 (Fig. 3B; Table 1). When exposed to 60 and 120 mM NaCl, shoot length declined by 17% and 28%, respectively, compared to unstressed plants in V1, and shoot length decreased by 15% and 35% in V2. However, foliar supplementation of B increased the shoot length by 31% and 62% at

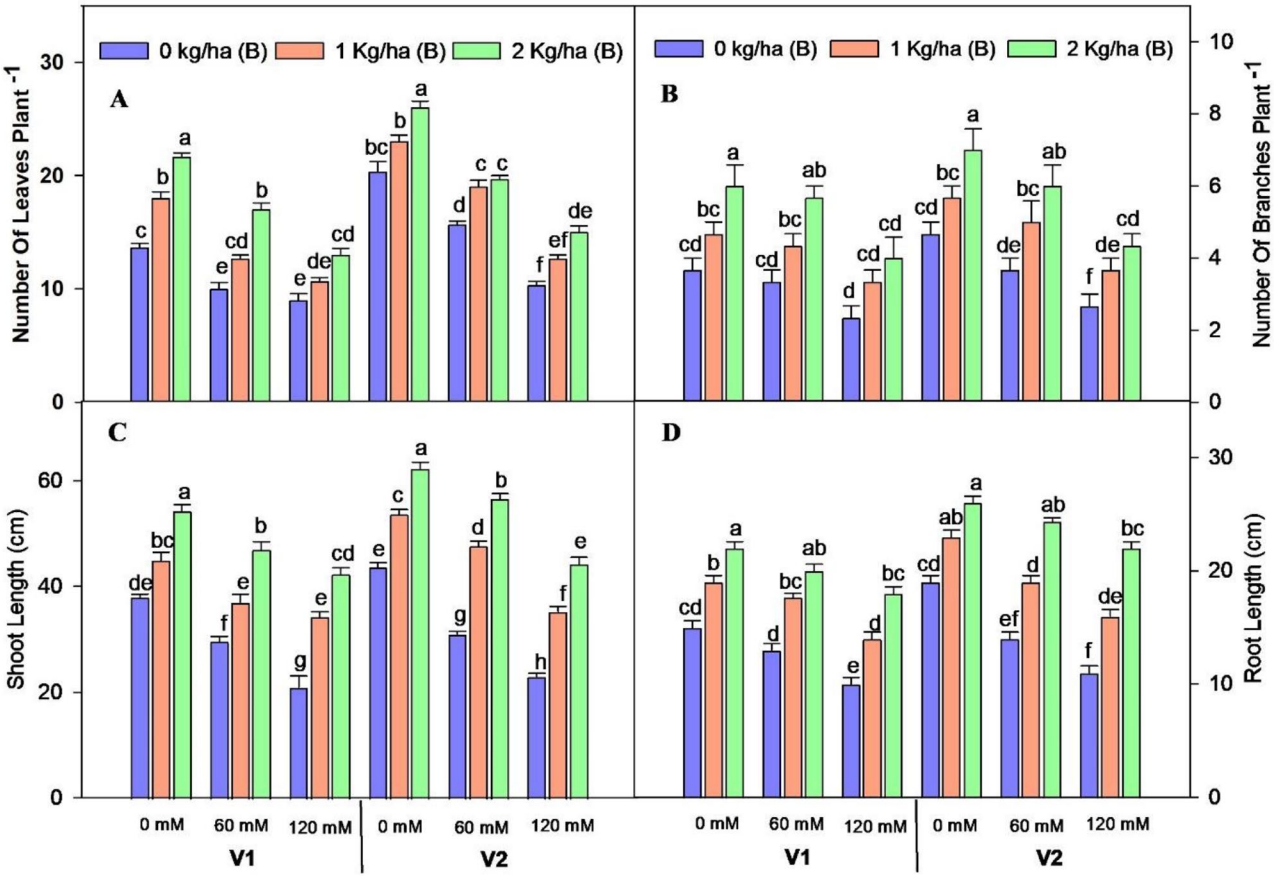


Fig. 3 Effect of exogenous B on number of leaves (**A**), number of branches (**B**), shoot length (**C**) and root length (**D**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

1 and 2 kg ha^{-1} B, respectively, in V1 and increased by 39% and 67% in V2 (Fig. 3C; Table 1). In 60- and 120-mM NaCl-stressed plants, root length was reduced by 9% and 25%, respectively, compared to untreated plants in V1 and reduced by 15% and 27% in V2. However, the B foliar spray increased the root length significantly by 33% and 56%, at 1 and 2 kg ha^{-1} B, respectively, in V1 and increased by 30% and 62% in V2 (Fig. 3D; Table 1).

Salt stress at 60 and 120 mM NaCl reduced SFW by 23% and 32%, RFW by 32% and 43%, SDW by 10% and 13%, and RDW by 31% and 43%, respectively, in V1, in comparison to unstressed plants. While foliar application of B at 1 and 2 kg ha^{-1} restored the reduction of both shoot and root FW in V1, with a significant increase in SFW by 34% and 65%, RFW by 54% and 97%, SDW by 8% and 17%, and RDW by 88% and 155% respectively. In V2, both mild and severe NaCl levels (60 and 120 mM) reduced SFW by 23% and 39%, RFW by 30% and 45%, SDW by 13% and 21%, and RDW by 25% and 45%. While 1 and 2 kg ha^{-1} B supplementation increased SFW by 34% and 75%, RFW by 44% and 76%, SDW by 12% and 21%, and RDW by 48% and 106%, respectively (Fig. 4; Table 1).

Physiological parameters

Compared to unstressed plants, a considerable decrease was noticed in Chl a, Chl b, carotenoid contents, and the salt-induced plants' total Chl and Chl ratio. Upon severe NaCl exposure at 120 mM, the Chl a, Chl b, and carotenoid content reduction were 29%, 34%, and 29%, respectively, in V1. While in V2, 120 mM NaCl reduced the Chl *a* content by 30%, Chl b content by 44%, and Car content by 34% (Fig. 5A, B, C; Table 1). In contrast, the total chlorophyll and chlorophyll ratio content declined by 36% and 11%, respectively; in V1 and V2, 48% and 21% reduction were noticed, compared with untreated plants (Fig. 5D, E, and Table 1). Likewise, Under NaCl exposure, foliar supplementation of B at 2 kg ha^{-1} increases the Chl *a*, Chl *b*, carotenoid, total Chl, and Chl ratio contents by 42%, 67%, 45%, 81%, and 19%, respectively, in V1. In V2, these contents were increased by 29%, 58%, 24%, 73%, and 25% (Fig. 5; Table 1).

Ionic analysis

Salinity treatments (60 and 120 mM NaCl) directed a significant increase in root Na^+ content by 11% and 26%, respectively, and a meaningful decrease in root K^+

Table 1 Effect of B foliar application on morpho-physiological attributes of soybean under salinity stress

Sources	df	NOL	NOB	SL	RL	SFW	RFW	TFW	SDW	RDW	TDW	Chl a	Chl b	Car	TChl	ChlR
Variety	1	216***	4.74**	400.16***	101.40***	4.73***	0.85***	9.60***	0.45***	0.03***	0.72***	5.61***	1.40***	2.52***	5.78***	3.76ns
NaCl	2	339.18***	16.72***	1168.07***	133.40***	5.35***	0.34***	8.42***	0.38***	0.02***	0.58***	4.92***	1.50***	3.49***	6.54***	0.10***
Boron	2	138.90***	20.05***	1826.74 ***	304.51***	6.87***	0.31***	10.11***	0.31***	0.03***	0.55***	3.59***	1.43***	1.92***	6.67***	0.12***
Variety*NaCl	2	16.88***	0.57ns	48.22***	3.85*	0.32***	0.02***	0.55***	0.03***	0.00***	0.05***	2.90*	5.26***	1.05***	3.14***	0.01*
Variety*Boron	2	3.38*	0.01ns	20.22*	5.40**	0.25**	0.01**	0.36***	0.00*	6.00*	0.00**	5.62**	1.93**	1.03***	1.17**	0.00ns
NaCl*Boron	4	2.43*	0.27ns	10.65*	3.24*	0.15**	0.00*	0.17**	0.00*	5.37**	0.00**	1.99*	1.92***	1.16ns	1.06**	0.00*
Variety*NaCl*Boron	4	2.36*	0.01ns	11.69*	2.68*	0.09*	0.00*	0.14*	0.01***	3.08*	0.01***	3.02**	6.32ns	2.60ns	2.77ns	0.00ns
Error	36	0.77	0.51	3.92	0.92	0.03	0.00	0.04	0.00	1.14	0.00	7.18	3.23	1.15	2.16	0.00

content by 5% and 25% in V1. Meanwhile, in V2, root Na + content increased by 18% and 39%, and root K^+ content decreased by 15% and 30% at both salinity treatments. Exogenous B at a rate of 2 kg ha^{-1} resulted in a remarkable rise of 35% in the K^+ content of the roots in V1 and a 27% increase in the K^+ content of the roots in V2. Conversely, the Na^+ content of the roots reduced dramatically by 30% in V1 and 26% in V2 (Fig. 6A, B; Table 2). In addition, the data demonstrated that the Ca^{2+} concentration was significantly decreased by 16% in V1 roots and 17% in V2 roots when subjected to severe stress conditions. On the other hand, administering B at a rate of 2 kg ha^{-1} led to an increase in the Ca^{2+} level that was 20% higher in V1 and 18% higher in V2 when assessed with un-stressed plants (Fig. 6C; Table 2).

Application of salt stress (60 and 120 mM NaCl) dramatically enhanced shoot Na^+ content of V1 by 16% and 38%, respectively, and by 52% and 86% in V2. Exogenous B supplementation at a rate of 1 and 2 kg ha^{-1} significantly decreased shoot Na^+ content, with a reduction of 19% and 39%, respectively, in V1 and 23% and 46% in V2 compared to the plants subjected to stress (Fig. 7A; Table 2). Exogenous boron (2 kg ha^{-1}) dramatically increased shoots' K^+ and Ca^{2+} content by 48% and 38%, respectively, in V1 and 36% and 45% in V2. Whether salt stress (120 mM NaCl) reduced both K^+ and Ca^{2+} content of shoots by 32% and 18%, respectively, in V1 and 39% and 21% in V2 (Fig. 7B, C; Table 2).

Activity of enzymatic antioxidant

In stress-treated plants (60 and 120 mM NaCl), the levels of CAT activity decreased by 16% and 25% in V1 and 21% and 31% in V2, respectively, compared to untreated plants. Additionally, enhancement of the CAT activity was observed at varying salinity levels when foliar B application was provided. Under salt stress, B (1 and 2 kg ha^{-1}) enhanced the CAT activity by 35% and 58%, respectively, in V1 and 37% and 57% in V2 (Fig. 8A; Table 2). All salt treatments significantly increased POD activity compared to untreated control. Compared to untreated plants, V1 POD activity decreased by 15% and 23% under mild to severe salinity, whereas V2 decreased by 9% and 38%. In contrast, B foliar spray (1 and 2 kg ha^{-1}) increased POD activity under salt stress by 24% and 42% in V1 and 15% and 32% in V2 (Fig. 8B; Table 2). SOD activity was decreased by 2% in V1 and by 7% in V2 in response to a stress of 120 mM NaCl. Conversely, foliar B application enhanced the SOD activity in both varieties by 7% in V1 and 10% in V2, compared to salt treatment solely (Fig. 8C; Table 2).

Lipid peroxidation (MDA) and H_2O_2 content

Oxidative stress, which is produced by salt, is the cause of lipid peroxidation. The concentration of MDA is a

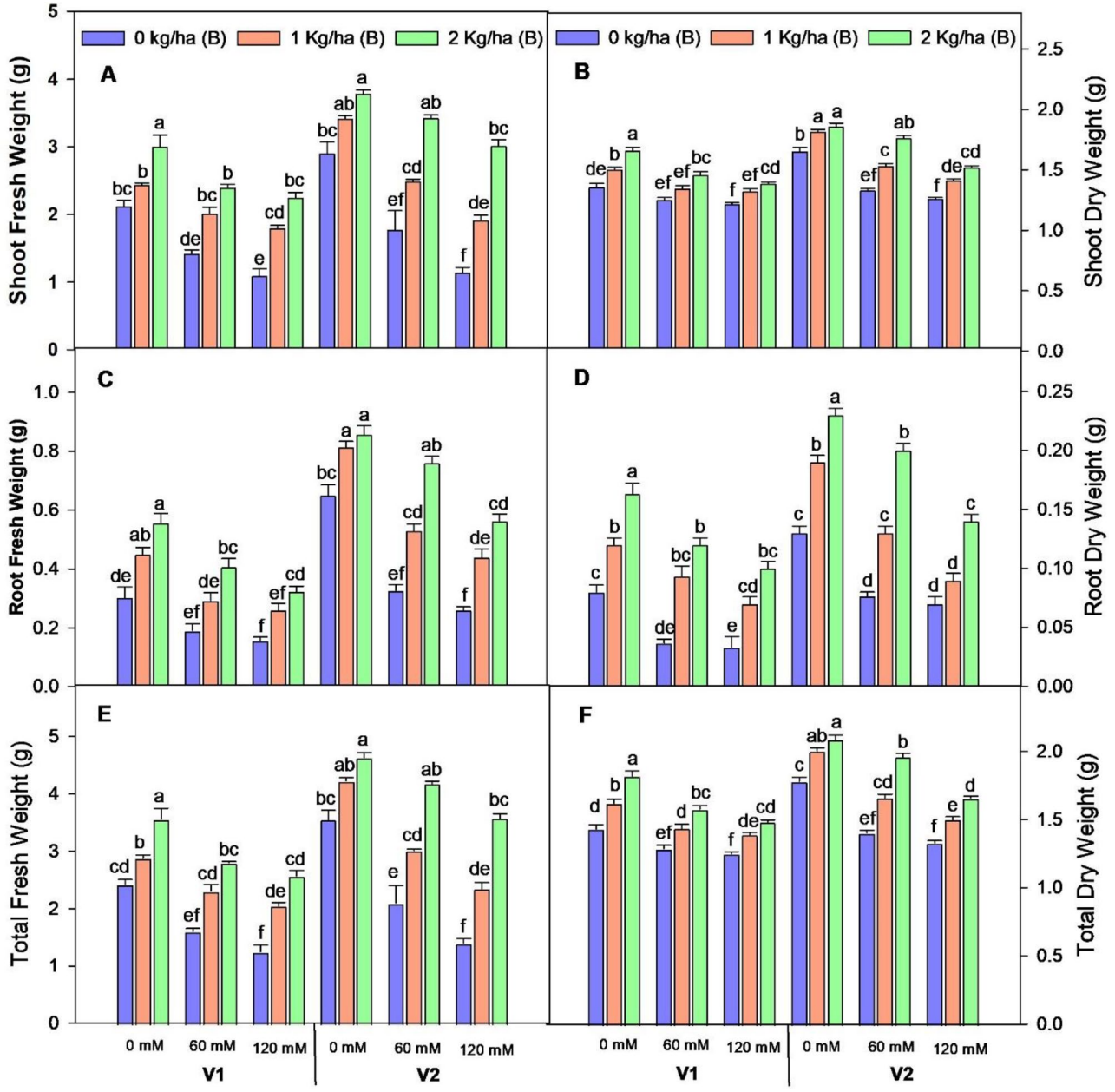


Fig. 4 Effect of exogenous B on shoot fresh weight (**A**), shoot dry weight (**B**), root fresh weight (**C**), root dry weight (**D**), total fresh weight (**E**) and total dry weight (**F**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

significant indicator of the level of lipid peroxidation. With increasing NaCl concentration, salt stress increased MDA content. Under mild and severe salinity (60 and 120 mM), MDA content was increased significantly by 38% and 80%, respectively, in V1 and 26% and 66% in V2. Under stress conditions, exogenous B (2 kg ha^{-1}) diminished the MDA content by 18% in V1 and 21% in V2 (Fig. 9A; Table 2). Salt-stressed plants increased H_2O_2 by 15% and 33% under moderate and severe salinity (60 and 120 mM), respectively, in V1 and 43% and 70% in V2. Moreover, H_2O_2 content decreased due to B foliar application (1 and 2 kg ha^{-1}) by 13% and 30%, respectively, in V1 and 16% and 38% in V2, in contrast to plants that have only been treated with salt (Fig. 9B; Table 2).

Secondary metabolites

As compared to control plants, NaCl treatment (120 mM) enhanced TPC, TFC, and ANTs by 31%, 27%, and 20%, respectively, in V1, while in V2, these contents increased by 35% in TPC, 37% in TFC and 23% in ANTs. Meanwhile, exogenous administration of B at 2 kg ha^{-1} concentration resulted in a substantial increase in the

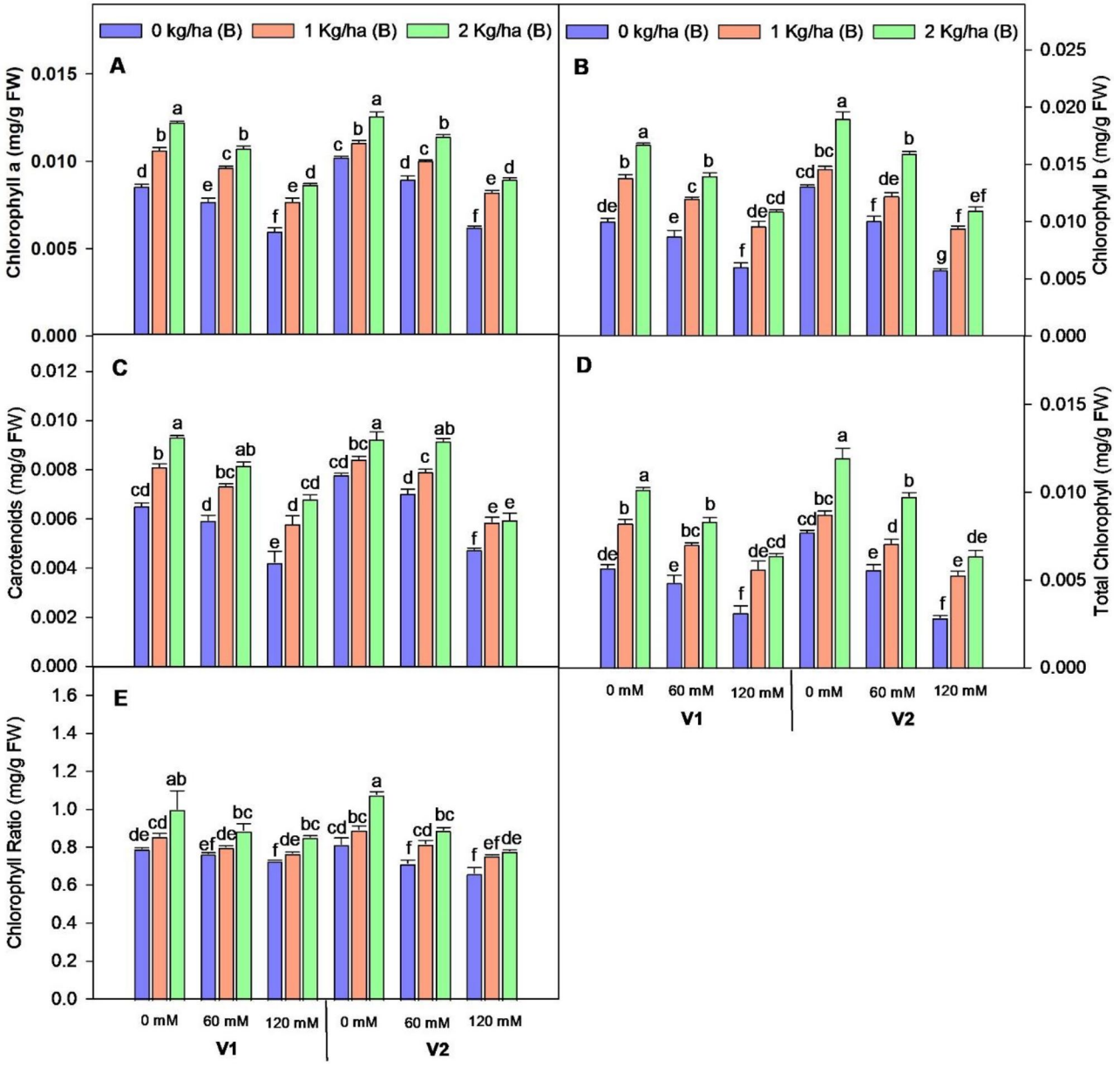


Fig. 5 Effect of exogenous B on chlorophyll *a* (**A**), chlorophyll *b* (**B**), carotenoids (**C**), total chlorophyll (**D**) and chlorophyll ratio (**E**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

level of secondary metabolites found in soybean plants by 52%, 27%, and 33% for TPC, TFC, and ANTs respectively, in V1 and 59% TPC, 21% TFC and 25% ANTs in V2 (Fig. 10; Table 2). The increase was significant under severe salinity conditions. High B treatment caused further gains, although salinity stress and 2 kg ha^{-1} B treatment produced the most important findings.

Heatmap

The heatmap (AARI-2021) was constructed among morpho-physiological and biochemical parameters under different salinity levels (0, 60, and 120 mM) and foliar application of Boron (0, 1, and 2 kg ha^{-1}). The central cluster showed three distinct sub-clusters. The first sub-cluster showed a strong positive association among MDA, H_2O_2, root, and shoot sodium ions at 120 mM NaCl (0B). In contrast, a negative association was observed among H_2O_2 and root and shoot sodium ions at 0 mM NaCl (2B) and 60 mM NaCl (2B), while MDA at 0 mM NaCl (2B), 0 mM NaCl (1B), and 0 mM NaCl (0B). The second subcluster had a positive association among total phenolics, anthocyanin, and total flavonoid contents at 120 mM NaCl (2B). In comparison, there was a strong negative association at 0 mM NaCl (0B). The third subcluster

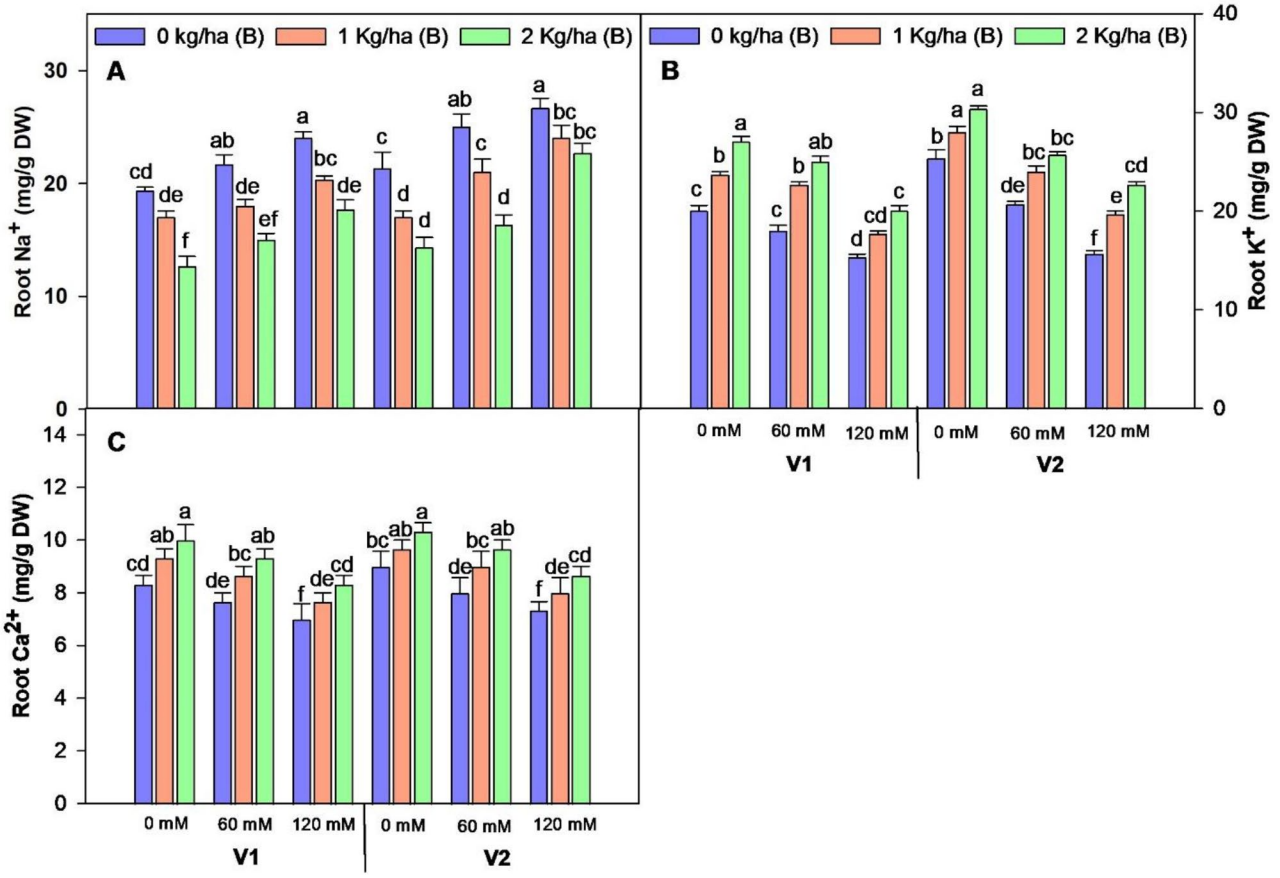


Fig. 6 Effect of exogenous B on Root Na^+ content (**A**), Root K^+ content (**B**) and Root Ca^{2+} content (**C**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

revealed a negative association of photosynthetic (chlorophyll *a*, chlorophyll *b*, carotenoids, chlorophyll ratio), ionic (root and shoot calcium and shoot potassium ions), and growth (root and shoot fresh and dry weights, root and shoot lengths) and antioxidants (superoxide dismutase, catalases, and peroxidases) traits at 0 mM NaCl (2B). In comparison, these parameters exhibited a negative association at 120 mM NaCl (0B) and 60 mM NaCl (0B) (Fig. 11).

The heatmap (Ajmeri) was constructed among morpho-physiological and biochemical parameters under different salinity levels (0, 60, and 120 mM) and foliar application of Boron (0, 1, and 2 kg ha^{-1}). The main cluster showed three distinct sub-clusters. The first sub-cluster showed positive association among plant photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids chlorophyll ratio, total chlorophyll), ionic contents (root and shoot potassium, and root calcium ions), growth parameters (root and shoot fresh and dry weights, root and shoot lengths, number or leaves and branches) and antioxidants (superoxide dismutase, and catalase) contents. In contrast, these parameters revealed strong negative associations at 120 mM NaCl (0B). In the second subcluster, there was a positive association between MDA, hydrogen peroxide, root and shoot sodium ions at 120 mM NaCl (0B), 60 mM NaCl (0B), and 120 mM NaCl (1B), while negative association at 0 mM NaCl (2B),0 mM NaCl (1B), and 60 mM NaCl (2B). In the third sub-cluster, antioxidant stress markers such as total phenolics contents, peroxidases, and total flavonoids showed a strong positive association at 120 mM NaCl (0B). These parameters showed a negative association at 0 mM NaCl (2B), 0 mM NaCl (1B), and 60 mM NaCl (2B) (Fig. 12).

Principal component analysis (PCA)

PCA biplot (AARI-2021) between morpho-physiological and biochemical traits showed four isolated clusters (Fig. 13).

In the first group, MDA contents were strongly associated with 120 mM NaCl (1B). Plant secondary metabolites as total flavonoids contents and total phenolics contents strongly related to each other's at 120 mM NaCl (2B) showed a weak association with photosynthetic contents (chlorophyll *b*, chlorophyll ratio, total chlorophyll), growth parameters (shoot fresh weight, root dry weight,

Table 2 Effect of B foliar application on biochemical and ionic attributes of soybean under salinity stress

Sources	df	RNa^+	RCa^{2+}	RK^+	SNa^+	SCa^{2+}	SK^+	SOD	POD	CAT	H_2O_2	MDA	TPC	TFC	ANTs
Variety	1	85.62***	1.85ns	85.62***	332.51***	101.40***	66.66***	3.71***	0.02***	1.52***	342.51***	160.16***	101.40***	78.24***	115.57***
NaCl	2	142.05***	11.72***	236.57***	261.72***	128***	254.46***	2.51***	0.06***	1.49***	298.29***	2144.01***	133.40***	259.12***	114.35***
Boron	2	193.55***	10.16***	160.35***	355.55***	304.38***	157.90***	4.30***	0.02***	2.42***	310.01***	373.46***	304.51***	161.90***	174.24***
Variety*NaCl	2	7.35*	0.01ns	11.12***	51.24***	3.85*	13.5***	0.21*	0.00*	8.69**	49.85***	5.05ns	3.85*	14.12***	2.46ns
Variety*Boron	2	0.29ns	0.01ns	0.35ns	24.51***	6.46**	0.5ns	0.05ns	1.13ns	2.44ns	21.24***	7.72*	5.40**	0.79ns	0.57ns
NaCl*Boron	4	2.69ns	0.05ns	0.43ns	6.19*	3.38*	0.9ns	0.01ns	3.06ns	1.84ns	16.74***	17.71***	3.24*	1.07ns	3.01ns
Variety*NaCl*Boron	4	2.60ns	0.01ns	2.43*	7.32*	3.07*	3.91**	0.15ns	6.50ns	5.75**	7.07*	1.69ns	2.68*	2.51*	2.46ns
Error	36	2.24	0.55	0.70	2.24	1.01	0.81	0.06	4.94	1.25	2.01	1.55	0.92	0.77	2.37

Where the *Significant at $p \leq 0.05\%$, **Significant at $p \leq 0.01\%$, ***Significant at $p \leq 0.001\%$, ns at $p > 0.05\%$, No. of leaves (NOL), No. of branches (NOB), Shoot length (SL), Root length (RL), Shoot fresh weight (SFW), Root fresh weight (RFW), Total fresh weight (TFW), Shoot dry weight (SDW), Root dry weight (RDW), Total dry weight (TDW), Chlorophyll *a* (chl *a*), Chlorophyll *b* (chl *b*), Carotenoids (car), Total chlorophyll (Tchl), Chlorophyll ratio (chlR), Root Na^+ (RNa^+), Root Ca^{2+} (RCa^{2+}), Root K^+ (RK^+), Shoot Na^+ (SNa^+), Shoot Ca^{2+} (SCa^{2+}), Shoot K^+ (SK^+), Superoxide dismutase (SOD), Peroxidase (POD), Catalase (CAT), Hydrogen peroxide (H_2O_2), Malondialdehyde (MDA), Total phenols content (TPC), Total flavonoids content (TFC), Anthocyanin's (ANTs)

root and shoot lengths) and SOD, CAT, and shoot calcium ions linked at 60 mM NaCl (1B). Plant hydrogen peroxide contents were strongly associated with root and shoot sodium ions linked at 60 mM NaCl (0B). plant photosynthetic contents (chlorophyll *a*, carotenoids) showed strong association with ionic contents (root and shoot potassium, and root calcium ions) and shoot dry weight, root fresh weight, total fresh and dry weights, POD weakly linked at 0 mM NaCl (2B) and 0 mM NaCl (1B).

Principal component analysis (Ajmeri) between morpho-physiological and biochemical traits showed four isolated clusters (Fig. 14).

In the first cluster, chlorophyll a, b, and carotenoids positively associated with POD, root calcium and potassium ions, shoot potassium ions, shoot dry weights, and number of leaves linked at 0 mM NaCl (1B). Root and shoot sodium ions were strongly associated with H_2O_2 contents linked at 60 mM NaCl (1B). Plant growth-related attributes (root length, shoot length, root fresh and dry weights, total dry weights, number of branches) showed a strong association with catalase, superoxide dismutase, shoot calcium ions, total chlorophyll, and chlorophyll ratio linked at 60 mM NaCl (2B). Plant total phenolic contents strongly linked at 120 mM NaCl (2B) showed a weak association with MDA contents linked at 120 mM NaCl (1B).

Correlation

Correlations between morpho-physiological and biochemical traits of AARI-2021 are exhibited in Fig. 15. Plants' photosynthetic contents (chlorophyll *a*, chlorophyll *b*, carotenoids, chlorophyll ratio, total chlorophyll) and ionic contents (root and shoot potassium and calcium ions) showed positive correlation with plant growth parameter (root and shoot fresh and dry weights, total dry and fresh weights, root and shoot lengths, number of leaves and branches) and superoxide dismutase while negative correlation with hydrogen peroxide, MDA, and root and shoot sodium ions. Correlations between the morpho-physiological and biochemical traits of Ajmeri are exhibited in (Fig. 16).

Plants root and shoot sodium ions showed positive correlation with MDA and H_2O_2 contents while showed strong negative association with plant photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, chlorophyll ratio, total chlorophyll, carotenoids), ionic contents (root and shoot calcium and potassium ions), with plant growth related attributes (root and shoot length, number of leaves and branches, root and shoot fresh and dry weights) and SOD, POD, CAT contents.

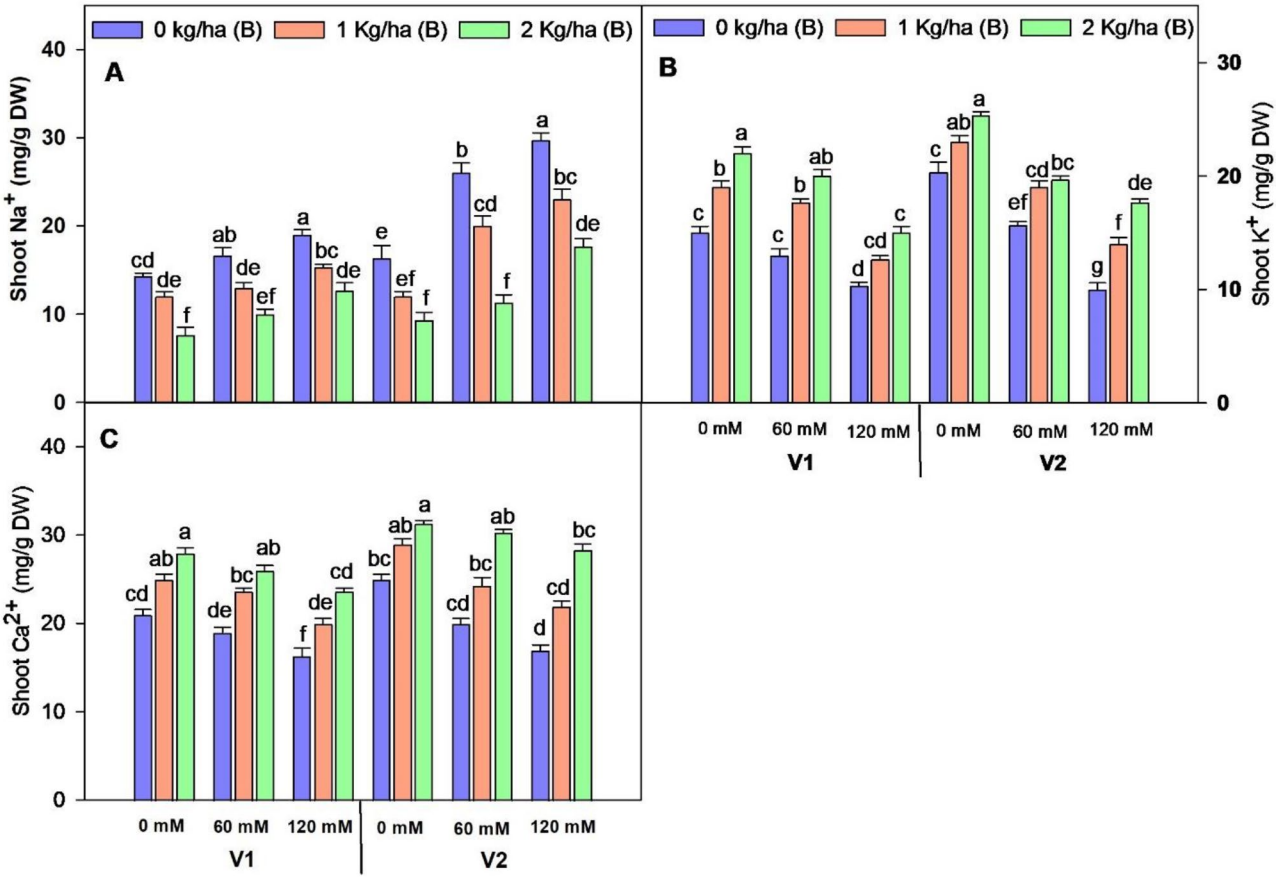


Fig. 7 Effect of exogenous B on Shoot Na^+ content (**A**), Shoot K^+ content (**B**) and Shoot Ca^{2+} content (**C**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

Discussion

Growth inhibition is the most prevalent negative effect of salt. All factors that caused this inhibition were the focus of our investigation. Saline conditions induce a decrease in plants' water absorption rate due to modifications to their osmotic potential. Alongside this is reduced absorption of vital mineral nutrients, partially attributed to competition with Na [31]. Salt stress inhibits nutrient and water absorption, in addition to transport, due to poor root development. Salinity hinders the process of photosynthesis. The primary sources of salt-induced growth inhibition include these modifications [32, 33]. Beneficial trace element supplements, such as B, enhance the antioxidant defense and plant's ability for stress tolerance. Through micronutrients, plants' antioxidant defense systems and physiological activities are boosted, ultimately increasing their stress response [34, 35].

Shoot growth is inhibited because of salinity stress. In soybeans, salinity reduced morphological parameters (shoot length, root length, fresh weight, and dry weight). Because salt stress causes plants to undergo ionic and osmotic stress, it impairs normal cell functioning, cell division, and elongation, thereby preventing plant growth [36, 37]. Boron is essential in various biological activities, including respiration, protein synthesis, sugar transport, and carbohydrate metabolism [38]. Wimmer, et al. [39] discovered that B promoted cell division in the region of active growth, particularly in the area adjacent to the stem and root tips. In the current investigation, B spray reversed the adverse effect that salt has on the qualities of growth. In the present research, the Chlorophyll content of the soybean plants decreased due to salt stress; however, the results showed that supplementing with B considerably alleviated the Chlorophyll losses. Boron synthesizes chlorophyll and nitrogen metabolism, which are necessary for cytoskeletal protein formation [40]. In salt-stressed roses, it has been found that Chlorophyll contents were effectively restored [41] and potato [42] plants that have been treated with boron.

Ionic stress is the fundamental constraint of salt stress, with sodium ion (Na^+) being the principal damaging ion accumulating excessively in the cytoplasm. This process results from moving potassium ions from the cytoplasm to the ectoplasm. Consequently, plant salt tolerance depends on the upkeep of cytoplasmic K^+/Na^+ balance as a critical component [43]. Our research findings

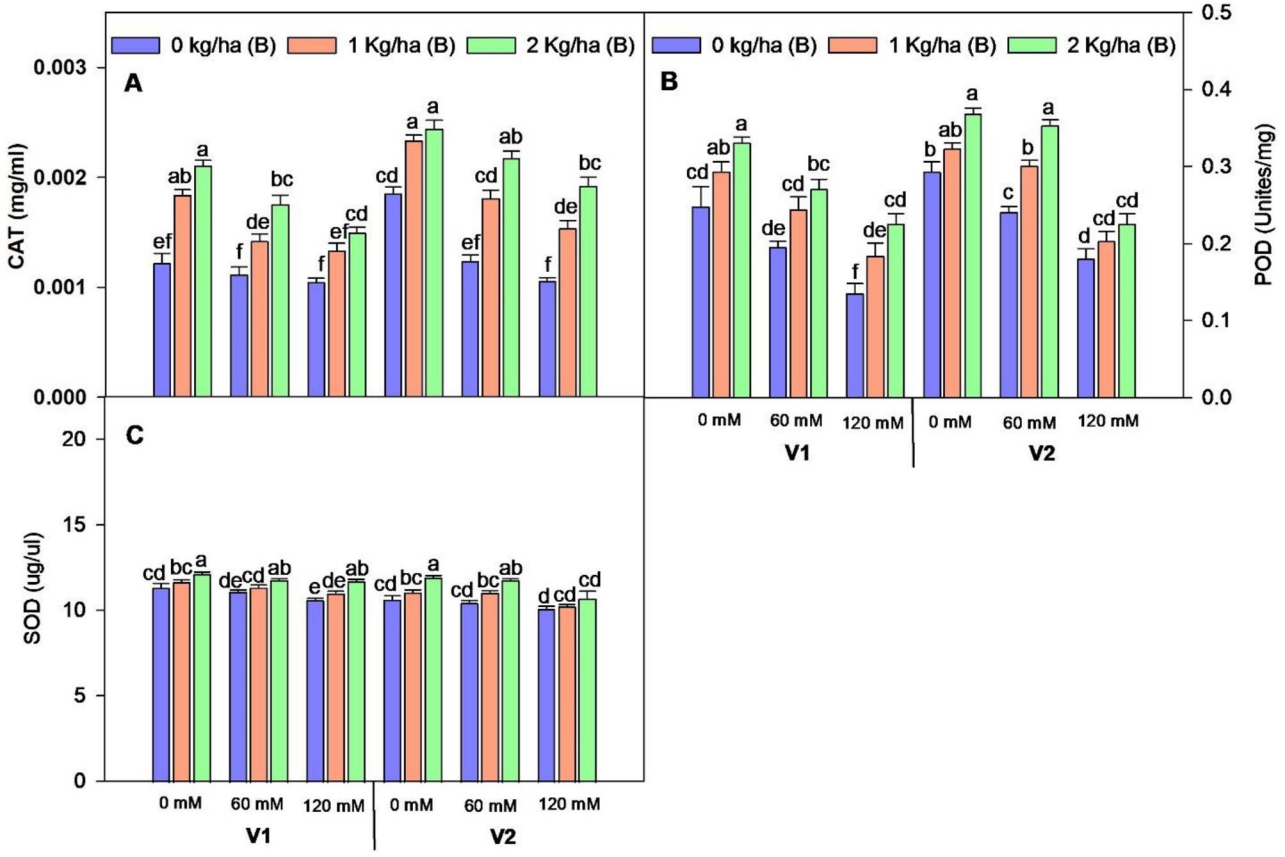


Fig. 8 Effect of exogenous B on CAT activity (**A**), POD activity (**B**) and SOD activity (**C**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

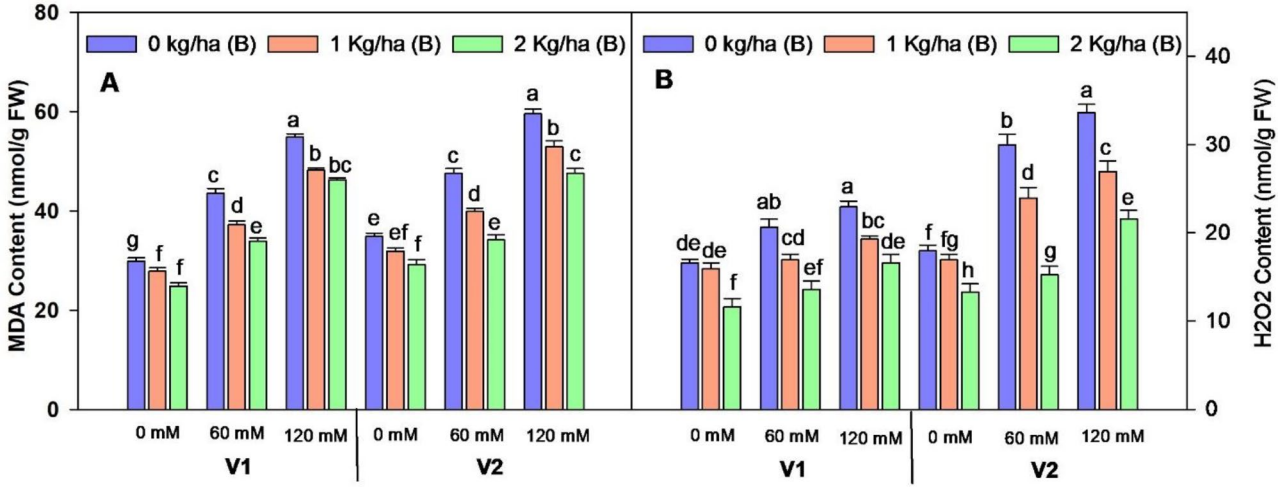


Fig. 9 Effect of exogenous B on MDA content (**A**) and H_2O_2 content (**B**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

demonstrated that salt stress in soybeans leads to significant Na^+ aggregation and a continuous decrease in K^+ levels. This resulted in K^+ and Na^+ entrance into plant cells being effectively inhibited. Excessive Na^+ ions that penetrate plant cells directly disturb cellular metabolism, subsequently leading to the efflux of K^+ ions [44]. An excessive amount of sodium ions in plants will cause Ca^{2+} to be displaced at the binding site, which will result in a reduction in the cross-linking of pectin and a delay in the expansion of the cell. The B treatment resulted in a

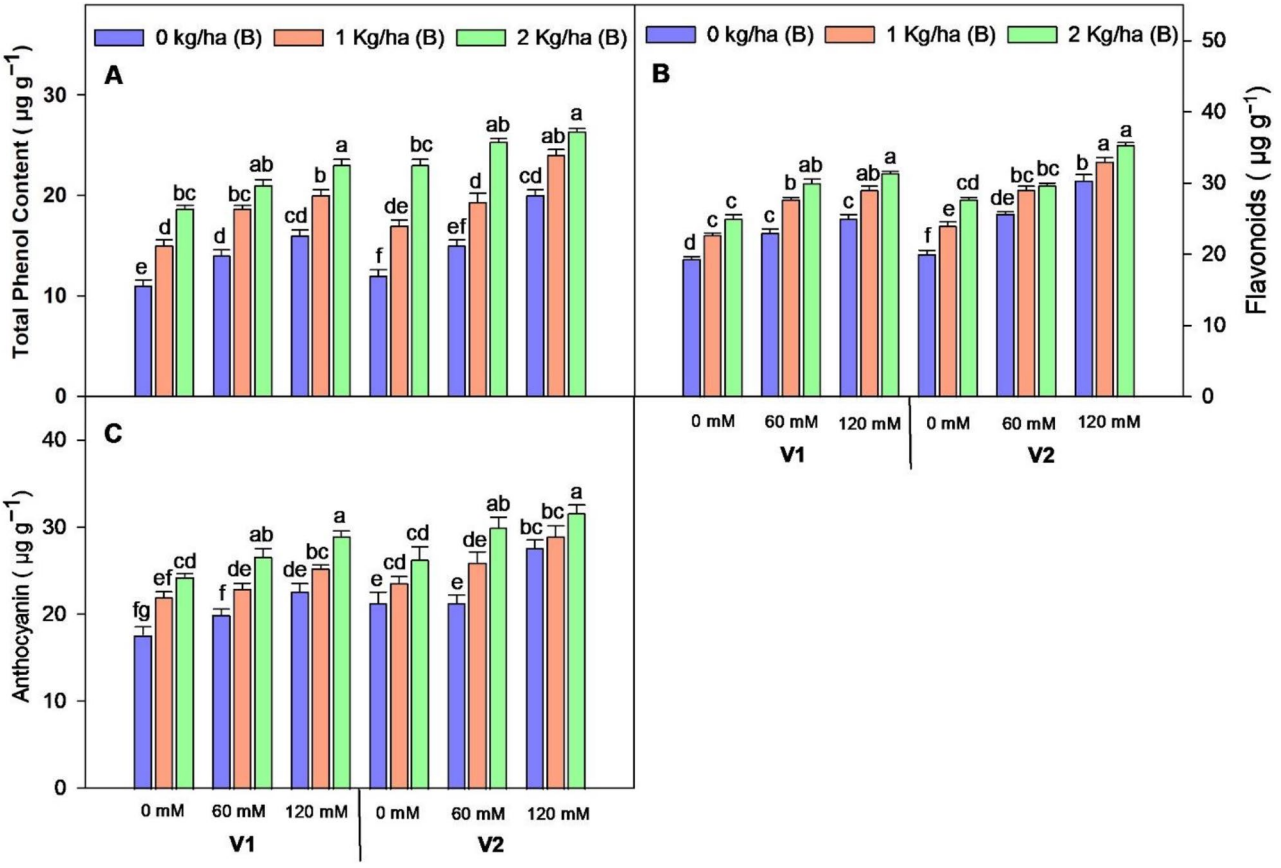


Fig. 10 Effect of exogenous B on Total Phenols content (**A**), Total Flavonoids content (**B**) and Anthocyanin Content (**C**) in two soybean cultivars (AARI-2021 and Ajmeri) under NaCl-induced salt stress. Different letters over the bars show significant differences at $p \leq 0.05$ applying Tukey's HSD test

significant decrease in the amount of sodium ions (Na^+) and an increase in the amount of potassium ions (K^+) in the roots, regardless of whether the treatment was salt or non-salt [45]. For resistance against adversity, the root system functions as the primary organ of the plant. Based on this, we hypothesized that B primarily exerted its effect on the roots of soybeans, thereby enhancing ion homeostasis and thus mitigating the negative impact that ionic stress has on the plant. Calcium is a fundamental constituent of the cell wall, with most Ca functioning as a pectineus structural element that maintains the cell wall's structure and function. Ca^{2+} may also be critically involved in salt damage mitigation, possibly by promoting K^+ absorption and reducing Na^+ toxicity [45, 46].

In disrupting H_2O_2, catalase produces both water and oxygen. Thus, CAT is essential to ROS detoxification when abiotic stress is present. According to prior research, several researchers found that the activity of CAT decreased in response to varying degrees of salt stress [47]. This may have resulted from increased hydrogen peroxide (H_2O_2), which triggers oxidative stress [48]. It was discovered that the activities of superoxide dismutase (SOD) and catalase (CAT) were at a lower level in soybean plants that had been damaged by salt. This may have resulted from increased hydrogen peroxide (H_2O_2), which stimulates the oxidative stress response [49]. POD activity was elevated in common beans under salt stress conditions [50]. Under salt-induced oxidative stress conditions, treating B at low concentrations resulted in an even more significant rise in the levels of POD activity [51].

Osmotic stress is brought on by salinity, which in turn brings about oxidative stress and limits the functioning of biological membranes via their inhibition. Biological membranes may also be damaged by oxidative damage when ion toxicity is present [52]. Increased malondialdehyde (MDA) levels were found in soybean plants subjected to salt. This led to a rise in hydrogen peroxide (H_2O_2) production, which in turn caused damage to the membranes. Numerous plant species have been seen to exhibit these typical responses to oxidative stress when they are subjected to salt [53]. Exogenous B supplementation was shown to reduce the oxidative stress caused by salinity in the present investigation. This was demonstrated by the reduced levels of hydrogen peroxide and malondialdehyde (MDA) in the soybean plants treated with salt. Studies conducted in previous years on the effects of B on salt stress responses have shown that B

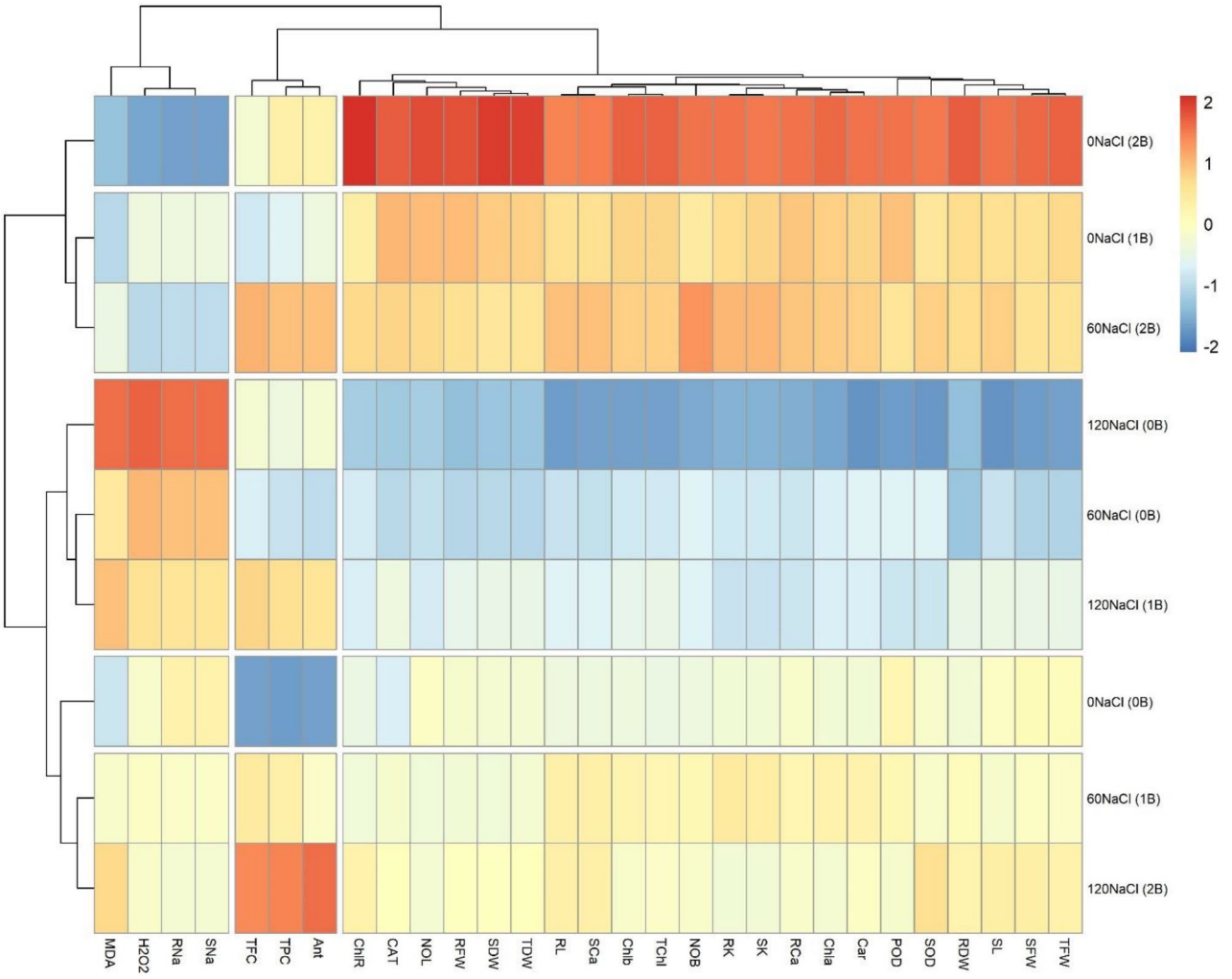


Fig. 11 Heatmap showing interaction of morpho-physiological, biochemical, and ionic attributes of soybean (AARI-2021) under salt stress. Where the 0NaCl = 0mM, 60NaCl = 60mM, 120NaCl = 120mM, 0B = 0 kg ha^{-1}, 1B = 1 kg ha^{-1}, 2B = 2 kg ha^{-1}

has a function in the reduction of oxidative stress. *Pistacia vera* leaves subjected to salt stress were shown to have lower levels of MDA and H_2O_2 when B was present [54]. Boron decreased MDA and H_2O_2 in aluminum-stressed plants. B supplementation in citrus plants under aluminum toxicity stress reduced H_2O_2 production and membrane lipid peroxidation [55].

To detoxify and adapt to stressful situations, plants exhibit physiological characteristics dependent on signaling and metabolic pathways. These characteristics allow plants to detoxify and adjust to the salinity stress [56]. According to the findings of this research, there is an interaction between significant amounts of secondary metabolites and the resistance of soybeans to salt treatments. The electron-donating abilities of phenols, flavonoids, and anthocyanins are responsible for the antioxidant characteristics. These characteristics contribute to reducing reactive oxygen species and protecting plants against the detrimental effects of stress [57, 58]. According to the results of our research, the rise in the levels of secondary metabolites in soybean plants that were treated with B at either low or high doses was significant. In line with our findings, Mahmoud, et al. [59] discovered that the presence of B in red radish root led to a considerable rise in the content of anthocyanins, phenols, and flavonoids.

Recent research by Gul, et al. [60] emphasizes the need for an effective stress mitigation method including increased sodium levels that lead to an increase in reactive oxygen species (ROS). The clear sub clusters of the heatmap analysis show the complex interactions between many different traits, displaying how some biochemical pathways (such as phenolic compounds pathways) respond differently to salinity and boron treatment. Boron might play a protective role at lower salinity levels, consistent with Cheng, et al. [61] who showed that boron application may protect against salt induced oxidative stress by elevating enzymatic antioxidant activities.

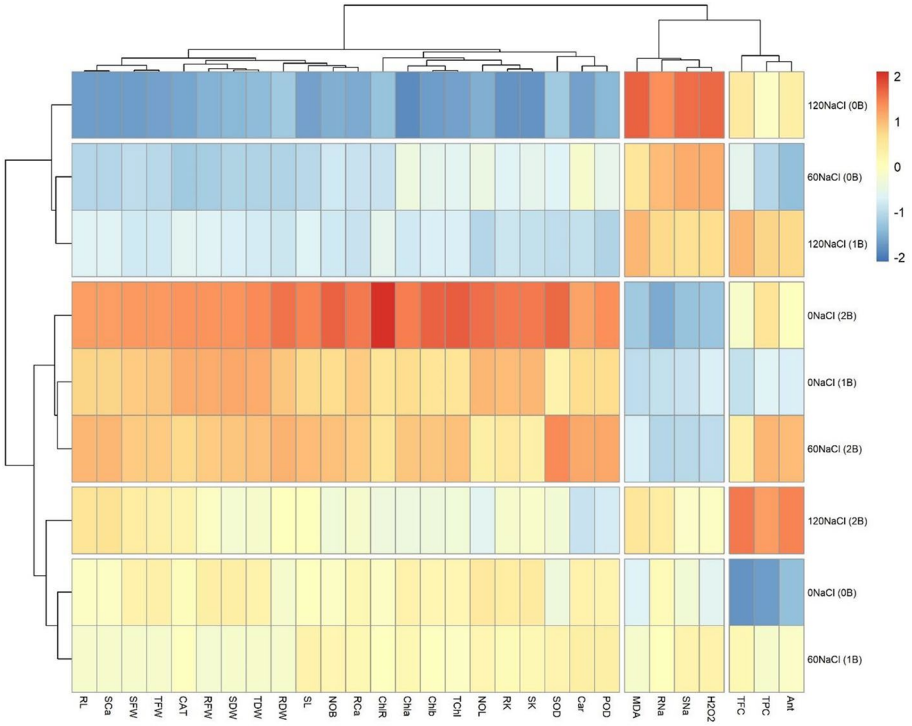


Fig. 12 Heatmap showing interaction of morpho-physiological, biochemical, and ionic attributes of soybean (Ajmeri) under salt stress. Where the 0NaCl = 0mM, 60NaCl = 60mM, 120NaCl = 120mM, 0B = 0 kg ha^{-1}, 1B = 1 kg ha^{-1}, 2B = 2 kg ha^{-1}

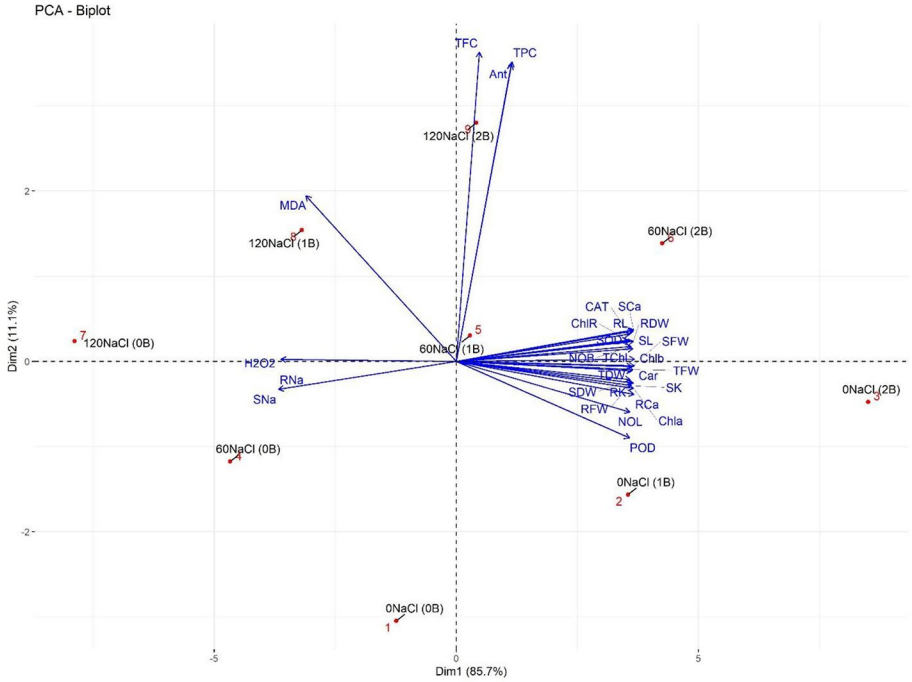


Fig. 13 PCA-Biplot of morpho-physiological, biochemical and ionic attributes of soybean (AARI-2021) under salt stress. Where the 0NaCl = 0mM, 60NaCl = 60mM, 120NaCl = 120mM, 0B = 0 kg ha^{-1}, 1B = 1 kg ha^{-1}, 2B = 2 kg ha^{-1}

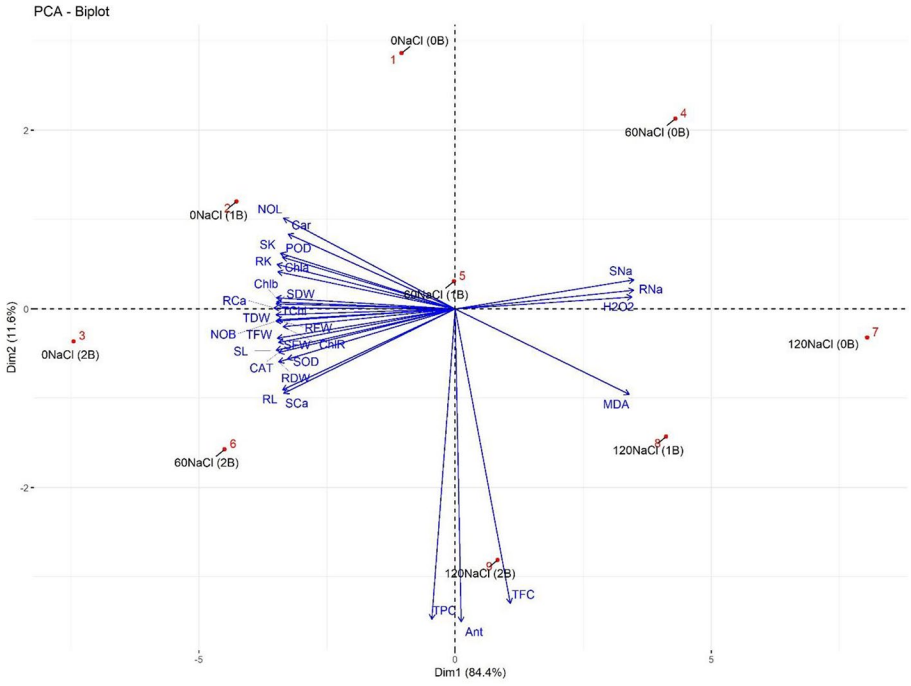


Fig. 14 PCA-Biplot of morpho-physiological, biochemical and ionic attributes of soybean (Ajmeri) under salt stress. Where the 0NaCl = 0mM, 60NaCl = 60mM, 120NaCl = 120mM, 0B = 0 kg ha^{-1}, 1B = 1 kg ha^{-1}, 2B = 2 kg ha^{-1}

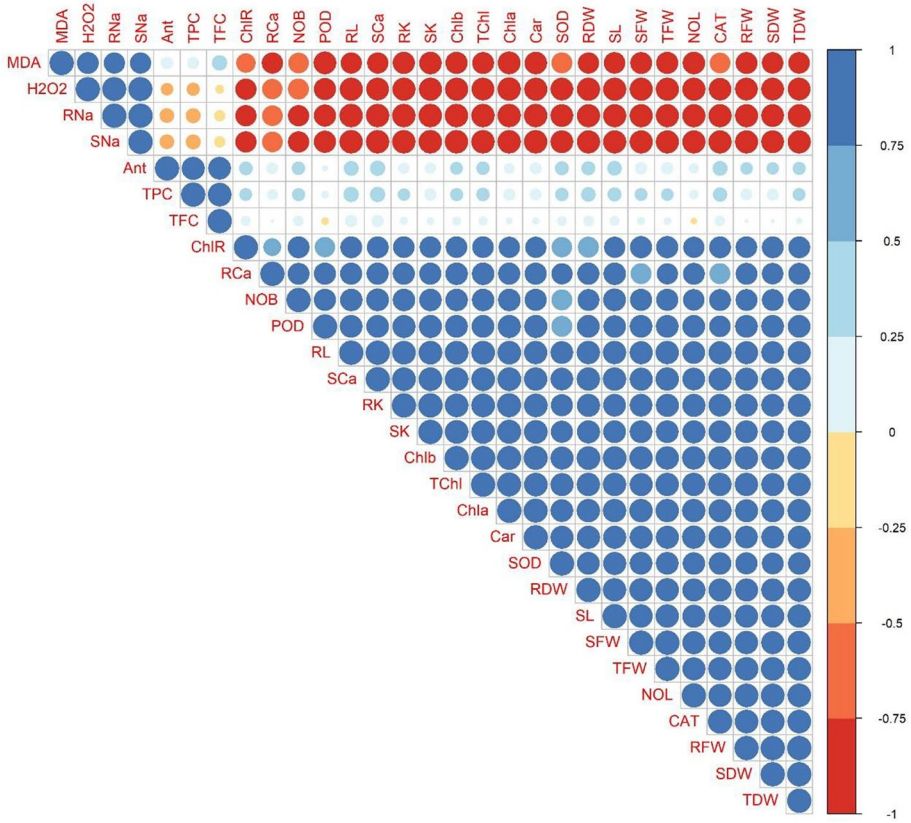


Fig. 15 Correlation-plot of morpho-physiological, biochemical and ionic attributes of soybean (AARI-2021) under salt stress

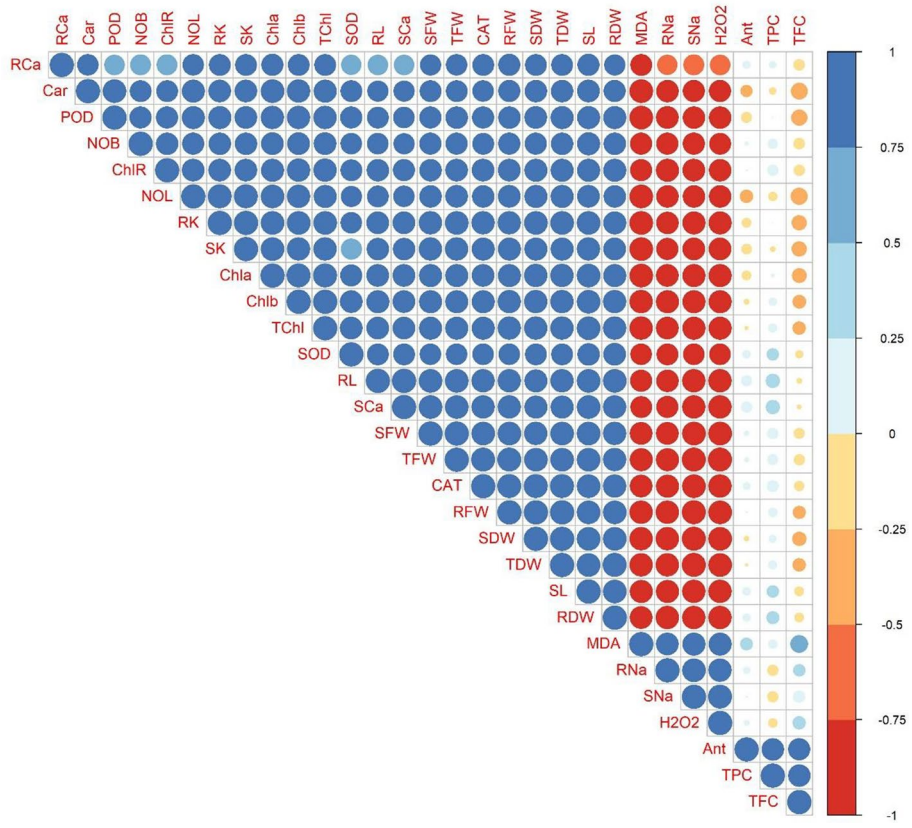


Fig. 16 Correlation-plot of morpho-physiological, biochemical and ionic attributes of soybean (Ajmeri) under salt stress

Moreover, the PCA results also reveal how different sets of traits, such as photosynthetic pigments and growth parameters, are altered by salinity and by boron treatment. This is consistent with recent work by Sivakumar, et al. [62] which employed PCA to identify salt tolerant traits in plants. Additionally, the correlation between stress tolerant induced secondary metabolites, namely total phenolics and total flavonoids, emphasizes the importance of secondary metabolites in providing oxidative damage protection.

Conclusion

The outcomes of the present research provide evidence that the administration of B foliar spray has a protective impact on both soybean cultivars, preventing the detrimental effects of salt stress and salt-induced oxidative stress. This is accomplished by increasing the number of enzyme activities that are part of the antioxidant defense system, which eventually reduces the production of reactive oxygen species. This reduction was indicated by the decreased levels of H_2O_2 and MDA in both cultivars. In the soybean plants stimulated by salt, adding foliar B increased plant growth, biomass, and photosynthetic pigment activity. Furthermore, ion homeostasis was preserved in plants treated with B by demonstrating increased K^+ and Ca^{2+} ions that accumulated in the root and shoot of soybeans when subjected to salt stress. Higher concentrations of boron help to mitigate the severe impact that salt stress has on both cultivars of soybean plants. This is accomplished by raising the synthesis of secondary metabolites, such as total phenols content, total flavonoids content and anthocyanins. However, B's specific function in salt stress tolerance and its intricate association with a diverse range of secondary metabolites and enzymatic antioxidant activity has not yet been wholly explored; hence, this leads the way for more investigation.

Abbreviations

NOL	Number of leaves
NOB	Number of branches
SL	Shoot length
RL	Root length
SFW	Shoot fresh weight
RFW	Root fresh weight
TFW	Total fresh weight
SDW	Shoot dry weight
RDW	Root dry weight
TDW	Total dry weight
chl a	Chlorophyll a
chl b	Chlorophyll b
car	Carotenoids
Tchl	Total chlorophyll
chlR	Chlorophyll ratio
RNa^+	Root sodium
RCa^{2+}	Root calcium
RK^+	Root potassium

SNa^+	Shoot sodium
SCa^{2+}	Shoot calcium
SK^+	Shoot potassium
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
H2O2	Hydrogen peroxide
MDA	Malondialdehyde
TPC	Total phenols content
TFC	Total flavonoids content
ANTs	Anthocyanin's

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Author contributions
MT; Conducted investigations and drafted paper, AM; supervised research, Conception and design, analyses and interpretation of the data, HFNA, AA, MMJ and BAK; analyses and interpretation of the data Drafting of paper; Application of Statistics Analyses and Software, MFS; Provide Resources and Revising it critically for intellectual content, AM, AW, FA and MFS; revising it critically for intellectual content; and the final approval of the version to be published. All authors agree to be accountable for all aspects of the work. All authors reviewed the manuscript.

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Data availability
Data will be provided if required.

Declarations

Ethics approval and consent to participate
N/A.

Consent for publication
All authors give consent to publish the data.

Competing interests
The authors declare no competing interests.

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