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RESEARCH ARTICLE

Impact of brassinosteroids on biochemical parameters of papaya (*Carica papaya* L. cv. Red Lady.) under salt stress

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Abstract

In fruit orchards, the excessive use of sodium chloride (NaCl) caused significant plant physiological stress and a decline in soil health. In order to counteract the negative effects of salinity stress, the current study was carried out in 2024 to assess how foliar spraying with brassinosteroids (BRs) affected the rate of expansion and biochemical functioning of *Carica papaya* L. cv. Red Lady. In fruit orchards, brassinosteroid foliar sprays were applied at concentrations of 0.05, 0.1 and 0.2 mg L^{-1} in combination with salinity treatments of 150, 200 and 250 mM NaCl, along with an untreated control. A number of physiological and biochemical parameters were evaluated in the study, such as membrane stability index, injury index, anthocyanin and carotenoid contents, proline accumulation, catalase activity, total chlorophyll, chlorophyll a, chlorophyll b and chlorophyll a/b ratio. The application of brassinosteroid was found to significantly mitigate the adverse consequences of salinity stress, as evidenced by better pigment and osmolyte levels as well as increased antioxidant enzyme activity. Biochemical parameters improved most noticeably with 0.1 mg L^{-1} and 0.2 mg L^{-1} BR in combination with 200 mM NaCl among the treatments. According to the research, papaya's ability to withstand salt can be successfully increased by foliar implementation of brassinosteroids, especially at 0.1 mg L^{-1}, which improves antioxidative defence and preserves membrane stability.

Keywords: brassinosteroids; foliar application; papaya; sodium chloride; salinity stress

Introduction

A highly important fruit crop, papaya (*Carica papaya* L.) is prized for their remarkable nutritional and therapeutic qualities. Because of its flexibility and simplicity of cultivation, it is commonly grown in tropical and subtropical regions. In the 16th century, Portuguese traders from Malacca introduced papaya to India; the crop originated in tropical America (1). Papaya is one of the most significant and delectable fruit crops cultivated in tropical and subtropical regions worldwide. It was developed in tropical America and subsequently spread to nearly all tropical regions. Papaya is rich in nutrients and is easy to cultivate. Its digestive and medicinal properties are attributed to the abundance of vitamins, minerals and catalysts found in its fruits, particularly papain (2). In order to overcome these obstacles, plant growth regulators-in particular, brassinosteroids (BRs), have drawn interest due to their capacity to increase abiotic stress tolerance. A class of naturally occurring polyhydroxylated steroidal phytohormones known as BRs controls a wide range of physiological and developmental activities in plants, including as pollen tube expansion, seed germination, cell division, cell elongation, embryogenesis and leaf senescence (3, 4). Numerous investigations have demonstrated that the exogenous administration of BRs, such as Epibrassinolide (EBL), can decrease membrane lipid peroxidation under stress, boost proline accumulation, improve antioxidant enzyme activity and raise chlorophyll content (5). This oxidative stress causes poor germination of seeds, stunted growth and decreased productivity by damaging cellular membranes, impairing the photosynthetic machinery and disrupting enzyme activity (6). However, abiotic stressors-among which salt stress is one of the most harmful-frequently limit papaya cultivation. Plant growth, physiological processes and biochemical markers are all negatively impacted by salinity, which eventually lowers crop output and quality. Elevated salt levels cause oxidative damage to plant tissues by upsetting ion homeostasis, water content and photosynthetic efficiency. They also encourage the overproduction of reactive oxygen species (ROS) (7). The ability of BRs to protect plants from salt-induced oxidative stress, along with increased activity of metabolite-scavenging systems and antioxidant enzymes within organelles, may be associated with BR-induced enhancement of plant growth under saline conditions (8). Additionally, it has been discovered that BRs reduce the negative effects of salinity by stabilizing chloroplast membranes, regulating ROS levels and enhancing photosynthetic system efficiency (9). BRs enhanced the light-harvesting capacity and the transcriptional and translational activities of enzymes implicated in chlorophyll synthesis, leading to improved chlorophyll production (10). By increasing apoplast hydrogen peroxide that stimulates the protective antioxidant system, BRs help cells tolerate oxidative stress. By reducing the damaging effects of ROS, preserving the makeup of proteins and lipids and reducing protein oxidation and lipid peroxidation, the exogenous application of BRs enhances membrane permeability and maintains the integrity of cellular membranes (11). On the other hand, exogenously applied EBL altered the activity of enzymes involved in proline metabolism (12). Examining practical methods to increase papaya's stress tolerance is crucial because of its sensitivity to salt stress. This study was conducted to assess the effects of varying brassinosteroid concentrations (0.05, 0.1 and 0.2 mg L^{-1}) on a number of biochemical parameters in papaya (cv. Red Lady) grown under varying salt stress levels (150, 200 and 250 mM NaCl). These parameters included membrane injury index, catalase activity, proline content, malondialdehyde (MDA), chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a/b ratio, anthocyanin and carotenoids. The purpose of this research is to determine whether using BRs can enhance both the physiological and biochemical functioning of papaya plants while reducing the negative impacts of salinity.

Materials and Methods

Experiment site

The current experiment was conducted at the Horticultural farm, Lovely Professional University and in the labs of its School of Agriculture in Phagwara, Punjab, in the period 2022–23. Phagwara is situated in the Doaba region of Punjab and lies at 31.25° N latitude and 75.70° E longitudes at an altitude elevation of 249 m on top of the ocean level.

Experimental material

The experiment was carried out under controlled greenhouse conditions. A commercial potting mixture of cocopeat, vermicompost and sand (1:1:1, v/v/v) was used to sprout papaya seeds (*C. papaya* L. cv. Red Lady). With an annual rainfall of roughly 957 mm, the greenhouse's average temperature was 40 °C during the summer (May–June) and just 4 °C during the winter (December–January). The sprinkler system was used to apply irrigation once every 8–10 days in the winter and once a week during the warm season. In order to guarantee steady growth, the seedlings were given consistent irrigation. Sodium chloride was added to irrigation water in three different concentrations and BRs were sprayed on leaves. For the treatments, plants with comparable vigor and morphology were chosen in order to reduce variability.

Treatment use and experimental design

BRs was sprayed on plants as a foliar spray at three different concentrations (0.05, 0.1 and 0.2 mg L^{-1}) and a control (water only) in a completely randomized design (CRD) with three replications. Plants were irrigated with 150 mM, 200 mM and 250 mM NaCl solutions to induce salinity stress (Table 1).

Forty days after planting, BRs were first applied foliarly and it was then repeated every five days. Every fifteen days, salinity treatments were administered. A hand sprayer was used to apply the product early in the morning and it was carefully cleaned prior to and following each use.

Table 1. Treatment details showing different levels of NaCl-induced salinity stress combined with varying concentrations of brassinosteroids.

Treatments	Treatments details
T_1	Control
T_2	NaCl (150 mM) + Brassinosteroids (0.05 mg L^{-1})
T_3	NaCl (150 mM) + Brassinosteroids (0.1 mg L^{-1})
T_4	NaCl (150 mM) + Brassinosteroids (0.2 mg L^{-1})
T_5	NaCl (200 mM) + Brassinosteroids (0.05 mg L^{-1})
T_6	NaCl (200 mM) + Brassinosteroids (0.1 mg L^{-1})
T_7	NaCl (200 mM) + Brassinosteroids (0.2 mg L^{-1})
T_8	NaCl (250 mM) + Brassinosteroids (0.05 mg L^{-1})
T_9	NaCl (250 mM) + Brassinosteroids (0.1 mg L^{-1})
T_{10}	NaCl (250 mM) + Brassinosteroids (0.2 mg L^{-1})

Biochemical parameter estimation

Proline Content

Proline content was estimated by standardazied protocol (13). In order to calculate the membrane stability index (MSI) and membrane injury index (MII), 200 mg of fully grown young leaves were homogenized in 10 mL of distilled water. A conductivity metre (Model ME977-C, Max Electronics, India) was used to test conductivity. For the initial conductivity ($C_{initial}$), the first set of leaf samples was incubated at 40 °C for 30 minutes and for the final conductivity (C_{total}), the second set was boiled at 100 °C for 10 minutes. Utilizing the ratio of $C_{initial}$ to C_{total}, MSI and MII were computed.

Peroxidation of lipids (MDA)

Using the thiobarbituric acid (TBA) method, the amount of MDA was used to measure lipid peroxidation. Trichloroacetic acid (TCA) 5 % (w/v) (5 mL) was used to homogenize about 0.5 g of leaf tissue, which was then centrifuged for 15 min at 12000 rpm. Supernatant and 0.5 % (w/v) TBA prepared in 20 % (w/v) TCA were combined in equal volumes and the mixture was heated to 96 °C for 25 min. The resulting mixture was centrifuged for 5 min at 10000 rpm after being quickly cooled. After accounting for indiscriminate turbidity at 600 nm, absorbance was measured at 532 nm. An extinction value of 155 mM^{-1} cm^{-1} was used to compute the MDA concentration, which was then reported as µmol MDA g^{-1} fresh weight (FW).

Catalase activity

Activity of catalase (CAT; EC 1.11.1.6) was measured in accordance with standardized protocol (14). After 100 mg of fresh leaf tissue has been homogenized in 5 mL of 0.1 M phosphate buffer (pH 7.0), it was centrifuged at 10000 × g for 20 min at 4 °C. There were 2.6 mL of 0.1 M phosphate buffer, 0.1 mL of enzyme extract and 0.1 mL of 1 % (v/v) H_2O_2 in the reaction mixture. For 2 min, the absorbance drop at 240 nm was measured at 15 s intervals. The attenuation coefficient of 43.6 mM^{-1} cm^{-1} was used to compute catalase activity, which was then represented in units of g^{-1} FW.

Chlorophyll content

Leaf tissue weighing 100 mg was extracted using 20 mL of 80 % acetone and centrifuged for 10 min at 5000 rpm (15). After collecting the supernatant, extraction was carried out once more until the residue had lost its hue. To make 100 mL of the combined extract, 80 % acetone was used. To measure absorbance, a spectrophotometer (Spectramax M2) was used at 645 and 663 nm. The amounts of chlorophyll a, b and total chlorophyll were determined using established formulas and reported as mg g^{-1} FW.

Anthocyanin content

The extract was filtered and centrifuged after fresh tissue was crushed in 1 % acidified methanol. 3 mL of methanolic HCl and 1 mL of anthocyanin reagent were combined with 1 mL of the extract. At 525 nm, absorbance was measured following a 15 min dark incubation period. Utilizing a cyanidin chloride standard curve, the amount of anthocyanin was measured and reported as µg g^{-1} FW (16).

Carotenoid content

To extract carotenoids, 2 g of fresh tissue was homogenized in 20 mL of acetone until it was colourless. The extract was separated three times using ether free of peroxide in a separating funnel after being filtered through Whatman No. 42 paper. The residue from the collected ether fraction was dissolved in ethanol after it was vaporized at 35 °C with lowered pressure. Overnight saponification using 60 % aqueous KOH (1:10 v/v) was performed. After a water wash, ether was used to extract the combination once more. The final extract's absorbance was measured at 450 nm and a β-carotene standard curve was used to quantify the carotenoid concentration.

Analytical statistics

Every experiment was run in triplicate. Software called SPSS (version 16.0) was used to examine the data. The effects of treatments were assessed using analysis of variance (ANOVA) and mean separation was carried out at a level of significance of $p \leq 0.05$ using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Brassinosteroids effect on the presence of MDA and proline content

The MDA concentration of papaya leaves under salinity stress rose noticeably in comparison to the control, suggesting increased oxidative stress-induced lipid peroxidation and damage to membranes. Treatments T_1 (control), T_2 and T_6 recorded MDA concentrations of 0.00206, 0.00211 and 0.00288 µmol g^{-1} FW respectively, but no significant differences were found between them. Similar MDA values (0.00001 µmol g^{-1} FW) were also shown by treatments T_7, T_8, T_9 and T_{10}, indicating that the use of BRs successfully reduced membrane lipid peroxidation at increasing salinity levels (Table 2, Fig. 1).

Proline accumulation under salt stress varied significantly. Proline content significantly increased in the presence of NaCl as compared to the untreated control, indicating that it plays a role as a suitable osmolyte that aids in osmotic adjustment. The most significant proline content (5.01 µmol g^{-1} FW) was reported by T_6 (0.1 mg L^{-1} BRs under salinity stress) out of all treatments, far surpassing the control and other BRs concentrations (0.05 and 0.2 mg L^{-1}). This implies that the plant's ability to withstand salt is improved when the BRs concentration is at its ideal level because it promotes the biosynthesis or accumulation of proline. In contrast to BRs-treated plants, plants subjected to NaCl alone displayed lower proline levels, suggesting that BRs administration mitigated the adverse effects of salt stress by promoting osmoprotectants accumulation (Table 2, Fig. 2).

Brassinosteroids effect on the presence of membrane stability and injury index

Plant reactions to salt stress were significantly influenced by the MSI. The findings demonstrated that the brassinosteroids treatment significantly improved the stability of membranes and the injury index. The treatment T_{10} showed its best results in MSI with 0.2 mg L^{-1} brassinosteroids and 250 mM NaCl as compared to the control (T_1) without the application of BRs and NaCl. BRs treatment significantly decreased the MII compared with the control under salt stress conditions. A slight increase in MSI and decrease in MII was detected in papaya leaves under T_{10} (BRs 0.2 mg L^{-1}) and T_9 (BRs 0.1 mg L^{-1}) was 99.53 and 57.89 respectively. (Table 2, Fig.3).

Brassinosteroids effect on the presence of catalase activity, anthocyanin and carotenoid content under salt stress

Salt stress resulted in decreased catalase activity and reduced anthocyanin and carotenoid contents compared with the control. However, T_6 and T_7 treatments (NaCl 200 mM) led to a significant increase in these parameters. Catalase activity was highest in T_6 (76.13 µmol g^{-1}) (Table 3, Fig. 4), anthocyanin content was highest in T_6 (0.44 mg g^{-1}) (Table 3, Fig. 5) and carotenoid content was highest in T_7 (18.92 mg g^{-1}) (Table 3, Fig. 6) compared with the NaCl 150 mM and 250 mM treatments.

Table 2. Brassinosteroids effect on the presence of MDA content, proline content, membrane stability index and membrane injury index under salt stress

Treatments		MDA content (µmol g^{-1})	Proline content (µmol g^{-1})	Membrane stability index	Membrane injury index
T_1	Control	0.00206^{b}	$3.83^{abc} \pm 1.05$	$98.96^{bcd} \pm 0.113$	$103.93^{abc} \pm 11.83$
T_2	NaCl (150 mM) + Brassinosteroids (0.05 mg L^{-1})	0.00211^{b}	$3.98^{ab} \pm 0.99$	$99.15^{abc} \pm 0.086$	$84.126^{bcd} \pm 8.46$
T_3	NaCl (150 mM) + Brassinosteroids (0.1 mg L^{-1})	0.00288^{a}	$2.57^{cde} \pm 0.37$	$99.28^{ab} \pm 0.900$	$71.54^{cd} \pm 9.16$
T_4	NaCl (150 mM) + Brassinosteroids (0.2 mg L^{-1})	0.00046^{c}	$3.70^{abcd} \pm 0.30$	$98.75^{d} \pm 0.145$	$123.76^{a} \pm 14.70$
T_5	NaCl (200 mM) + Brassinosteroids (0.05 mg L^{-1})	0.00023^{d}	$2.45^{de} \pm 0.28$	$99.28^{ab} \pm 0.111$	$71.25^{cd} \pm 10.90$
T_6	NaCl (200 mM) + Brassinosteroids (0.1 mg L^{-1})	0.00211^{b}	$5.01^{a} \pm 0.79$	$98.84^{cd} \pm 0.254$	$115.33^{ab} \pm 25.25$
T_7	NaCl (200 mM) + Brassinosteroids (0.2 mg L^{-1})	0.00001^{e}	$4.53^{a} \pm 0.79$	$98.99^{bcd} \pm 0.153$	$100.04^{abc} \pm 15.33$
T_8	NaCl (250 mM) + Brassinosteroids (0.05 mg L^{-1})	0.00001^{e}	$2.39^{de} \pm 0.41$	$99.04^{bcd} \pm 0.262$	$95.15^{abc} \pm 26.17$
T_9	NaCl (250 mM) + Brassinosteroids (0.1 mg L^{-1})	0.00001^{e}	$1.93^{e} \pm 0.46$	$99.41^{a} \pm 0.179$	$57.89^{d} \pm 18.14$
T_{10}	NaCl (250 mM) + Brassinosteroids (0.2 mg L^{-1})	0.00001^{e}	$2.72^{bcde} \pm 1.06$	$99.53^{abc} \pm 0.192$	$84.10^{bcd} \pm 19.07$

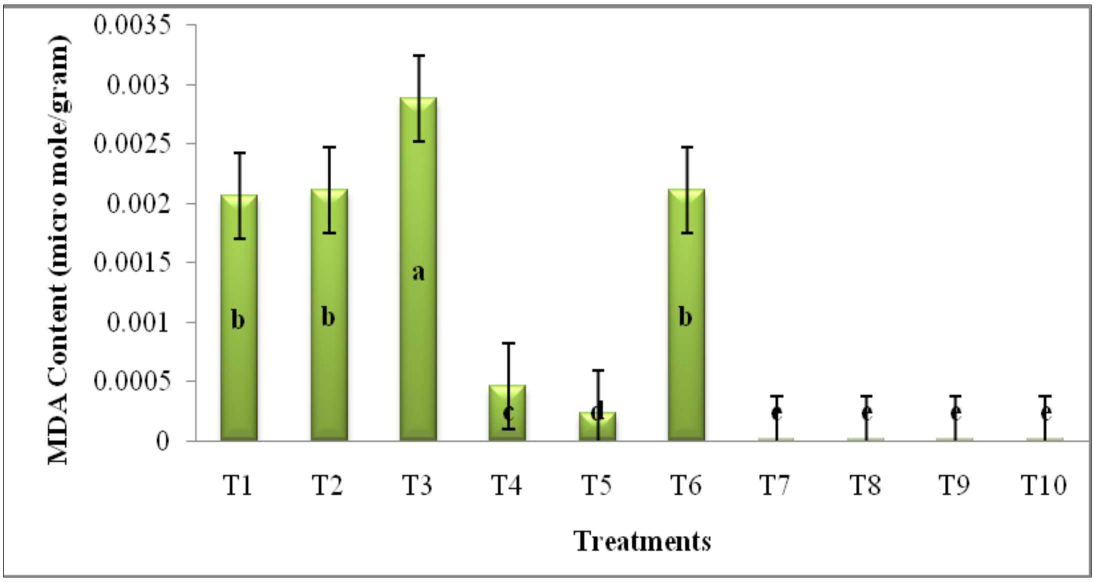

Fig. 1. Brassinosteroids effect on the presence of MDA content under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars ($p < 0.05$). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

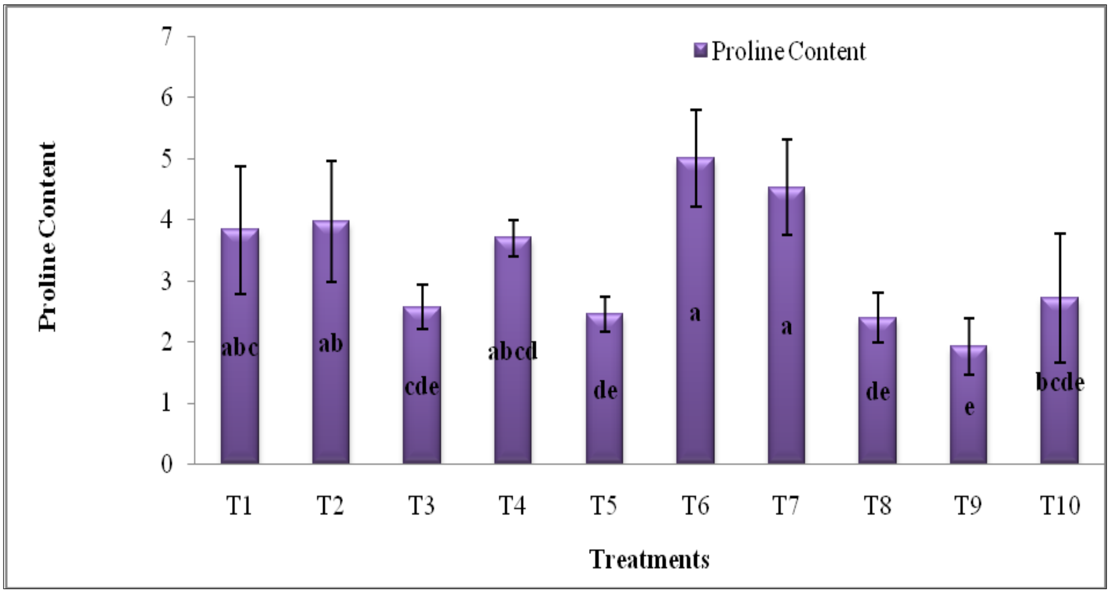

Fig. 2. Brassinosteroids effect on the presence of proline content under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars ($p < 0.05$). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

Table 3. Brassinosteroids effect on the presence of catalase activity, anthocyanin content and total carotenoids under salt stress

	Treatments	Catalase activity	Anthocyanin content	Total carotenoids
T_1	Control	$50.71^{e} \pm 0.25$	$0.24^{cd} \pm 0.07$	$8.46^{cd} \pm 2.78$
T_2	NaCl (150 mM) + Brassinosteroids (0.05 mg L^{-1})	$50.34^{e} \pm 0.10$	$0.27^{abc} \pm 0.03$	$8.44^{cd} \pm 2.74$
T_3	NaCl (150 mM) + Brassinosteroids (0.1 mg L^{-1})	$40.17^{g} \pm 0.25$	$0.25^{cd} \pm 0.03$	$5.54^{d} \pm 0.36$
T_4	NaCl (150 mM) + Brassinosteroids (0.2 mg L^{-1})	$69.53^{b} \pm 0.46$	$0.26^{cd} \pm 0.09$	$6.34^{cd} \pm 0.63$
T_5	NaCl (200 mM) + Brassinosteroids (0.05 mg L^{-1})	$14.88^{i} \pm 0.10$	$0.30^{abc} \pm 0.01$	$9.04^{cd} \pm 0.46$
T_6	NaCl (200 mM) + Brassinosteroids (0.1 mg L^{-1})	$76.13^{a} \pm 0.15$	$0.44^{a} \pm 0.05$	$17.12^{a} \pm 2.35$
T_7	NaCl (200 mM) + Brassinosteroids (0.2 mg L^{-1})	$64.45^{c} \pm 0.21$	$0.35^{b} \pm 0.05$	$18.92^{a} \pm 2.68$
T_8	NaCl (250 mM) + Brassinosteroids (0.05 mg L^{-1})	$17.07^{h} \pm 0.40$	$0.31^{bc} \pm 0.05$	$17.60^{a} \pm 1.83$
T_9	NaCl (250 mM) + Brassinosteroids (0.1 mg L^{-1})	$47.51^{f} \pm 0.20$	$0.21^{d} \pm 0.01$	$11.37^{bc} \pm 1.15$
T_{10}	NaCl (250 mM) + Brassinosteroids (0.2 mg L^{-1})	$52.22^{d} \pm 0.21$	$0.28^{abc} \pm 0.06$	$15.62^{ab} \pm 6.38$

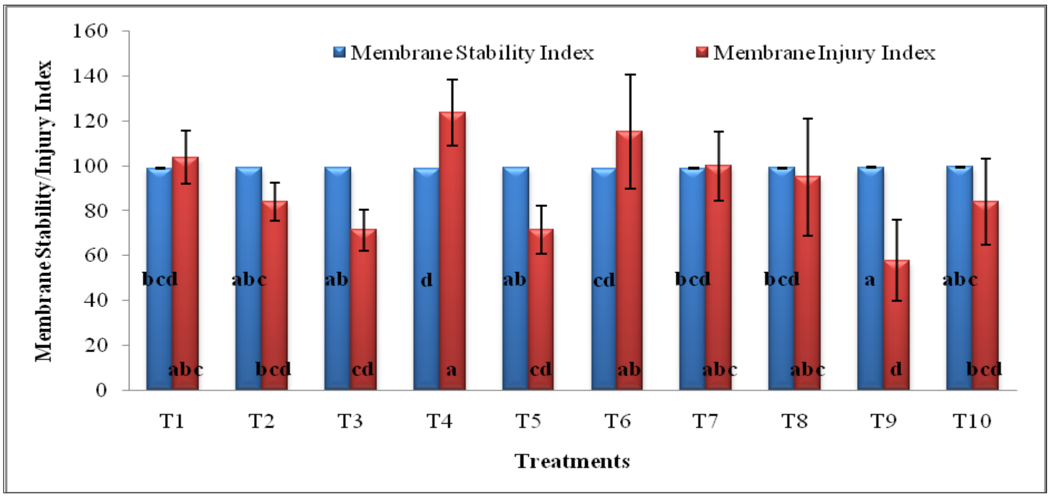

Fig. 3. Brassinosteroids effect on the presence of membrane stability and injury index under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars ($p < 0.05$). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

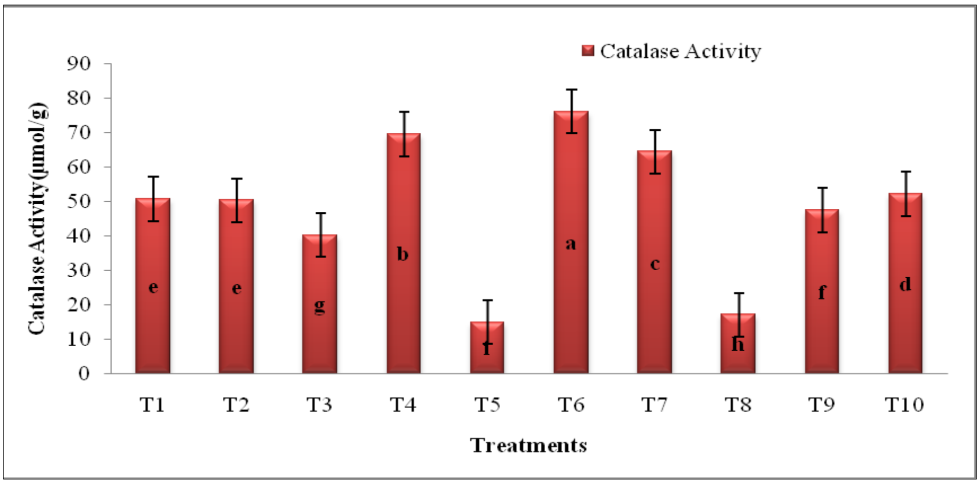

Fig. 4. Brassinosteroids effect on the presence of catalase activity under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars ($p < 0.05$). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

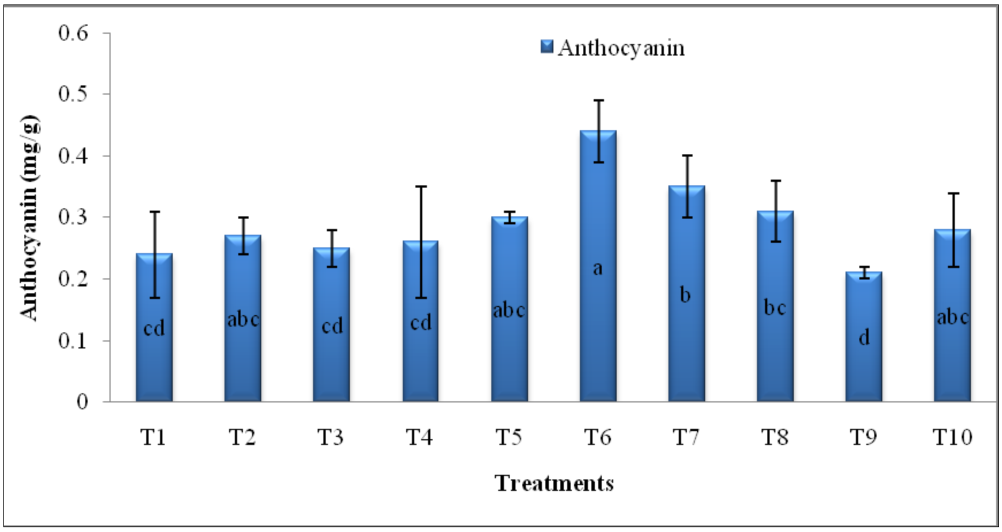

Fig. 5. Brassinosteroids effect on the presence of anthocyanin content under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars ($p < 0.05$). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

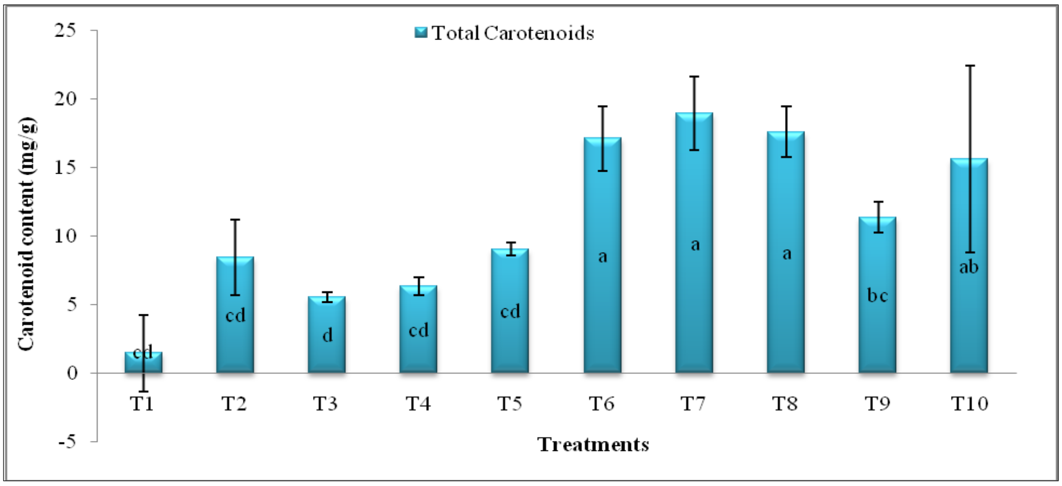

Fig. 6. Brassinosteroids effect on the presence of carotenoid content under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars ($p < 0.05$). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg

Brassinosteroids effect on the presence of chlorophyll b, chlorophyll a, total chlorophyll and chlorophyll a/b ratio under salt stress

Under salt stress conditions over-exploitation of NaCl will cause the papaya plant damage, while the contents of chlorophyll b, chlorophyll a and total chlorophyll showed the highest value in T_7 compared to the T_1(control). Treatment T_7 showed the highest chlorophyll content, with values of 9.07 mg g^1 (Table 4, Fig. 7), 13.67 mg g^1 (Table 4, Fig. 8) and 10.02 mg g^1 under 200 mM NaCl + 0.2 mg L^{-1} BRs (Table 4, Fig. 9). The chlorophyll a/b ratio did not differ significantly among treatments T_1, T_8, T_9 and T_{10}. Similarly, no significant differences in the chlorophyll a/b ratio were observed among the values 1.51, 1.51, 1.55 and 1.55 respectively (Table 4, Fig. 10).

BRs are typically believed to serve a variety of advantageous roles regarding plant physiology, biochemistry and expansion, especially in plants exposed to multiple sub-optimal constraints (17). BRs not only controls different processes associated with development and growth but also has a significant impact on reducing both abiotic and biotic stress in plants. Catalase is an antioxidant enzyme that can be enhanced by BRs in tomato plants that are grown in highly salinized soil (18). BRs can increase the production of other antioxidant enzymes in plants under salt stress, including superoxide dismutase, hydrogen peroxidase and ascorbate peroxidase (19). BRs can boost the enzymatic activity of H ± ATPase and the enzymatic process that breaks down cell walls, boosting cell development by encouraging proliferation and expansion (20). BRs can improve membrane stability and reduce lipid peroxidation under saline conditions (21). By reducing the extent of ion accumulation, BRs can improve membrane stability and maintain membrane integrity, leading to an increase in the MSI (22). BRs can reduce membrane damage and increase membrane stability under salt stress. It can be hypothesized that the application of BRs can decrease the MII in plants under salt stress (23). Proline can stabilize lipids and protein molecules, buffer physiological redox activity and serve as ROS scavenger. Proline is believed to accumulate more in cell cytosol under stressful circumstances, enhancing the cell's capacity for ionic changes (24). Its buildup is inversely correlated with plants' ability to withstand stress. One of its most widely used compounds for protection is proline, found in stressful environments, such as those with high salinity or brassinolide (BL) application (25). BRs have been shown to increase the activity of genes involved in proline biosynthesis, thereby enhancing proline accumulation in plants (26). Plant cells generate and circulate proline in their cytoplasm to other parts of the plant through specialized transporters. Salt exposure resulted in cellular membrane wreckage (more electrolyte leakage), peroxidation of lipids (more MDA) and reduced activity of photosynthesis (27). Implementation of 24-epibrassinolide (EBR) at 0.05, 0.1 and 0.2 mg L^{-1} reduced damage to the membrane of cells and the action of photosynthesis was restored by saltwater. Higher salinity conditions caused an upsurge in MDA levels and lipid peroxidation, while greater levels of BRs caused a decrease in MDA content and peroxidation of lipids in the leaves (28). Chlorophyllase, an enzyme that catalyzes the breakdown of chlorophyll molecules, has

Table 4. Brassinosteroids effect on the presence of chlorophyll b, chlorophyll a, chlorophyll a/b ratio under salt stress

	Treatments	Chlorophyll b	Chlorophyll a	Total chlorophyll	Chlorophyll a/b
T_1	Control	$4.73^{cd} \pm 1.52$	$7.12^{cd} \pm 2.18$	$5.22^{cd} \pm 1.68$	$1.51^{a} \pm 0.03$
T_2	NaCl (150 mM) + Brassinosteroids (0.05 mg L^{-1})	$4.55^{cd} \pm 1.22$	$6.46^{cd} \pm 1.84$	$5.01^{cd} \pm 1.35$	$1.41^{b} \pm 0.02$
T_3	NaCl (150 mM) + Brassinosteroids (0.1 mg L^{-1})	$2.80^{d} \pm 0.26$	$3.61^{d} \pm 0.38$	$3.07^{d} \pm 0.29$	$1.29^{c} \pm 0.04$
T_4	NaCl (150 mM) + Brassinosteroids (0.2 mg L^{-1})	$3.21^{cd} \pm 0.12$	$4.20^{d} \pm 0.20$	$3.52^{cd} \pm 0.13$	$1.30^{c} \pm 0.01$
T_5	NaCl (200 mM) + Brassinosteroids (0.05 mg L^{-1})	$5.10^{cd} \pm 0.38$	$7.08^{cd} \pm 0.65$	$5.61^{cd} \pm 0.42$	$1.38^{b} \pm 0.02$
T_6	NaCl (200 mM) + Brassinosteroids (0.1 mg L^{-1})	$8.53^{a} \pm 1.53$	$11.97^{ab} \pm 2.15$	$9.38^{a} \pm 1.66$	$1.40^{b} \pm 0.006$
T_7	NaCl (200 mM) + Brassinosteroids (0.2 mg L^{-1})	$9.07^{a} \pm 0.35$	$13.67^{a} \pm 0.12$	$10.02^{a} \pm 0.37$	$1.50^{a} \pm 0.04$
T_8	NaCl (250 mM) + Brassinosteroids (0.05 mg L^{-1})	$8.60^{a} \pm 1.76$	$13.01^{a} \pm 2.61$	$9.50^{a} \pm 1.94$	$1.51^{a} \pm 0.01$
T_9	NaCl (250 mM) + Brassinosteroids (0.1 mg L^{-1})	$5.70^{bc} \pm 0.66$	$8.88^{bc} \pm 1.25$	$6.31^{bc} \pm 0.74$	$1.55^{a} \pm 0.04$
T_{10}	NaCl (250 mM) + Brassinosteroids (0.2 mg L^{-1})	$8.15^{ab} \pm 3.37$	$12.61^{ab} \pm 4.95$	$9.01^{ab} \pm 3.71$	$1.55^{a} \pm 0.03$

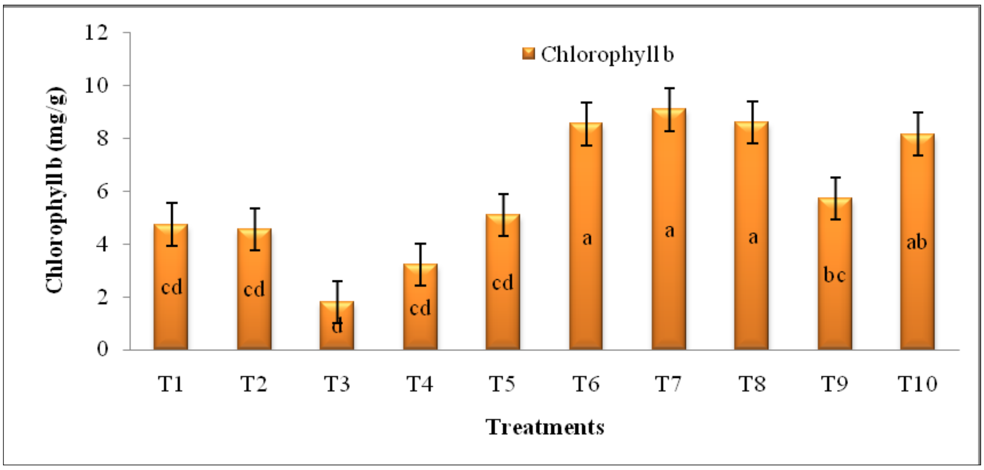

Fig. 7. Brassinosteroids effect on the presence of chlorophyll b under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars (*p* < 0.05). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

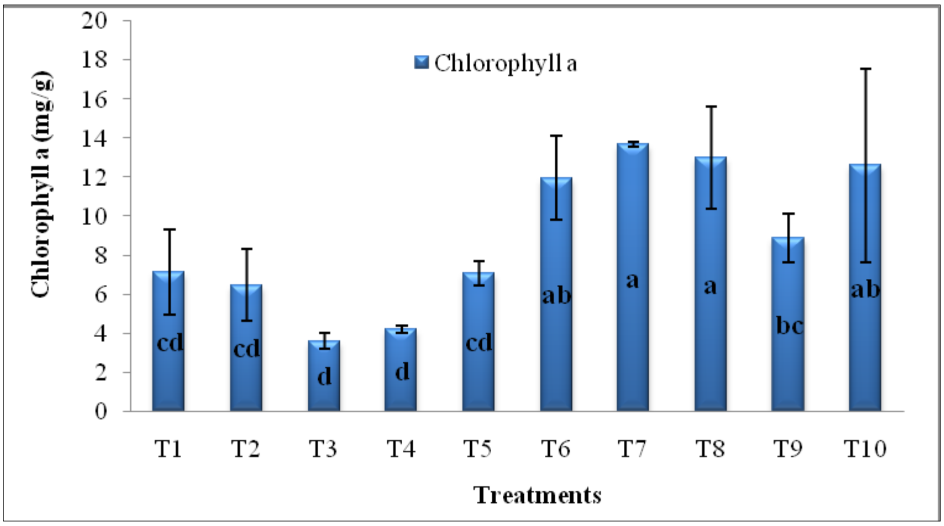

Fig. 8. Brassinosteroids effect on the presence of chlorophyll a ratio under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars (*p* < 0.05). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

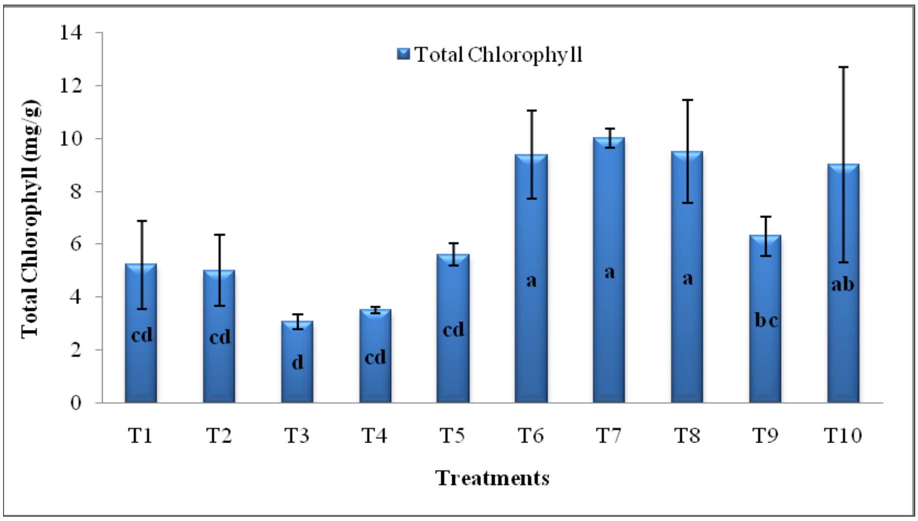

Fig. 9. Brassinosteroids effect on the presence of total chlorophyll under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars (*p* < 0.05). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

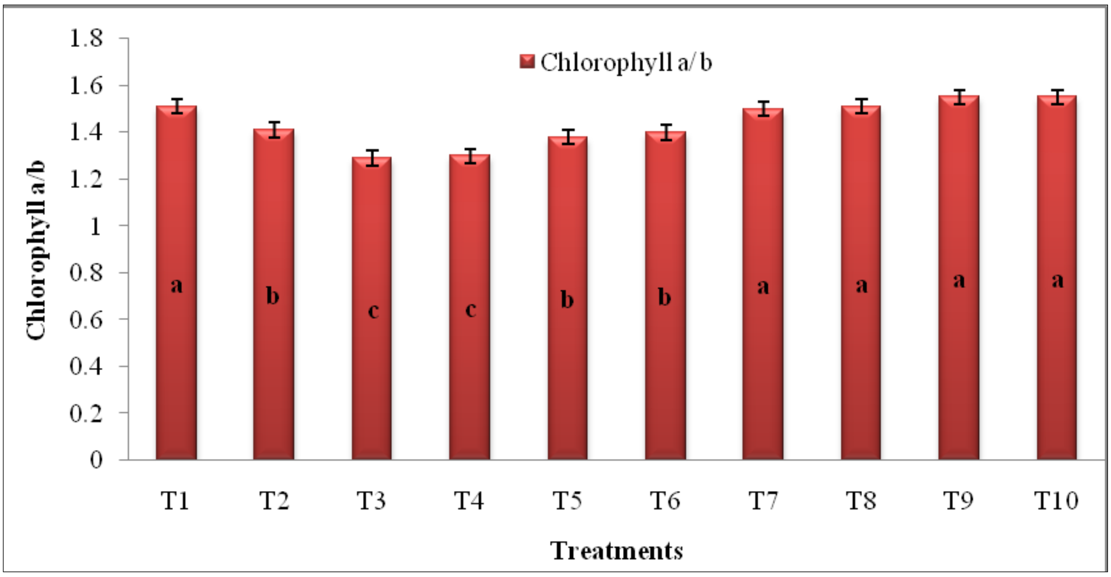

Fig. 10. Brassinosteroids effect on the presence of chlorophyll a/b ratio under salt stress.

According to Duncan's Multiple Range Test, significant differences are indicated by different letters in the bars ($p < 0.05$). (T1 = Control, T2 = NaCl 150 mM + Brassinosteroids 0.05 mg L^{-1}, T3 = NaCl 150 mM + Brassinosteroids 0.1 mg L^{-1}, T4 = NaCl 150 mM + Brassinosteroids 0.2 mg L^{-1}, T5 = NaCl 200 mM + Brassinosteroids 0.05 mg L^{-1}, T6 = NaCl 200 mM + Brassinosteroids 0.1 mg L^{-1}, T7 = NaCl 200 mM + Brassinosteroids 0.2 mg L^{-1}, T8 = NaCl 250 mM + Brassinosteroids 0.05 mg L^{-1}, T9 = NaCl 250 mM + Brassinosteroids 0.1 mg L^{-1}, T10 = NaCl 250 mM + Brassinosteroids 0.2 mg L^{-1})

increased activity. It is possible that control of translation and transcription in restitution of the amount of chlorophyll after exogenous BL injection is caused by processes involving BRs signaling, which in turn increases chlorophyll formation and prevents the destruction of chlorophyll molecules (29). By increasing chlorophyllase activity, BRs preserve the integrity of the thylakoid membrane and control chlorophyll molecules (30). Anthocyanin is a pigment that imparts a red, blue or purple color to plants. They have been demonstrated to play a role in defending plants from a variety of abiotic stressors, including intense light, drought, salt and cold (31). The ability to withstand environmental challenges is made possible by anthocyanin. Since, in reaction to abiotic events, anthocyanin either functions as a source of radicals that are hydroxyl or as an organic compound that prevents lipid peroxidation and promotes the activity of antioxidant enzymes. The synthesis of anthocyanin increased with salinity and EBR treatments. By stabilizing macromolecules and boosting antioxidant activity, they control cellular osmotic, ionic and oxidative equilibria. They protect the photosynthetic apparatus, boost chlorophyll synthesis and photosynthetic efficiency, control plant metabolic pathways and promote food and water intake, all of which contribute to a considerable increase in salt tolerance (32). Carotenoids are important pigments that are active in several plant physiological procedures, such as photosynthesis, photo protection and signalling. BRs promote the accumulation of carotenoids in plants under saline conditions. This is thought to occur via the over-expression of genes associated with the synthesis of carotenoids, such as phytoene synthase (PSY), phytoene desaturase (PDS), and ζ-carotene desaturase (ZDS) (33). PSY is a vital enzyme in the biochemical pathway that was responsible for enhancing carotenoid production with EBR administration (34). Plants exposed to salt stress may exhibit reduced carotenoid concentrations due to increased activity of chlorophyllase, an enzyme responsible for chlorophyll degradation. When EBR was applied, plants under salt stress exhibited carotenoid content. Chlorophyll degradation was slowed down as a result of EBR-protecting pigment-protein complexes.

Conclusion

The ability of BRs to lessen the adverse impacts of salt exposure on *C. papaya* cv. Red Lady was well proven in this study. The foliar administration of BRs at various levels (0.05, 0.1 and 0.2 mg/L) considerably enhanced important biochemical and physiologic parameters of papaya, particularly when paired with 200 mM and 250 mM NaCl. These included decreased levels of MDA and MII, which are markers of oxidative damage and increased levels of catalase activity, proline content, MSI and chlorophyll a, chlorophyll b, total chlorophyll, anthocyanins and carotenoids. The treatments that had the greatest beneficial impact on the plant's physiological status in saline circumstances were 0.1 mg L^{-1} and 0.2 mg L^{-1} BRs in combination with 200 mM NaCl. By stabilizing membranes, controlling pigment synthesis and strengthening their antioxidant defense system, BRs increased the efficiency of photosynthesis and stress tolerance. These results demonstrate that BRs are efficient, environmentally benign bio-regulators that increase papaya's resistance to salt stress. As a result, BRs can be regarded as a durable agronomic strategy to improve papaya quality and performance in salty conditions. To confirm its practical applicability and adjust dosages for commercial growth, more field-scale experiments are advised.

Authors' contributions

AD, KS and SD prepared the concept and design of the work. AD, SD and RK[1] completed the analysis process and interpretation of data. AD, KS and RK[2] had done the drafting of the paper. AD, KS, RS, RK[1], RK[2], RJ and LTH revised the manuscript. SD and MB had done final approval of the version to be published. All Authors read and approved the manuscript.

[RK[1] stands for Rupesh Kaushik and RK[2] for Ranjeesh Kumar]

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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