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Methyl jasmonate mitigates biochemical and phytochemical changes in salt stressed *Stevia rebaudiana* plants

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Abstract

This study explored the effects of salt stress on growth, oxidative stress, and steviol glycoside content in *Stevia rebaudiana* Bertoni plants cultivated in soil supplemented with methyl jasmonate (MeJA). Stevia plants were cultivated under normal and salt stress conditions, with and without MeJA supplements of 30 µM, 60 µM, and 120 µM. Samples were harvested after the 1st, 3rd, 5th, 10th, and 15th day of treatment. The levels of chlorophyll ($p<0.0001$), carotenoids ($p<0.0001$), and antioxidant enzymes such as catalase ($p<0.0001$), superoxide dismutase ($p<0.0001$), APX ($p<0.0001$), and glutathione reductase ($p<0.0001$) were observed. The quantification of steviol glycosides, including stevioside ($p<0.0001$) and rebaudioside-A ($p<0.0001$), was studied by the most advanced hyphenated technique, LC-MS/MS. The study revealed that the oxidative stress responses were significantly improved in MeJA-treated plants compared to salt-stress control plants. The level of production of phenols ($p<0.0001$), flavonoids, total sugar, reducing sugar, and steviol glucosides was significantly altered in salt-stress plants. MeJA showed a dose- and time-dependent significant effect on the improvement of these factors over salt stress. In conclusion, MeJA not only improves the growth of plants but also reduces oxidative stress and enhances the level of phytochemicals under saline stress.

Keywords

Methyl jasmonate; salt stress; *Stevia rebaudiana* Bertoni; oxidative stress; proline

Introduction

Stevia rebaudiana Bertoni belongs to the family "Asteraceae (Compositae)" and is considered as a sweet tree due to its sweet glycosides. Stevia plants are also known as candy leaf, sweet leaf, or sugar leaf. Stevia plants are well known for their pharmacological properties, including diabetes (1), immunomodulatory (2), antioxidant (3), anti-cancer (4), antimicrobial (5), etc. Due to its diverse pharmacological properties, particularly its calorie-free sweet taste and well-studied antioxidant properties, it has garnered significant attention. Because of antidiabetic activity, its demand remains high in daily consumption among diabetic and health-conscious people. The sweetness of plant leaves is due to compounds called stevioside and rebaudioside (6). The presence of flavonoids (7, 8), polyphenols (9), vitamins (10), alkaloids (10), phytosterols (11), essential oils (10) were reported in stevia. It is highly delicate plant; numerous studies suggest that high saline levels in soil alter the growth and levels of phytochemical properties in various

plants. The issue of soil salinity has reached a critical point, as large areas of land, both globally and in India, are being severely affected by the damaging effects of salt. This issue not only affects the productivity of our lands but also puts a burden on our food supply chains, necessitating urgent and innovative actions to restore soil health. Inadequate irrigation methods have exacerbated the issue, resulting in a buildup of salt that hampers the growth of plants by obstructing the roots' access to water, even when it is abundant beneath the surface (12). Salt stress not only affects the growth of the plant externally but also affects the plant biochemically. Salt stress enhances oxidative stress (13), reduces chlorophyll content, and alters the production of phytochemicals (14). Stress-induced reactive oxygen species (ROS) were converted into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) at the primary level in plant cells (14, 15). The resulting hydrogen peroxide is further converted into oxygen and water by either catalase (CAT) or guaiacol peroxidase (GPX) (16). A change in the levels of SOD, CAT, GPX, glutathione reductase (GR), etc. is a natural phenomenon for plants under salt stress, inducing high ROS.

In plant, oxidative metabolism of polyunsaturated fatty acids makes jasmonates (JAs), which are cyclopentanones. Methyl jasmonate (MeJA) is one of the active compounds occurring in the metabolic pathway of JAs. MeJA is an important cellular regulator that is involved in diverse developmental processes such as seed germination, root growth, fertility, fruit ripening, and senescence. MeJA is well studied for its beneficial role in plant development under salt stress. Yoon and co-workers have studied the beneficial effects of MeJA on soybean plants under salt stress (17). Beneficial effect of MeJA on salt stress *Brassica napus*, strawberries (15), *Pisum sativum*, *Limonium bicolor*, tomato plants, etc. were well described in the literature. Numerous studies suggest that MeJA restores oxidative stress to its normal level in saline-stressed plants (17).

Considering the adverse effects associated with high salt concentrations, such as reduction in chlorophyll, phenolic, and flavonoid content, elevated oxidative stress, and reduced phytochemical production, the present work was designed to study the dose-dependent effect of MeJA (30 µM, 60 µM, and 120 µM) at different time intervals (1st day, 3rd day, 5th day, 10th day, and 15th day) on biochemical and phytochemical parameters including total chlorophyll content, oxidative markers like SOD, catalase, APX, GPX, MDA, total flavonoids, total phenolics, and total and reducing sugar levels in stevia plants. The study was also exploring the role of MeJA in the production of steviol glycoside, which was quantified by a hyphenated technique called LC-MS/MS on salt-stressed stevia plants.

Materials and Methods

Plant materials

The nursery's stock in Manawar, Madhya Pradesh, supplied stevia plants. The plants were acclimatised at an experimental location by putting all the plants under controlled conditions with an equal day and night cycle. The plant was identified by a botanist at Department of Botany, The Maharaja Sayajirao University of Baroda, Vadododra, Gujarat, India (herbarium number 19461). Plants selected for experiments contain seedlings with similar characteristics, such as leaf count, age, and height. The plants were snapped off under controlled laboratory circumstances, the leaves were kept at -80°C for future study.

Growing stevia seedling and treatment protocol

The randomised block design was adopted to carry out the experiments. Plants were treated with varying concentrations of MeJA. All groups except the control group received 100 mM NaCl stress before MeJA treatment (18). Leaves were collected at different time intervals, such as on the 1st, 3rd, 5th, 10th, and 15th days of treatment. Leaves were selected based on their size and location on the plant. The experiment was performed in three replicates in the month of September 2022. Treatment was performed as per Table 1.

Table 1. Treatment of different concentrations of methyl jasmonate.

Sr. No.	Methyl Jasmonate	100 mM NaCl for 7 Days + Methyl
1	0.0 µM	0.0 mM NaCl for 7 Days + 0.0 µM MJ
2	0.0 µM	100 mM NaCl + 0.0 µM MJ
3	30 µM	100 mM NaCl + 30 µM MJ
4	60 µM	100 mM NaCl + 60 µM MJ
5	120 µM	100 mM NaCl + 120 µM MJ

Determination of chlorophyll

Determination of chlorophyll on the same day of harvesting. Estimation was done by following the method reported by Wellburn using dimethyl sulfoxide (DMSO) (19). Final estimation was done by following equations:

$$Chlorophyll\ A = 12.47 \times A_{665} - 2.62 \times A_{649}$$

.....(Eqn. 1)

$$Chlorophyll\ B = 25.06 \times A_{665} - 6.5 \times A_{649}$$

.....(Eqn. 2)

$$Total\ Chlorophyll = Chlorophyll\ A + Chlorophyll\ B$$

.....(Eqn. 3)

$$C\ (x + c) = \frac{(1000 \times A_{480} - 1.29\ (Ca - 53.78Cb)}{220}$$

.....(Eqn. 4)

Determination of proline

Determination of proline was done by following the process described by Bates *et al.* (20). 500 mg plant material was homogenized in 3% sulfosalicylic acid. Filtrate was collected and mixed with double volume of glacial acetic acid and acid-ninhydrin (1:1 v/v) in a test tube and incubated for 1 hr at 100°C. Resultant solution was fractionated with 4 mL toluene. Absorption of organic fraction was taken at 520 nm using spectrophotometer (UV visible spectrophotometer, Shimadzu 1800).

Determination of lipid peroxidation (LPO)

The level of lipid peroxidation in plant leaves was determined by quantitative estimation of malondialdehyde (MDA) using published protocol (21). Briefly, plant material was homogenized in trichloroacetic acid solution. Supernatant was collected by centrifuging the homogenate. Half mL of supernatant was treated with 1.5 mL TCS solution containing 0.5% thio-barbituric acid (TBA) (w/v). The resulting solution was incubated in a boiling water bath for a period of 10 min. After incubation, the solution was allowed to cool down, and absorbance was measured at 3 different wavelengths (450 nm, 532 nm, and 600 nm) using a UV spectrophotometer. MDA levels were determined using equation 5.

$$TBARS\ (\mu M/gm\ leaf) = 6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}$$

.....(Eqn. 5)

Determination of antioxidant enzymes

Beauchamp and Fridovich's technique was used for estimating the SOD concentration (22). Nakano and Asada's process was used for the estimation of ascorbate peroxidase (APX) (23). Level of guaiacol peroxidase (GPX) was estimated by previously published literature (24). Catalase enzyme concentrations were determined using the standard method described in the literature (25). D'Antuono and Moretti's protocol was used to determine the extent of PAL activity (26).

Determination of relative water content

The relative water content (RWC) of leaves was estimated using the technique of Weatherley (27). The following equation (equation no. 6) was used to calculate the RWC:

$$RWC = \frac{fresh\ weight - dry\ weight}{saturated\ weight - dry\ weight} \times 100$$

.....(Eqn. 6)

Determination of sugars

The dinitrosalicylic acid (DNSA) technique was used to estimate reducing sugars (28), while anthrone methods reported by Yemm and Willis were used to measure total sugar (29).

Quantitative determination of phytochemical

The quantitative estimation of total flavonoids was done using aluminium chloride colorimetric technique (7). The total amount of phenolic compounds is determined using the modified Folin-Ciocaltu method (30). To calculate the amount of anthocyanin, the Wagner's method was followed (31).

Qualitative determination of steviol glycoside and by LC-MS/MS

A new LC-MS/MS method was developed for the determination of stevioside and rebaudioside A concentration in salt-stressed and methyl jasmonate-treated leaves. A HPLC (Model: LC-20AD, Make: Shimadzu Corporation, Japan) system with a MS/MS detector LC-MS 8030 (Shimadzu Corporation, Japan) and was used to estimate the stevioside. Separation was performed using a Kintex 2.64u PFP (50 × 4.6 mm) column. Data acquisition and processing were performed using lab solution software (version 5.53 SP3C) from Shimadzu Corporation, Japan. Optimization of tuning parameters was carried out for steviol glycoside through the infusion of a solution with a concentration of 250 ng/mL for each analyte. Nitrogen served dual roles, acting both as the nebulizing agent at a flow rate of 3 L/min and as the drying gas at 15 L/min. Control over the spray voltage and interface temperature was exercised meticulously, being set at 4500 volts and 350 °C, respectively, for the duration of the study. In the process of collision-induced dissociation (CID), argon was employed as the collision gas, with its pressure finely adjusted to 2.5 kPa. This experiment's mobile phase consisted of a 92:8 (v/v) methanol: ammonium sulphate solution combination. A flow rate of 0.5 mL/min was used to get higher resolution and a more rapid response. For mass detection, the ions were scanned in a negative polarity mode, which needed a collision energy of 25.

Statistical analysis

The data was presented as mean ± standard error of the mean (SEM). To determine statistical significance, a two-way analysis of variance (ANOVA) followed by Tukey-test was performed using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA). At a 95% and higher level of confidence, a p-value of less than 0.05 was declared as statistically significant.

Results

Determination of chlorophyll content

In plants subjected to salt stress, chlorophyll-a and total chlorophyll levels were significantly reduced at the 1st, 3rd, and 15th day of harvest ($p<0.001$). However, at the 5th and 10th day of harvest, although levels of chlorophyll-a and total chlorophyll were lower, the differences were not statistically significant (Table 1). Level of chlorophyll-b was found significantly low at 10th and 15th day of harvest ($p<0.001$ and $p<0.0001$) compared to control (Table 2). Improvement in the level of chlorophyll-a was noticed from the 1st day of treatment at higher concentration (120 µM MeJA) ($p<0.005$), and further statistically significant improvement was noticed at 60 µM and 120 µM MeJA on 3rd day treatment ($p<0.005$ and $p<0.0001$, respectively). Xanthophyll and carotenoid (x+c) content was significantly reduced by saline stress compared to control plants ($p<0.0001$) in all the leaves collected at different days. MeJA treatment showed significant improvement ($p<0.0001$) in salt stressed leaves x+c content at 60 µM and 120 µM concentrations (Table 2). The level of x+c did not show any significant improvement in lower dose (30 µM) treatment (Table 2).

Determination of proline

Proline is well known for its protective effect under diverse stress conditions, including high salt concentrations. Elevation of proline level under salt stress is well reported

Table 2. Effect of MeJA treatment on Chlorophyll-a, Chlorophyll-b, total Chlorophyll, and carotenoids + Xanthophylls contents in leaves of *S. rebaudiana* Bertoni grown under saline condition.

	Harvest on	Control	100 mM NaCl	30 µM MeJA + 100 mM NaCl	60 µM MeJA + 100 mM NaCl	120 µM MeJA + 100 mM NaCl
Chlorophyll-a	Day 1	14.9±0.21	11.95±0.08****	12.36±0.27	12.83±0.17	13.22±0.11$^{\#\#}$
	Day 3	14.39±0.15	12.67±0.28****	12.99±0.57	13.97±0.28$^{\#\#}$	14.43±0.49$^{\#\#\#\#}$
	Day 5	14.93±0.5	14.08±0.25	13.72±0.23	14.77±0.39	14.56±0.85
	Day 10	14.52±0.52	14±0.55	14.18±0.15	14.51±0.45	14.53±0.49
	Day 15	15.78±0.49	14.27±0.28***	14.29±0.51	13.71±0.4	13.6±0.31
Chlorophyll-b	Day 1	2.72±0.23	2.67±0.19	2.55±0.12	2.52±0.03	3.09±0.06
	Day 3	3.28±0.18	3.15±0.15	3.25±0.61	2.86±0.19	3.29±0.15
	Day 5	2.95±0.08	2.81±0.03	3.17±0.15	3.16±0.09	3.85±0.11$^{\#\#\#\#}$
	Day 10	3.43±0.51	3.11±0.34**	3.46±0.19$^{\#\#}$	3.59±0.05$^{\#\#\#}$	3.53±0.04$^{\#\#\#}$
	Day 15	3.27±0.3	3.11±0.27***	3.39±0.12	3.23±0.02$^{\#\#}$	3.14±0.04$^{\#\#\#}$
Total Chlorophyll	Day 1	17.62±0.02	14.62±0.21****	14.92±0.24	15.35±0.18	16.31±0.1$^{\#\#\#}$
	Day 3	17.66±0.06	15.82±0.39***	16.61±0.77	16.83±0.11	17.73±0.63$^{\#\#\#}$
	Day 5	17.88±0.5	16.9±0.22	16.9±0.37	17.93±0.4	18.4±0.92$^{\#\#}$
	Day 10	17.95±0.86	17.11±0.21	17.64±0.34	18.1±0.49$^{\#\#\#}$	18.06±0.48$^{\#\#}$
	Day 15	19.3±0.55	17.67±0.48**	17.67±0.59	16.93±0.4	16.75±0.28$^{\#\#\#\#}$
Carotenoids + Xanthophylls	Day 1	3.63±0.03	3.33±0.05****	3.42±0.02$^{\#}$	3.46±0.02$^{\#\#\#}$	3.55±0.01$^{\#\#\#\#}$
	Day 3	3.61±0.03	3.44±0.03****	3.47±0.01	3.57±0.03$^{\#\#\#}$	3.52±0.01
	Day 5	3.6±0.06	3.19±0.07****	3.36±0.03$^{\#\#\#\#}$	3.43±0.01$^{\#\#\#\#}$	3.53±0.02$^{\#\#\#\#}$
	Day 10	3.76±0.03	3.32±0.09****	3.34±0.04	3.42±0.01$^{\#}$	3.51±0.01$^{\#\#\#\#}$
	Day 15	3.87±0.08	3.31±0.01****	3.39±0.01	3.41±0.01$^{\#}$	3.49±0.02$^{\#\#\#\#}$

Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. Results are expressed as mean ± standard deviation, where **n**= **3**. Significantly different from control plants, if p<0.0001= ****; p<0.001= ***; p<0.01= **. Significantly different from salt stressed plants, if p<0.0001= ####; p<0.001= ###; p<0.01= ##; p<0.05 = #

in numerous publications (32–34). In our study, we found that a highly significant accumulation of proline levels was found in salt stressed stevia plants compared to control plants (p<0.0001) (Fig. 1A). Lower dose of MeJA (30 µM) showed significant improvement (p<0.0001) in the level of proline content in stevia plants from 3rd day of treatment (Fig. 1). 60 µM and 120 µM of MeJA showed excellent activity in normalizing the level of proline in salt stressed stevia plants (p<0.0001) (Fig. 1A).

Determination of lipid peroxidation

The LPO was enhanced by saline stress. Fig. 1B provides a clear illustration of effect of salt stress and beneficial effect of MeJA on salt stressed stevia plant. When comparing normal and salt-stressed stevia plants, we found that the former had a statistically significant increase in LPO (p<0.0001). MeJA at all doses showed a highly significant (p<0.0001) improvement in levels of LPO during the experiment when compared to salt-stressed stevia plants.

Determination of antioxidant enzymes

Determination of SOD

The production of reactive oxygen species (ROS) increased in response to extreme salt stress. Plant defence systems respond to an increase in ROS by increasing the production of antioxidant enzymes. An increase in SOD is a main indicator of increased ROS in plants. Our results showed that the SOD levels in salt-stressed stevia plants were notably higher than those in non-stressed stevia plants (p<0.0001). The level of superoxide dismutase (SOD) was significantly restored in salt-stressed stevia plants after MeJA was applied. MeJA protects stevia plants from the harmful effects of salt stress by decreasing superoxide dismutase (SOD) (p<0.0001). The results were well described in Fig. 2A.

Determination of APX and GPX

The APX and GPX responses play a crucial role in safeguarding plant cells from unfavourable environmental circumstances, and high salinity is one of them. Results indicated in Fig. 2B and 2C revealed that the level of APX and GPX were significantly high (p<0.0001) in salt-treated stevia plants compared to normal plants. The treatment with MeJA normalised (p<0.0001) the elevated APX and GPX level in salt-stressed stevia plants at all doses and time intervals.

Determination of catalase

The findings pertaining to the catalase activity were effectively elucidated in Fig. 2D. In contrast to the levels of SOD, APX, and GPX, the catalase levels in salt-stressed plants exhibited a considerable reduction when compared to those of non-stressed plants (p<0.0001). The use of MeJA has been shown to assist in the restoration of catalase levels in salt-stressed stevia plants, therefore enabling them to reach a state of equilibrium comparable to that of non-treated salt-stressed plants (p<0.0001).

Determination of relative water content

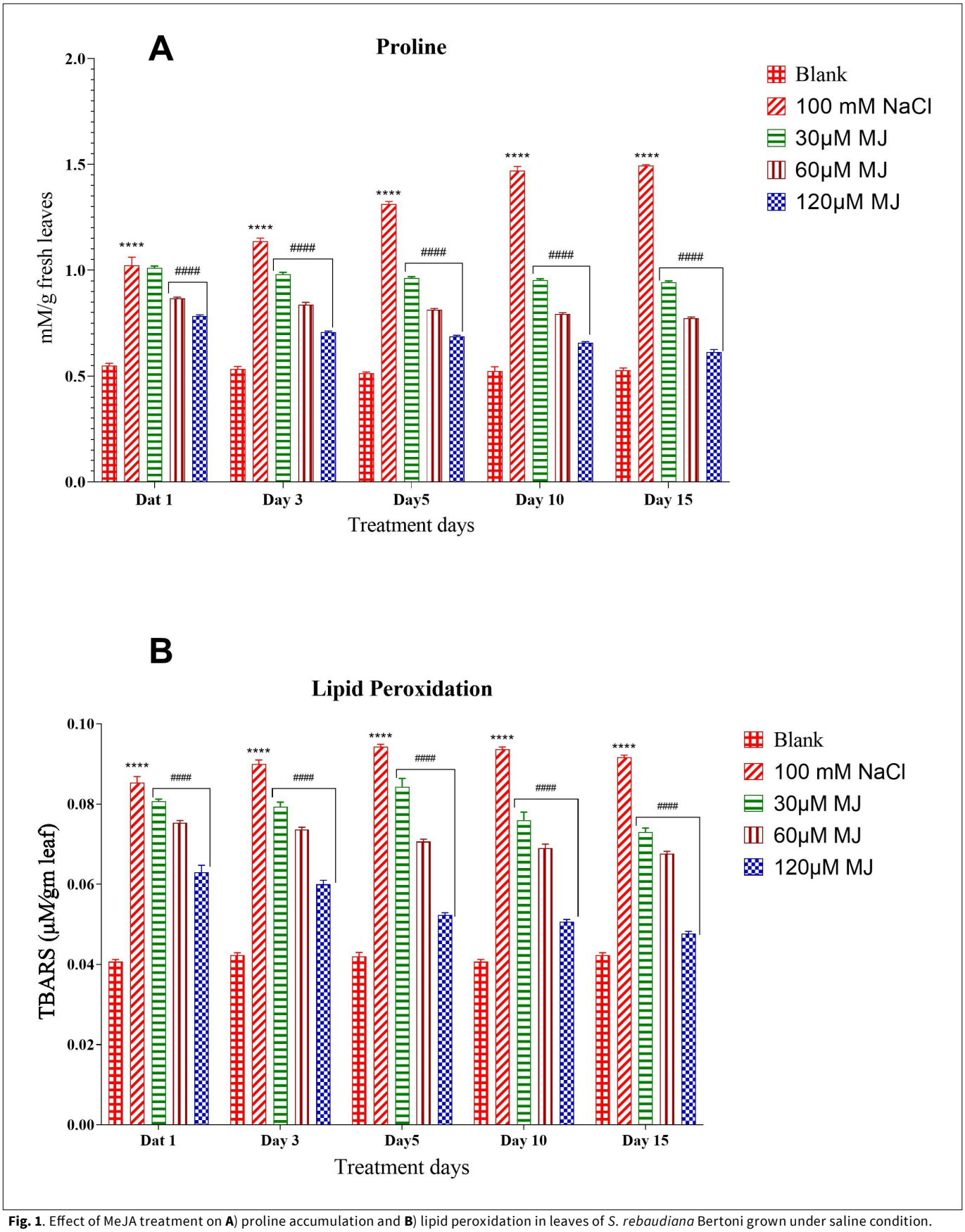


Fig. 1. Effect of MeJA treatment on **A**) proline accumulation and **B**) lipid peroxidation in leaves of *S. rebaudiana* Bertoni grown under saline condition. Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. Results are expressed as mean ± standard deviation, where n= 3. Significantly different from control plants, if p<0.0001= ****. Significantly different from salt stressed plants, if p<0.0001= ####.

Saline stress had a considerable impact on the RWC of plant leaves. As the stress increased, RWC decreased. In our research, it was discovered that stevia plants subjected to salt stress exhibited a noteworthy decrease in RWC as compared to the control group (p<0.0001). Results are well illustrated in Fig. 3A. Treatment of MeJA significantly improved the level of RWC in salt stressed plants compared to non-treated salt stressed plants (p<0.0001). The positive impact is seen to be statistically significant across various concentrations and throughout different harvest days. This observation highlights the beneficial impact of MeJA in maintaining leaf RWC in saline

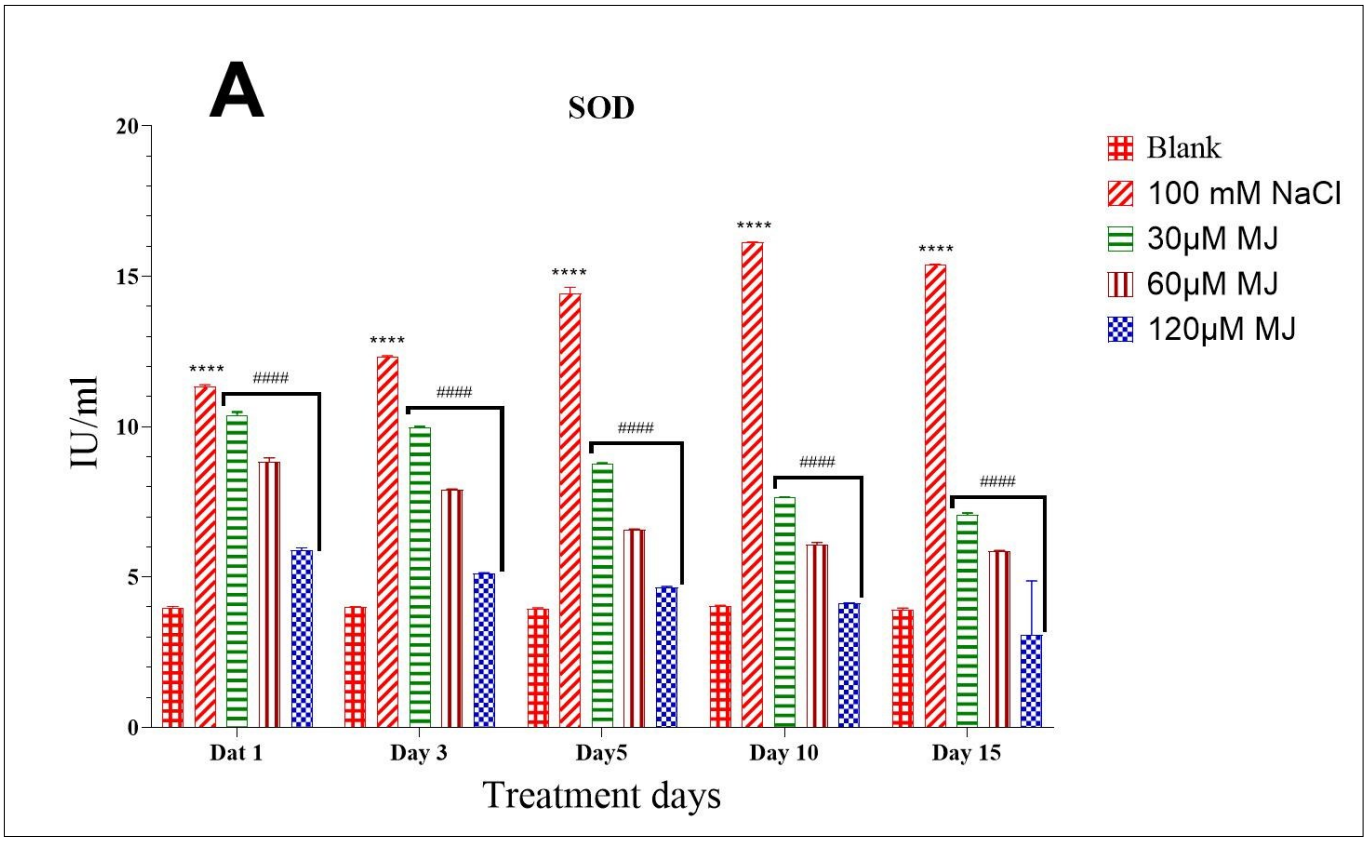
condition.

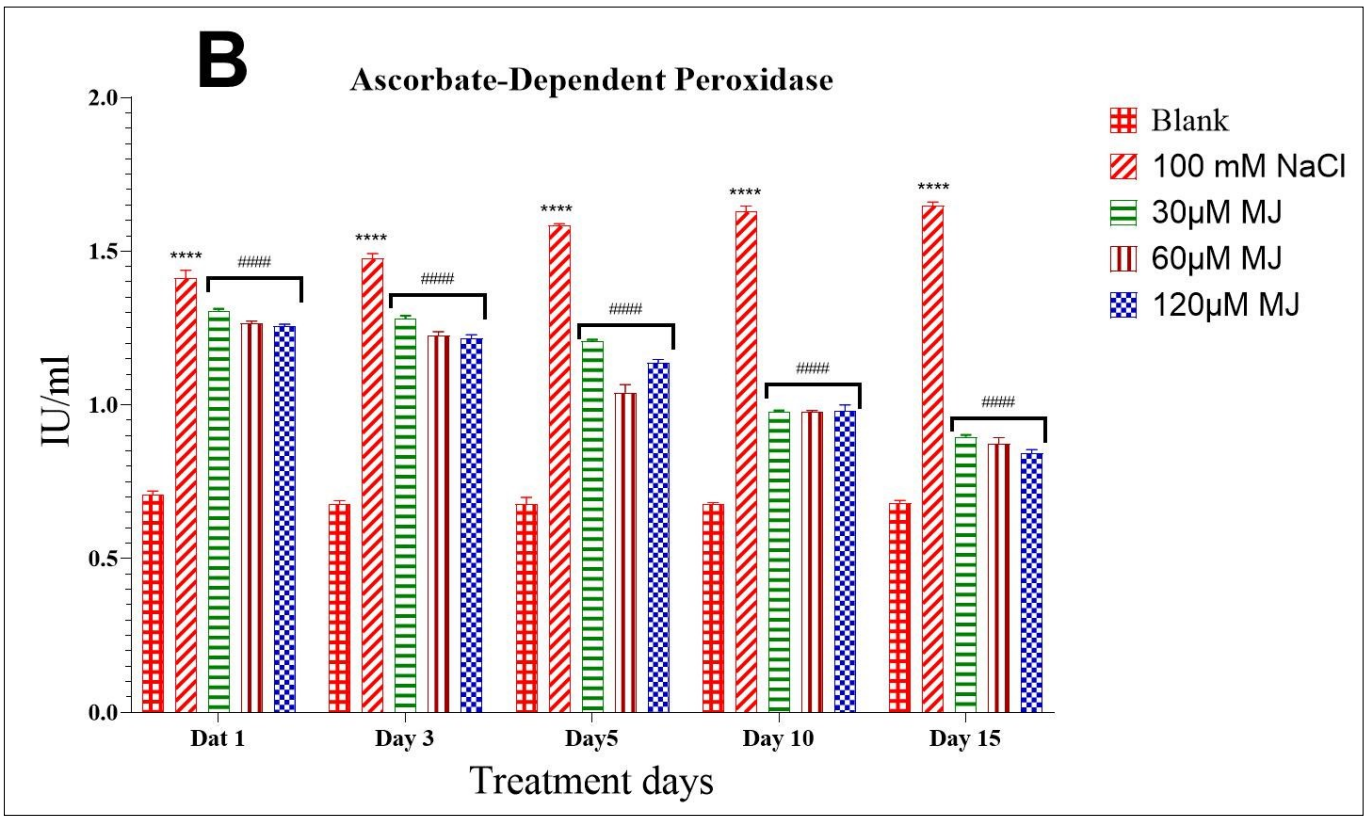
Determination of phenylalanine ammonia lyase (PAL) enzyme activity

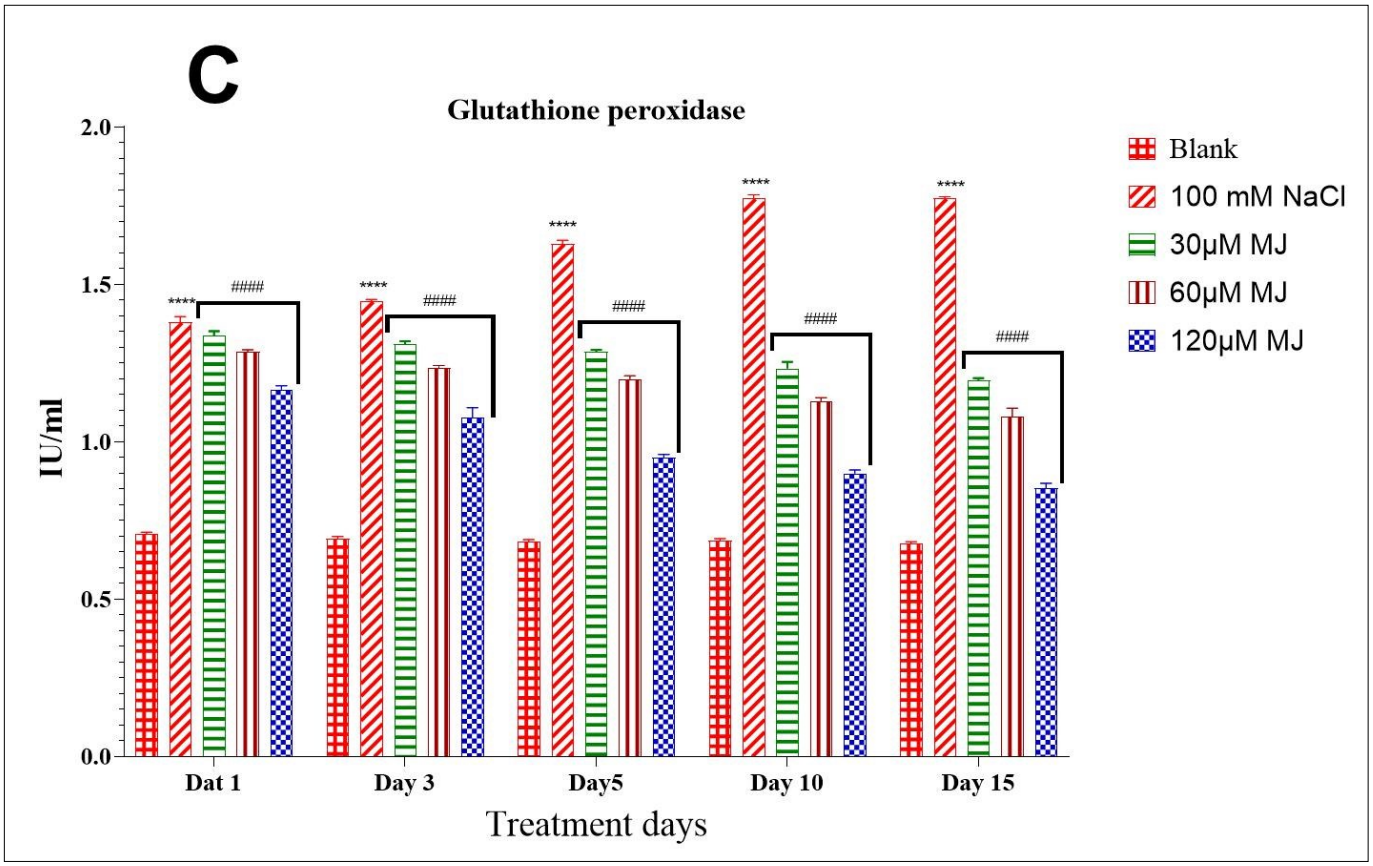
Phenylalanine is a member of the phenylpropanoid pathway, which plays a crucial role in the defensive mechanisms of plants. Therefore, the measurement of PAL activity is regarded as an indicator of stress in plants. In our findings, we observed that PAL activity was significantly elevated ($p<0.0001$) in salt-stressed stevia plants compared to control plants. MeJA treatment significantly reduced the PAL activity in salt-stressed stevia plants ($p<0.0001$) compared to non-treated salt-stressed stevia plants. The positive effect was found to be dose-dependent and time-dependent. Results are expressed in Fig. 3B.

Determination of sugars

Due to altered metabolism and oxidative state of cells, production of sugars is affected in plants. Salt stressed







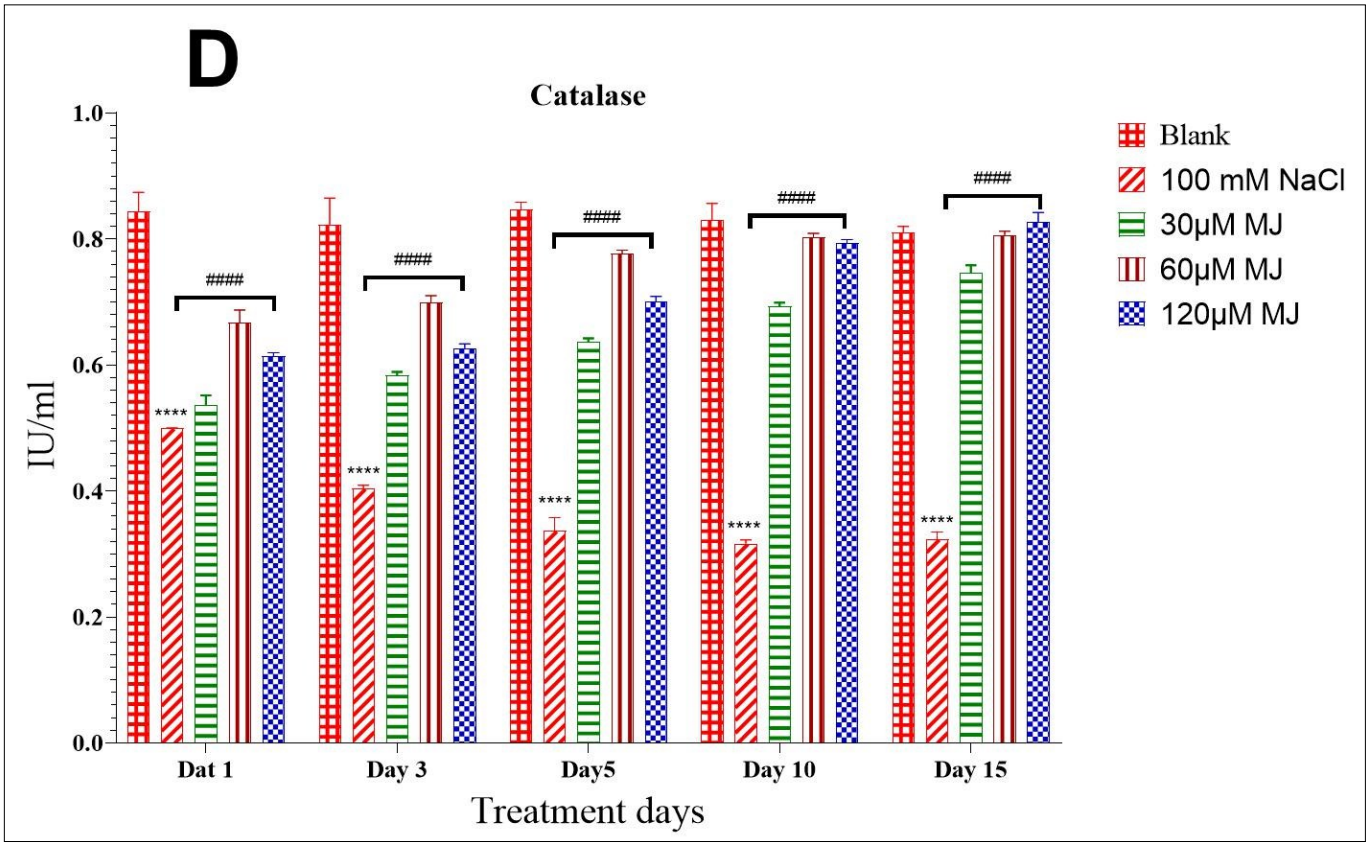


Fig. 2. Effect of MeJA treatment on **A**) SOD, **B**) APX, **C**) GPX, and **D**) catalase enzyme activity in leaves of *S. rebaudiana* Bertoni grown under saline condition. Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. Results are expressed as mean ± standard deviation, where n= 3. Significantly different from control plants, if p<0.0001= ****. Significantly different from salt stressed plants, if p<0.0001= ####.

stevia plant leaves showed statistically significant reduction in total (Fig. 3C) and reducing sugar (Fig. 3D) levels compared to control plant leaves (p<0.0001). The application of MeJA treatment has been seen to enhance the level of both total and reducing sugars in the leaves of salt-stressed stevia plants (p<0.0001), with the magnitude of this effect being dependent on the dosage administered.

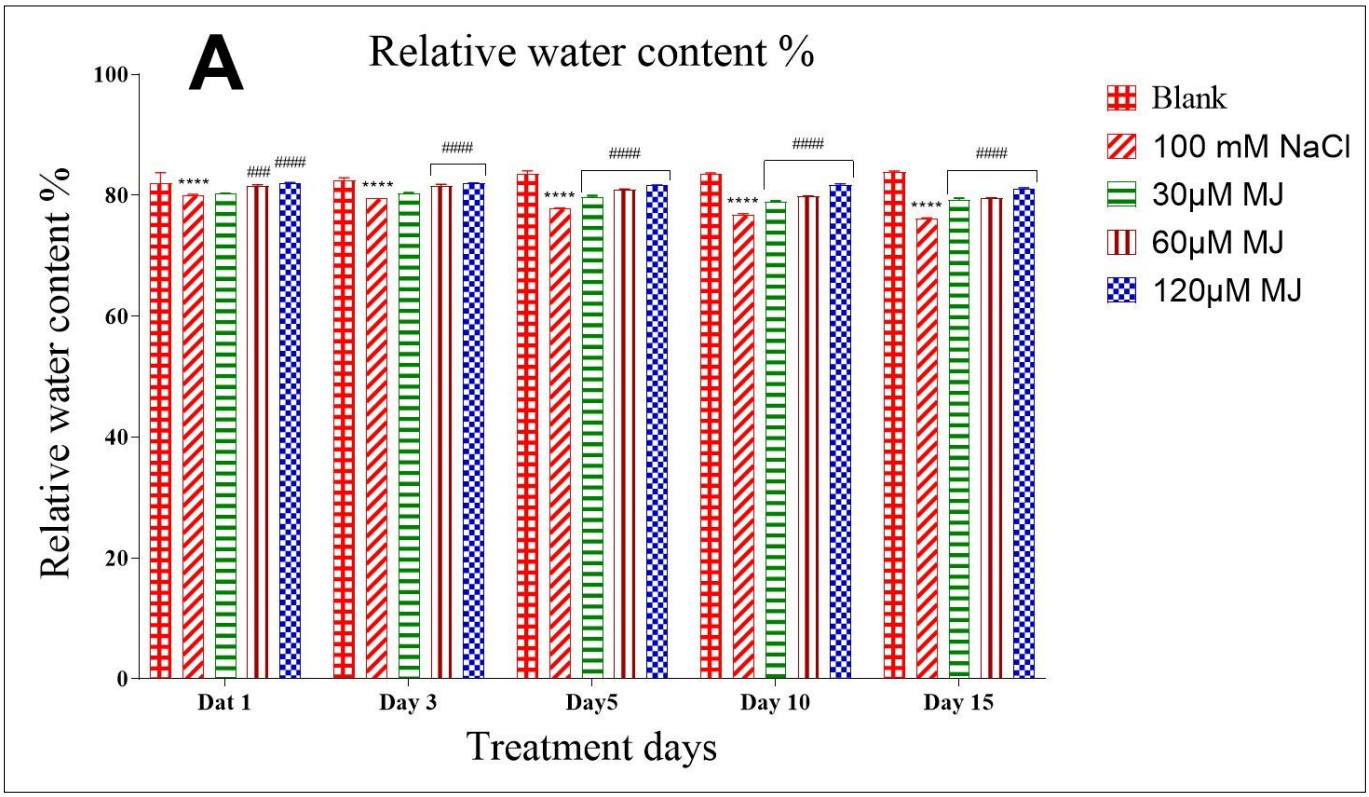
Quantitative determination of phytochemical

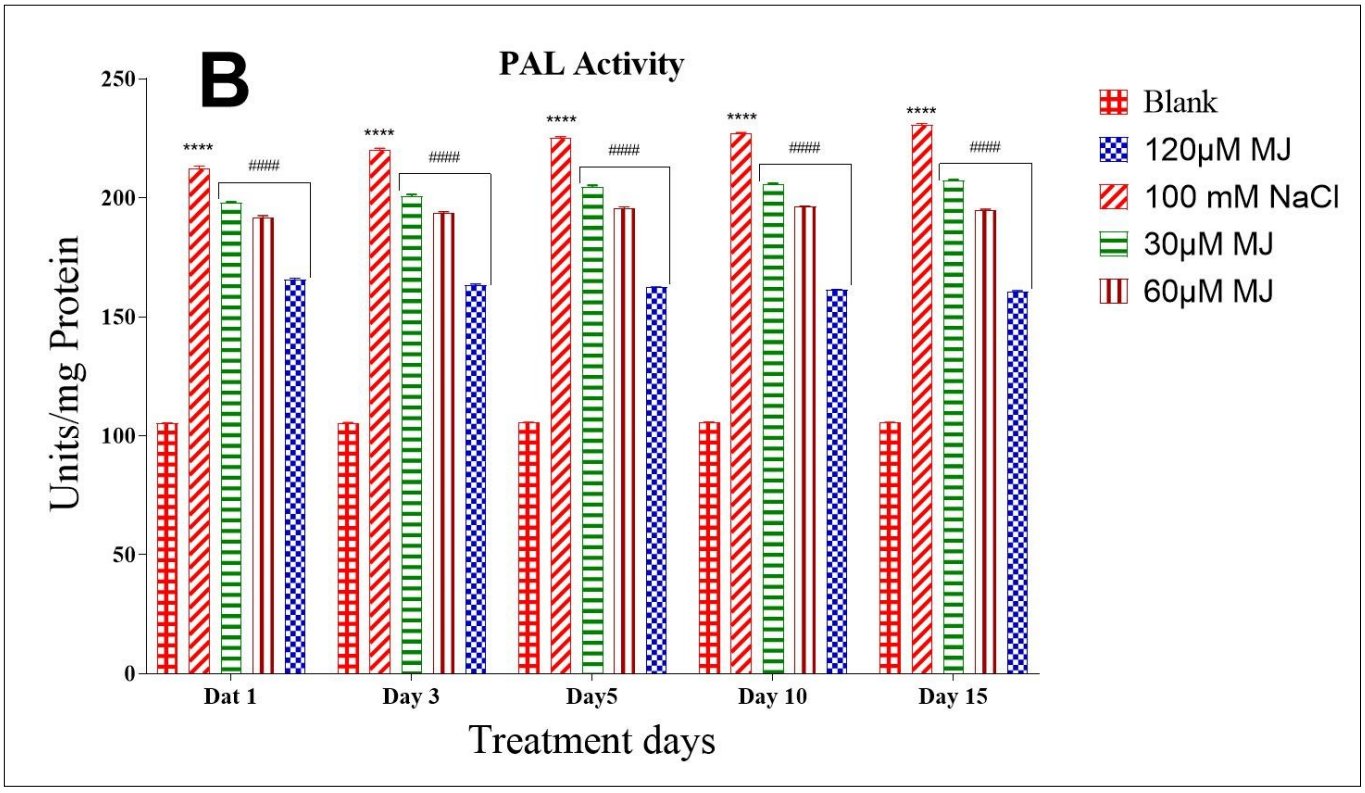
The concentrations of phytochemicals undergo substantial changes in conditions of salt-induced stress. In

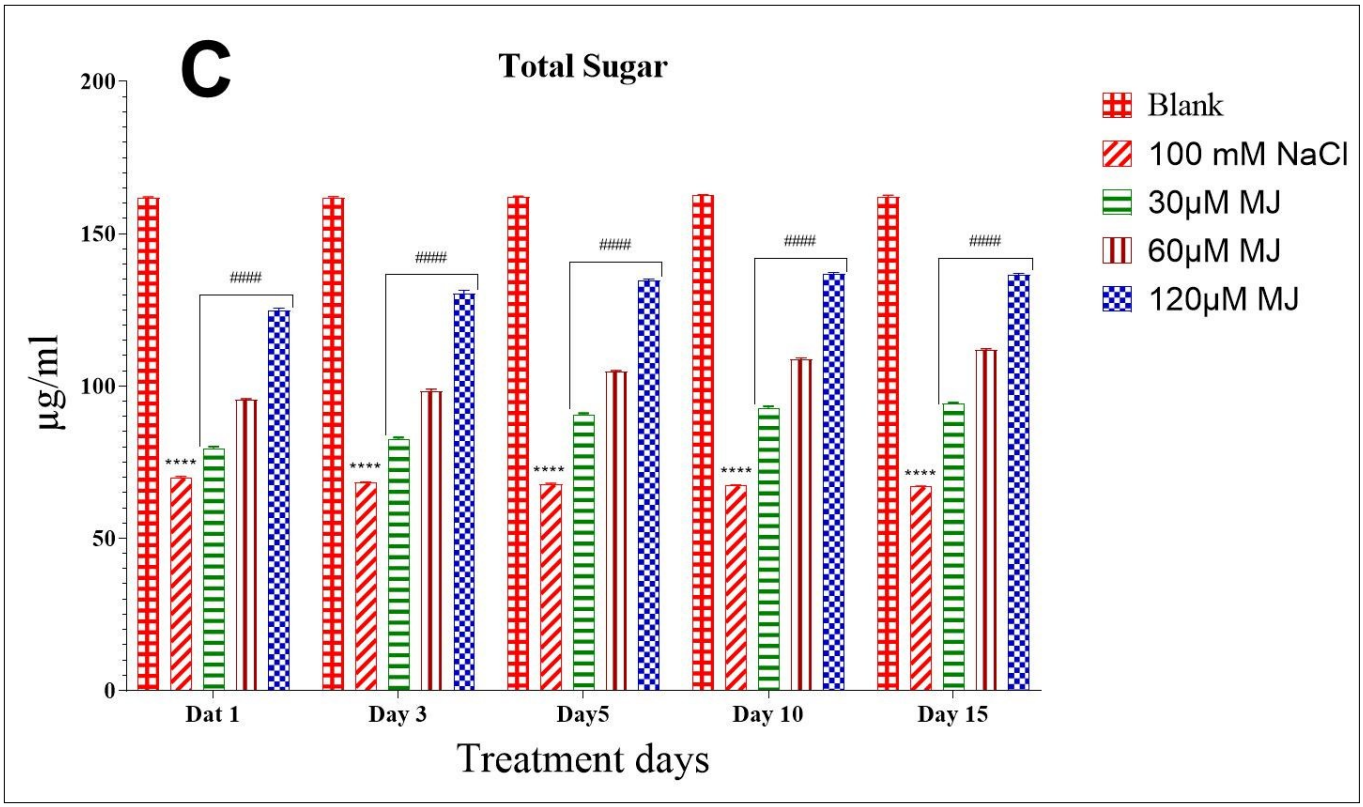
our research, we found that the amount of phenol and flavonoids in salt-stressed stevia plants was significantly higher than in non-stressed plants ($p<0.0001$) (Fig. 4A and 4B). In comparison to phenols and flavonoids, the amount of anthocyanin exhibited a notable drop in plants subjected to salt treatment as opposed to those in a normal state ($p<0.0001$) (Fig. 4C). The MeJA treatment has been shown to have a positive effect on the production of phenols, flavonoids, and anthocyanins in plants subjected to salt stress in comparison to non-treated salt-stressed plants ($p<0.0001$). The MeJA exhibited a dose-dependent relationship, with the degree of improvement commencing from the start of therapy and steadily increasing over time.

Qualitative determination of steviol glycoside by LC-MS/MS

In contrast with normal plants, stevia plants subjected to salt stress exhibited a significant increase in the concentrations of stevioside (Fig. 5A) and decreased accumulation of rebaudioside-A ($p<0.001$) (Fig. 5B). The application of MeJA resulted in a considerable elevation in the concentration of stevioside and rebaudioside-A in plants subjected to salt stress, as compared to salt-stressed plants that







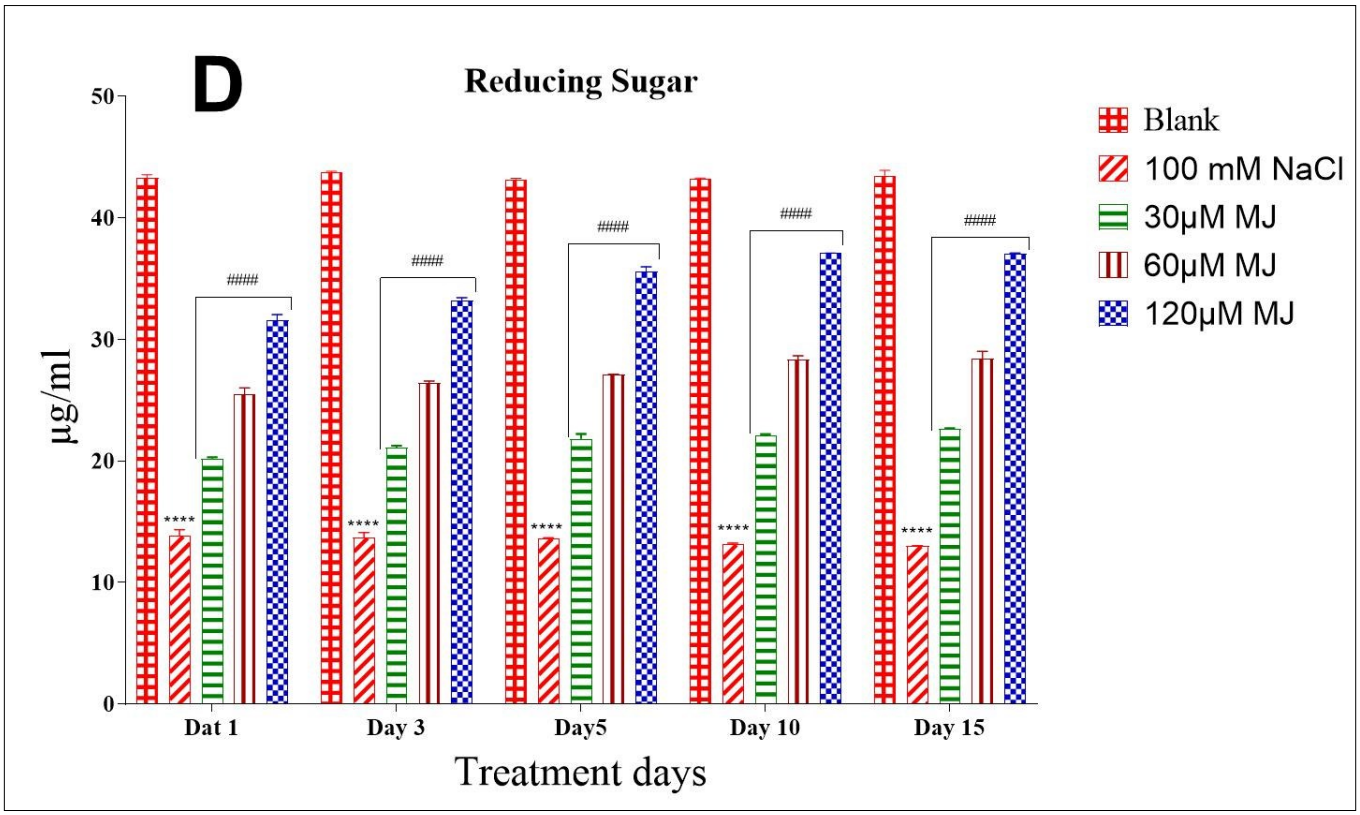
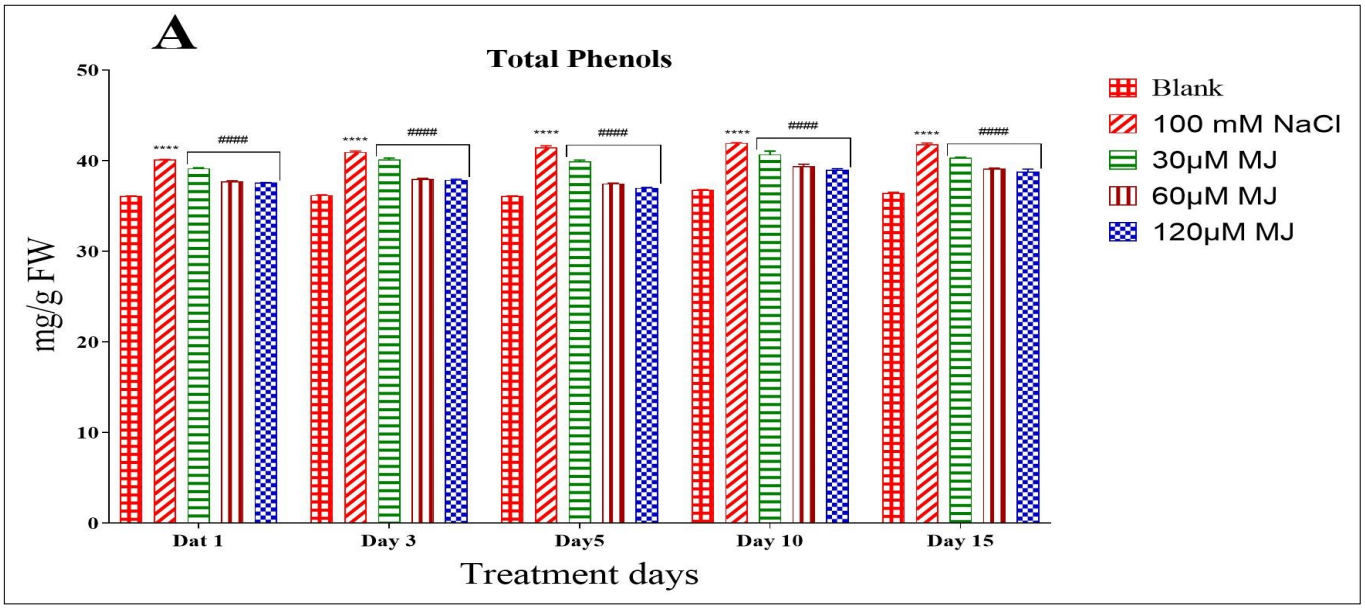


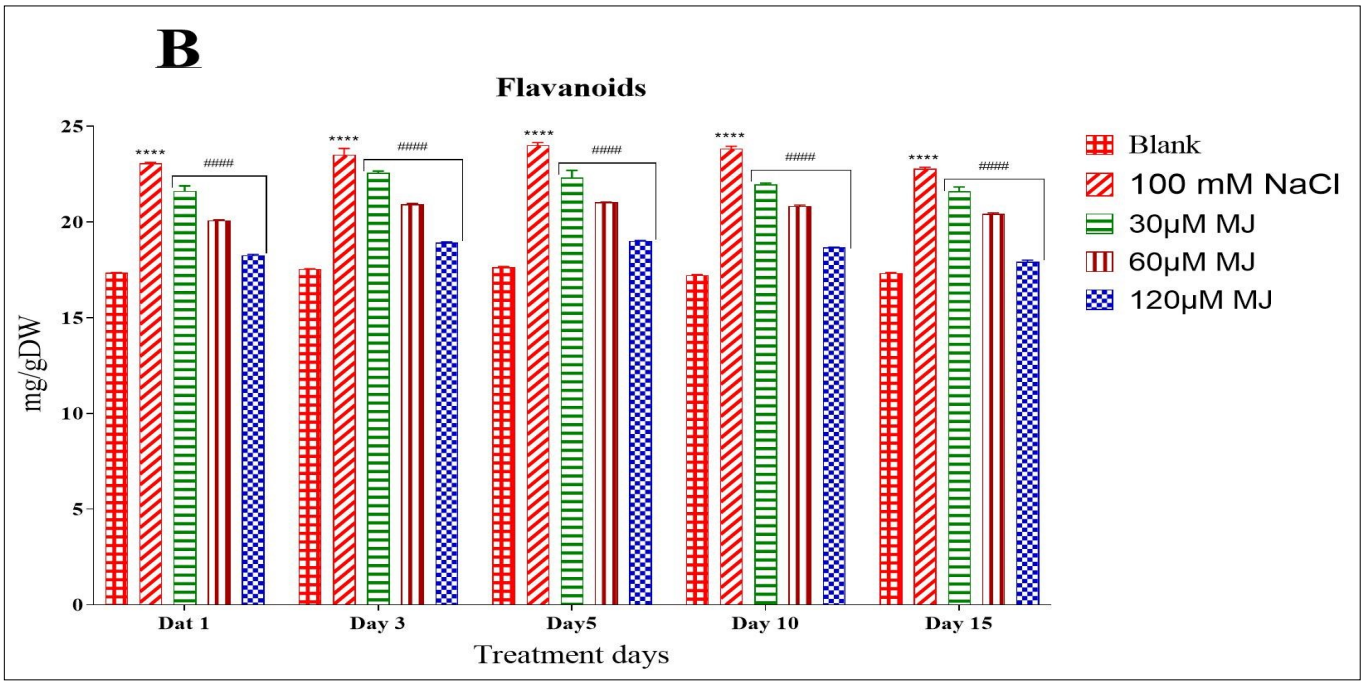
Fig. 3. Effect of MeJA treatment on **A**) relative water content, **B**) PAL enzyme activity, accumulation of **C**) total sugar and **D**) reducing sugar in leaves of *S. rebaudiana* Bertoni grown under saline condition. Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. Results are expressed as mean ± standard deviation, where n= 3. Significantly different from control plants, if p<0.0001= ****. Significantly different from salt stressed plants, if p<0.0001= ####.

were not treated with MeJA (p<0.0001) (Fig. 5A and 5B).

Discussion

Chlorophyll-a and -b, which are the principal pigments involved in photosynthesis, play a crucial role in photoreaction and the synthesis of plant nutrients. Published research has shown that salt stress has a significant impact on both the thylakoid membrane and the level of photosynthetic pigments in several plant species (35, 36). A significant amount of research has been dedicated to exploring the correlation between stress and photosynthetic pigments. Yoon and Hamayun (17) have studied the adverse effects of salt stress on soybean plants. The findings of the research indicated that salt stress significantly re-





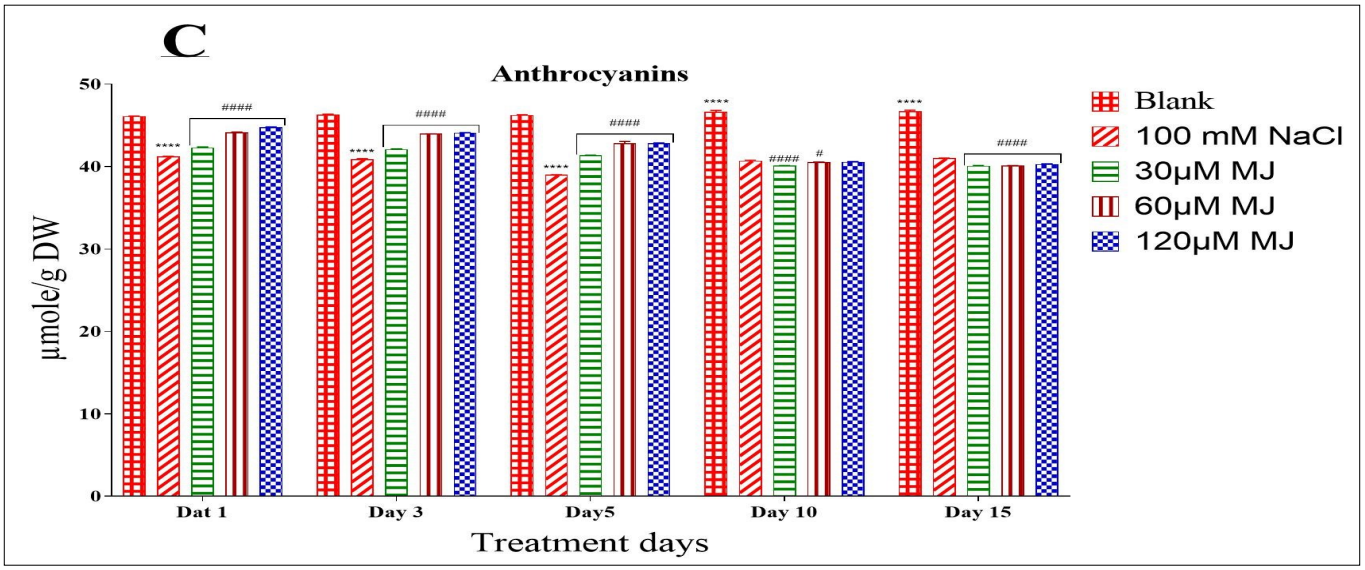


Fig. 4. Effect of MeJA treatment on accumulation of **A**) total phenol, **B**) total flavonoid, and **C**) anthocyanin in leaves of *S. rebaudiana* Bertoni grown under saline condition. Statistical analysis was performed by two-way ANOVA followed by Tukey's multiple comparisons test. Results are expressed as mean ± standard deviation, where n= 3. Significantly different from control plants, if p<0.0001= ****. Significantly different from salt stressed plants, if p<0.0001= ####.

duced the levels of chlorophyll-a, chlorophyll-b, and total

chlorophyll, however MeJA showed positive effect on level of chlorophyll-a, -b and total chlorophyll in soyabean plants under salt stress. Our results support the previous findings and prove the beneficial effect of MeJA in improving the chlorophyll level under salt stress.

Proline has a high solubility in water. It is nontoxic to plants and protects plants against stress through different mechanisms, such as contribution towards osmotic adjustment, detoxification of reactive oxygen species, stabilisation of membranes, and native structures of enzymes and proteins (37). Saline condition triggered significant rise in the level of proline (17). Numerous studies suggest the positive effect of MeJA in improving the level of proline in salt stressed plants such as soyabean (17, 38), *German chamomile* (37), *Pisum sativum* (39), etc. In our study, we found that MeJA treatment to salt stressed stevia plants showed significant reduction in proline levels compared to non-treated salt stressed stevia plants. Our study results are in line with the previous reports.

Saline stress is primarily responsible for the generation of ROS. ROS harmed the cell membrane, specifically its lipids. Due to the high amount of ROS, lipid molecules undergo peroxidation, and malondialdehyde (MDA) is generated. Due to peroxidation, the fluidity of the membrane decreases, making cells leaky and secondarily damaging proteins (40). Kukreja *et al*. (41) reported the high level of lipid peroxidation in roots of *Cicer arietinum* under salt stress. Pan *et al.* reported the elevated level of MDA in *Glycyrrhiza uralensis* under salinity and drought stress (42). In the present investigation, we have seen a comparable result. Nevertheless, it is noteworthy that the application of MeJA treatment resulted in a considerable decrease in the level of MDA in salt-stressed stevia plants as compared to salt stressed plants that did not receive any treatment.

In the presence of high salinity, plants undergo many physiological adaptations, such as the production of endogenous hormones and secondary metabolites. These alterations contribute to an enhanced antioxidant capacity inside plants, enabling them to better withstand the adverse conditions of their environment. Plants have developed an advanced antioxidant metabolism, including enzymes such as SOD, catalase, and APX, in order to mitigate the harm caused by ROS (43). Reduction of catalase enzyme and increased SOD, APX and GPX enzyme activity were reported by Faghih *et al.* (43). In their study, they studied the effect of MeJA on salt stressed strawberry plants and found a significant positive effect of MeJA in normalizing antioxidant enzymes. Beneficial effects of MeJA on salt stressed pea plants were studied by Fedina and Tsonev (39). They found that MeJA improves the level of antioxidant enzymes in salt stressed pea plants compared to non-treated plants. Our findings revealed the significant improvement of SOD, APX, GPX, and catalase in MeJA treatment in salt stressed stevia plants.

The RWC is a significant parameter used to assess the water status in plants, which is closely associated with both water absorption and transpiration rate. Elevated levels of salt in the soil solution induce osmotic stress, resulting in less water absorption by the roots and subsequent disruption of the plant's water balance. The current research observed a decrease in RWC during salt stress, which aligns with the findings of Sadeghipour (44) on cowpea, Gulmezoglu *et al.* (45), on green bean and Li *et al*. (46) on tomato. In the current investigation, the application of MeJA shown to enhance water absorption in salt-stressed stevia plants, leading to an improvement in RWC under conditions of salt-induced stress.

Sugar production has a positive correlation with level of chlorophyll. Salt stress significantly reduced chlorophyll level in stevia plants. Low level of chlorophyll indicated low sugar production. Significant reduction in both total and reducing sugar level was found in salt stressed stevia plants. However, MeJA treatment improves the level of both total and reducing sugar. Our results are in line with the previous findings. Cha-um *et al.* (47) reported that the salt sensitive rice plants showed poor total sugar level in leaf and root when exposed to salt stress compared to salt tolerant rice plants.

The research, led by Lucho *et al*. (48), conducted to investigate the impact of salt-induced stress on the overall phenolic and flavonoid levels in stevia plants cultivated in a controlled environment in the laboratory. The study results indicate that there is a positive correlation between the level of salt stress and the content of phenolics and flavonoids in stevia plants. The results obtained in our study were consistent with the findings reported in earlier research. The use of MeJA has been shown to significantly reduce the elevated concentration of phenolic and flavonoid compounds in stevia plants subjected to salt-induced stress. The extent of improvement is contingent upon the dosage and duration of treatment. Sakamoto and Suzuki (49) performed detailed study on effect of salt stress and effect of MeJA on anthocyanin accumulation in radish sprouts. Study results suggest that MeJA treatment significantly elevate the level of anthocyanin in radish sprouts in saline stress. In our study we found that, salt stress significantly reduces the anthocyanin level in stevia plants. MeJA treatment improved the anthocyanin accumulation in stevia plants and restores the metabolic activities.

Stevia plants are well recognised for their ability to provide a calorie-free sweet taste. The popularity of stevia is on the rise, with its consumption expected to show substantial growth in the future. The sweet taste of the stevia plant is derived from the presence of steviol glycosides, particularly stevioside and rebaudioside-A. Multiple studies have shown that salt stress significantly impedes the buildup of steviol glycoside (48). In the conducted investigation, it was shown that the presence of salt stress has a notable impact on the accumulation of stevioside and rebaudioside-A (50). Our findings suggest that salt stress significantly increases the accumulation of stevioside compared to non-treated plants. The accumulation of stevioside was time dependent. On the other hand, the level of rebaudioside A was significantly decreased in salt-stressed plants compared to normal plants. Nevertheless, the application of MeJA resulted in a significant enhancement in the concentrations of both stevioside and rebaudioside- A in

plants subjected to salt stress, as compared to salt-stressed plants that were not treated with MeJA. Salt stress and higher dose of MeJA produced highest stevioside when compared to normal and salt treated stevia plants.

Conclusion

In conclusion, our study demonstrates the significant impact of salt stress on various physiological and biochemical parameters in stevia plants, including chlorophyll content, proline levels, lipid peroxidation, antioxidant enzyme activities, relative water content, phenolic compounds, sugars, and steviol glycosides. The application of MeJA has shown a protective effect against salt-induced stress in stevia plants by improving chlorophyll levels, reducing lipid peroxidation, enhancing antioxidant enzyme activities, maintaining relative water content, modulating phenolic compound levels, and stabilizing sugar content. Notably, MeJA treatment also positively influenced the accumulation of steviol glycosides, particularly stevioside and rebaudioside-A, under salt stress conditions. These findings suggest that MeJA could be a potential mitigating agent for alleviating salt stress in stevia plants, thereby improving their growth and metabolic activities. Further research is warranted to explore the underlying mechanisms of MeJA's protective effects and its practical applications in agriculture.

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Authors' contributions

ZD planned the design of the study, carried out the experiments, performed statistical analysis, and drafted the manuscript. GS conceptualized the work and review the manuscript. All the authors approved and read the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

Ethical issues: None.

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