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Mitigating Salt Stress in Mustard (Brassica Juncea L.) through Foliar Application of Ascorbic Acid: Morphological, Physiological, and Biochemical Responses

Ifrah Nawaz[1], Ghulam Murtaza Kaleri[2], Mudassar Nadeem[3] & Nabila Maryam[4]

[1]*Department of Botany, University of Agriculture Faisalabad, Email: ifrahnawaz@gmail.com*
[2]*Institute of Plant Sciences, University of Sindh, Jamshoro, Email: murtazanifa@gmail.com*
[3]*Department of Botany, University of Agriculture Faisalabad, Email: mudassarnadeem187@yahoo.com*
[4]*Agriculture Research, Sindh, Tandojam, Email: nabilagmkaleri@gmail.com*

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Corresponding Author:
Ghulam Murtaza Kaleri
Email:
murtazanifa@gmail.com

ABSTRACT

Mustard (Brassica juncea L.) is a member of the Brassicaceae family has great economic and medicinal importance. It is third important crop in world while in Pakistan it is second after the soya bean production. Excess amount of salt changes the soil composition and also effects soil microbial activity and plants roots are damage thus productivity of crop reduced. Ascorbic acid increases growth and productivity of mustard under the saline and normal conditions. The Experiment was conduct to check the use of ascorbic acid to alter negative effects of saltiness on mustard using two cultivar of mustard super Raya and Faisalabad mustard. The salt concentrations used were 0 mM and 100 mM of sodium chloride, applied by dissolving it in Hoagland's solution after 10 days interval till vegetative stage. Ascorbic acid (AsA) was applied to the leaves at concentrations of 0 mM and 10 mM to counteract the harmful effects of salt stress. Statistical analysis showed that there was significant reduction in morphological parameters in both varieties under salt stress, while ascorbic acid treated plants exhibited an increase in morphological growth attributes. Saline conditions had significant decrease effects on chlorophyll a, chlorophyll b and carotenoids (40%, 34%, 37%) in V1 (47%, 40%, 39%) in V2 while ascorbic acid application increased (34%, 32%, 29%) in V1 (56%, 40%, 34%) in V2 the content in the current research. Substantial increases were recorded by enzymatic antioxidants (SOD, CAT and POD) in V1 and V2 and total soluble proteins, flavonoids, total soluble sugars content under salinity. There was significant increase in ROS production such as MDA and H_2O_2 under salt stress. While application of ascorbic acid showed remarkable decrease in MDA and H_2O_2 production. Salinity marked much increase in Na^+ (40% and 40%) accumulation in leaf and root (52% and 55%) by thereby reducing K^+ and Ca^{2+} level ions under stress or increase under treatment of foliarly applied ascorbic acid. In contrast, ascorbic acid application enhanced these nutrient uptakes of ions to maintain the ion balance in mustard cultivar under saline stress.

Introduction

One of the biggest challenges of the twenty-first century is the negative impact of climate variation on global food security (Syed *et al*., 2022). Salt stress is a significant agricultural problem in Pakistan. Salinity can also affect soil structure, reducing its porosity and water- holding capacity (Syed *et al*., 2021). The estimate of 1125 million hectares; area of world is affected with salt and with human induce salinization and sodification affecting area is roughly 76 million hectares (Mustafa *et al*., 2020). The area that is affected with salinity in Pakistan is around 2.5 million hectares and salinity affected area 3% in Punjab, 18% is in Sindh, 2% is in NWFP and remaining area is distributed in other regions of country (Syed *et al*., 2021).

Salinity causes decrease in cell division and expansion, decrease photosynthetic rate, low water level in all plant sections and lowered leaf growth rate are some of the disadvantages (Trusca *et al*., 2023). It may cause disturbance at genetic level, high accumulation of ROS that alter absorptivity of membrane thus the overall yield is greatly reduced (Patel *et al*., 2020). Salinity has two key types one is primary salinity and other one is secondary salinity. Primary salinity occurred naturally to buildup of salts in soil and ground water. While secondary salinization is caused by human activities. Use of low quality water and chemical fertilizers is the cause of secondary salinization by altering soil chemistry and increasing salt levels (Pena *et al*., 2020). Absorption of water and nutrients is decreased by salt concentration in soil solution, leading to water deficiency, nutrient imbalances and osmotic stress (Phour and Sindhu, 2020).

Ascorbic acid is an important cofactor in many enzymes reactions (Foyer *et al*., 2020). By acting as antioxidant Ascorbic acid scavenges (ROS) Reactive oxygen species can be detrimental to plant cells as they have the potential to harm cellular constituents such as DNA, protein and lipids (El-Beltagi *et al*., 2020). Application of AsA raises the rate of photosynthesis by improving the activity of enzymes Rubisco, which in turn boosts carbohydrate accumulation, this results enhances the production of plants (Alayafi, 2020). Ascorbic acid is essential for preventing oxidative damage and enhancing abiotic processes because it effectively scavenges accumulated ROS through direct or indirect mechanisms (Broad *et al*., 2020).

Mustard (*Brassica juncea* L.) is a member of the family of essential oilseed crops. Brassicaceae (Kang *et al*., 2021). Mustard is annual or biennial herbs and it is native plant in China (Huang *et al*., 2022).Composition of mustard includes several vitamins, chlorophylls, minerals, glucosinolates, fibers and volatile compounds (Tian and Deng, 2020). Typical fatty acid composition of mustard oil is 14% linoleic, 5% palmitic, 15% oleic, 1% stearic, 9% linolenic, and 45% erucic acid (Rathnakumar and Sujatha, 2022). Mustard crops can be more susceptible to a wide range of insect pests compared to other oilseed crops (Shah *et al*., 2020). It is hardy crop that can tolerate extensive range of soil and climatic situations making it well-suited for farming in many regions of the country (Sateesh *et al*., 2022). The foliar application of AsA on maize cultivars under salinity stress was previously checked and it showed remarkable results by improving plant growth that was hampered due to salt stress (Jamil *et al*., 2015). There was no recent application of AsA on mustard cultivars against salinity stress and to evaluate the efficacy of AsA in mustard plants grown under salty conditions this experiment was set up.

Mustard (*B. juncea* L.) cultivar survival potential and tolerance capacity to salinity-induced impairments and ascorbic acid counteraction were the focus of this study. Under salinity stress, we investigated the antioxidant metabolism and scavenging of reactive oxygen species (ROS). Mustard morphological responses and quality characteristics were also observed in order to assess the degree of damage and the protection against salinity toxicity provided by ascorbic acid. This study demonstrates that ascorbic acid has the potential to enhance plant growth and salinity tolerance particularly its ability to survive salinity's toxicity.

Material and Methods

Experimental design

The experiment was performed to determine whether mustard's salt stress can be alleviated by ascorbic acid. Experiment was conducted in the Old Botanical Garden of the University of Agriculture Faisalabad, Pakistan (Altitude and latitude). Filled plastic pots with 7 kg of freshly washed river sand. In this experiment, 12 seeds were sown in each pot and six plants survived after being thinned. The pots were arranged in a completely random design (CRD) with four replications for each treatment. Hoagland's nutrient solution was given to plants after every 10 days. After 4 weeks of sowing salt stress (control and 100 mM) and exogenously AsA (control and 10 mM) were applied. Plants harvested two weeks after treatment application. Morphological, physiological and biochemical parameters were examined.

For the experiment, seeds of the cultivars were taken from the Ayub Agricultural Research Institute, Faisalabad (AARI). For the purpose of the experiment, only healthy seeds of similar sizes were chosen to guarantee consistency. There are 32 pots in the experiment (six for each variety), each with four replicates:

T_0 (0 mM AsA + 0 mM NaCl)

T_1 (10 mM AsA + 0 mM NaCl)

T_2 (0 mM AsA + 100 mM NaCl)

T_3 (10 mM AsA + 100 mM NaCl)

Determination of morphological and growth characteristics

Took two plants from each pot and the uprooting process was carried out carefully to prevent any damage to plants. The fresh and dry weights of the shoots and roots were determined by an electronic weighing balance in grams. First dry sample under sunlight, then put them in oven at a temperature of 65°C. Plant shoots and root length were measured by a measuring tape, and values were recorded in centimeters.

Determination of the photosynthetic pigments

For this purpose, Arnon (1949) proposed a method, which is used to determine photosynthetic contents in leaves.

Chlorophyll contents of plant were measured by taking, plant sample 0.1 g was taken after weighing an electronic weighing balance. The leaf was then cut into small pieces and dipped in 5 ml of 80% of acetone. The samples of leaves were then kept in the dark for 10 to 12 hours. Enzyme extract used to take readings on a spectrophotometer (IRMECO U2020) (GmbH Germany) at a wavelength of 480 nm, 663 nm and 645 nm.

Chlorophyll *a* (mg/g) = $V/1000 \times W \times [12.7(OD663)-2.69(OD645)]$

Chlorophyll *b* (mg/g) = $V/1000 \times W \times [22.9(OD645)-4.68(OD663)]$

Carotenoids = $V/1000 \times W \times [(OD663)-0.638(OD645)]$

Were,

V = leaf extract volume in (ml)

W = fresh weight of leaves in grams

Determination of Enzymatic Antioxidants

Using a mortar and pestle 0.25 g of the leaf sample was ground in 5 ml of potassium phosphate buffer to measure the antioxidant content. Using a centrifuge machine the resulting extract was centrifuged for 15 minutes at 12000 rpm.

Chance and Maehly's method was used to measure catalase (CAT). In the cuvette, add 0.1 ml of leaf sample extract and 1ml of hydrogen peroxide for each sample. incorporated 1.9 ml of cold potassium phosphate buffer. Using a spectrophotometer, the absorbance at 240 nm was measured at specific intervals of 0, 30, 60 and 90 seconds.

The method of Chance and Maehly (1955) was used for POD measurement. Added 750 µl of potassium phosphate buffer into the ependrop with the help of micropipette. 100 µl of guaiacol into the solution. Then added 50 µl of enzyme extract. Added 100 µl of H_2O_2 into the solution. Absorbance was recorded at 470 nm at 0, 30, 60 and 90 seconds intervals.

To determine superoxide dismutase (SOD) activity method proposed by Spitz and Oberley (2001) was employed. Take 2 ml plastic cuvette, 50 µl of (NBT) was added. 100 ml of Triton-X, 100 ml of L-methionine, 400 ml of distilled H_2O_2 and 50 ml of leaf extract were combined with 250 ml of potassium phosphate buffer. Then added 50 µl of riboflavin in the cuvette. The cuvette was exposed to an electric lamp for 15 minutes. Readings were taken at 560 nm.

Determination of Non-Enzymatic Antioxidants

The Bradford (1976) technique was used for protein extraction A sample of 0.25 grams of fresh leaves was ground with 5 ml of pH 7.8 potassium phosphate buffer and kept in an ice bath. Solution was centrifuged for 10 minutes at 12,000 rpm. In a test tube, 0.1 ml extract and 5 ml of Bradford reagent dye were combined to extract the protein. The mixture was carefully vortexed for 30 seconds before being left at room temperature for half an hour. The reading was then taken with a spectrophotometer at 595 nm.

Method proposed by Stark and Wray (1989) was utilized for anthocyanin the purpose of the experiment. 0.25 g of fresh sample was ground with 5 ml of acidified methanol. The resulting mixture were covered with aluminium foil then incubated at 88°C for duration of 1 hour. After incubation periods, A spectrophotometer made by IRMECO U2020 (GmbH Germany) was used to measure the absorbance at 535 nm.

Sample 100 mg were ground up with 80% acetone to create an extract that was then put into an Eppendorf tube to measure the amount of phenol in the mixture. 100 µl of supernatant was mixed with 1 ml of Folin-Ciocalteu phenol reagent, 2 ml of distilled water, and 5 ml of 20% Na2CO3 solution in each labeled test tube. The volume increased to 10 ml when distilled water was added. A spectrophotometer made by IRMECO U2020 (GmbH Germany) was used to measure absorbance at 750 nm.

Method of Zhishen *et al.* (1999) used for determining flavonoid content, 100 mg of fresh sample placed in plastic bottles with 5ml acetone. After the sample was allowed to sit for an entire night, 4 ml of distilled water was added to 1 ml of the sample. After 5 minutes, 600 µl of 5 percent Na_2NO_2 and 500 µl of 10 percent aluminium chloride were added. 2 ml of 1 mM H_2O_2 and 2.4 ml of distilled water were added after one minute. A reading was taken with a spectrophotometer at 510 nm.

A 0.1 g leaf sample was ground with 5 ml of a 6 percent TCA solution to determine its ascorbic acid content. In a centrifugation machine, the obtained extract was centrifuged for ten minutes at 10,000 rpm. Each labeled test tube contained 2 ml of the supernatant and 1 ml of dinitrophenylhydrazine (2 %) solution for ascorbic acid evaluation. In 70% ethanol, a ten percent reduction in thiourea was added. The tubes were then immersed for 15 minutes in a water bath at 90 °C. After that, 5 milliliters of 80% sulfuric acid were added. A spectrophotometer made by IRMECO U2020 (GmbH Germany) was used to measure absorbance at 530 nm.

The total soluble sugars were determined using the Yoshida *et al.* (1976) method. Test tubes were filled with 10 ml of distilled water and 0.1 g of fresh leaves. After being covered with aluminum foil, test tubes were submerged for one hour in water that was 90 °C. After the test tubes had cooled to room temperature, 50 ml of water were added to dilute them. A pipette was used to extract 1.5 ml of the diluted solution. Each sample test tube was then filled with 5 milliliters of anthrone reagent, which was made by combining 1 g of anthrone with 1 L of sulfuric acid. The tubes were then left to cool at room temperature after 20 minutes of incubation at 90°C. The 620 reading of the samples was measured.

Reactive oxygen species

For this procedure, 0.25 grams of fresh sample were ground with 3 ml of 0.5% trichloroacetic acid (TCA) solution. By adding 0.5 g of TCA to 100 ml of distilled water, the TCA solution was ready. A centrifugation machine was used to centrifuge the obtained extract for ten minutes at 12000 rpm. Velikova, *et al*. (2000) introduced a method for measuring hydrogen peroxide concentration in fresh leaves. Add 1 ml of plant extract was taken in a test tube. In addition, a second labeled test tube containing 500 ml of extracted supernatant was used. 500 ml of ice-cold phosphate buffer and 1 ml of potassium iodide solution were added to this test tube. The sample was gently swirled. A reading was taken with a spectrophotometer at 590 nm.

A method for determining the amount of malondialdehyde in a sample was suggested by Cakmak and Horst (1991). One ml of the plant extract was taken to measure the amount of malondialdehyde present. The test tube was then filled with 1 ml of a TBA (thiobarbituric acid) solution containing 0.5% TBA and 20% TCA (trichloroacetic acid) solution. Test tubes were covered with aluminum foil, which was then immersed in an ice bath after 15 minutes in a 95°C water bath. A spectrophotometer was used to measure the enzyme extract at wavelengths of 532 nm and 600 nm.

Ion analysis

Method proposed by Kowalenko and Lower (1973) was employed to determine the concentration of nitrate ions. In labeled test tubes, 3 ml of digestion shoot sample was taken for the valuation of nitrate. Added 7 ml of chromotropic acid (CTA) and mixture was carefully vortexed, resulting in a yellowish color formation. A reading at 430 nm was taken with a spectrophotometer from IRMECO U2020 (GmbH Germany) after an 80-minute period to determine the concentration of nitrate ions.

The Yoshida *et al*. (1976) method was used to ascertain the phosphate content. In order to measure phosphorus, a digestion shoot sample of 3 ml was taken and placed in test tubes. Two milliliters of Barton's reagent were added to each sample test tube, thoroughly mixed and the mixture turned yellow. A spectrophotometer at 420 nm was used to measure absorbance after 80 minutes.

Mineral nutrients were measured by the following method given by (Allan *et al*., 1986). Dried shoot and root samples were added to a digestion flask with 0.1 g and 2 ml of sulfuric acid, covered with aluminum foil, and left overnight. The flasks were placed on a hot plate after 24 hours, and H_2O_2 was added until the mixture was clear or colorless. Using Whatman filter paper and plastic bottles, the solution was filtered through the filtrate after being diluted with distilled water to a final volume of 50 milliliters. Using a flame photometer, the concentration of Ca^{2+}, K^+ and Na^+ ions was measured.

Statistical analysis

Recorded data were studied by using CRD with four replications. CO-STAT used to examine collected data from various treatments by applying which test? (Lavezo *et al*., 2017) for statistical analysis.

Results

Morphological Parameters

Both cultivars of brassica shown notable decrease in morphological parameters under the treatment of salt stress (Fig.). Maximum reduction was recorded in root dry weight 55% at T_2 (Fig.) Application of ascorbic acid mitigate this toxic impact of salt 55% at T3 in V2. Similar reduction was observed in shoot fresh weight, shoot dry weight, root fresh weight root dry weight, shoot length and root length (47%, 49%,43%, 53%,47%,39%) in V1 and (50%, 51%,45%, 55%,49%, 40%) in V2. This harmful effect of salinity was overcome by exogenous application of ascorbic acid. in shoot fresh weight, shoot dry weight .root fresh weight root dry weight shoot length and root length (50%, 40%, 27%, 63%, 45%, 42%) in V1 and (55%, 40%, 35%, 73%, 51%, 52%) in V2.

Photosynthetic pigments

Exposure of salt stress to the canola varieties leads to reduction in chlorophyll attributes. Much decline recorded in chl *a* (40% and 47%), chl *b* (34% and 40%), carotenoids (37% and 39%), total chl (33% and 37%) and chl *a*/ chl *b* ratio (51% and 55%) in V_1 and V_2 respectively over control plants.

However seedlings of both varieties exhibited enhancement in chl *a* in V_1 (24% and 34%) and in V_2 (26% and 56%), chl *b* in V_1 (23% and 32%) and in V_2 (22% and 40%), carotenoids in V_1 (22% and 29%) and in V_2 (25% and 34%), total chl in V_1 (25% and 32%) and in V_2 (25% and 30%) and chl *a*/chl *b* ratio in V_1 (20% and 57%) and in V_2 (20% and 63%) at T_1 and T_3 respectively over T_0 plants.

Enzymatic Antioxidants

Enzymatic antioxidants activity was significantly increased under the salinity treatment in Brassica specie (Fig.). Salinity enhanced the concentration of superoxide dismutase, peroxidase, catalases 35%, 45%, 29% in V1, and 38%, 52% 30% in V2 respectively compared to control. Foliar application of AsA enhance activities of SOD, POD and CAT 23% 16% 13% in V1, similarly in 24% 16% 15% in V2 at T3.

Reactive oxygen species

Imposition of salinity significantly enhanced the reactive oxygen specie in brassica cultivars as compared with non-saline conditions at T_0 (Fig.). Maximum increased was observed for H_2O_2 in V2 at T2 as compared to control conditions. Salinity caused escalation for MDA and H_2O_2 at T_2

(38% and 65%) and (71% and 42%) in V1 and V2 respectively. Application of ascorbic acid significantly reduced the reactive oxygen species in both cultivars. Maximum reduction was recorded for H_2O_2 19% in V2 and 15% in V1, while for MDA 19% in V2 and 18% in V1 at T3.

Non enzymatic antioxidants

The implementation of 100mM NaCl proposed remarkable influence on various contents of various osmolytes/metabolites. Finding indicated increase in flavonoids content (30 and 34), total phenolics (34% and 38%), ascorbic acid (41% and 37%), anthocyanin (24% and 33%), total soluble sugars (41% and 43%) and total soluble proteins (40% and 43%) in V_1 as well as V_2 respectively relative to non-saline plants.

Similarly, 10 mM of AsA caused further accumulation of these above mentioned attributes. Maximum buildup was recorded of flavonoids in V_1 (34 and 28) and in V_2 (34 and 29), total phenolics in V_1 (19% and 35%) and in V_2 (23% and 44%), ascorbic acid in V_1 (26% and 34%) and in V_2 (24% and 24%), anthocyanin in V_1 (19% and 18%) and in V_2 (16% and 24%), total soluble sugars in V_1 (24% and 44%) and in V_2 (24% and 43%) and total soluble proteins in V_1 (27% and 55%) and in V_2 (21% and 59%) at both T_1 and T_3 plants corresponding to T_0 plants.

Mineral ions

Mineral ions were significantly influenced by the imposition of salinity in both cultivars of brassica (Fig.). Maximum increased was observed in root sodium at T_1 and minimum decreased recorded for leaf calcium also at T_1. Exposure of salinity diminished, calcium and potassium (38%, 56%) in V1 (39%, 59%) in V2 increased for root sodium (52% and 55%) at T2 as compared to control T0. Supplication of exogenous ascorbic acid suppressed toxicity for root sodium, calcium and potassium (19%, 34%, 61%) in V1 (20%, 37%, 74%) in V2 at T3. Mineral profile for plant leaf also exhibited similar kind of trend under salinity stress (Fig.). At T2 considerable decrease was recorded for, leaf calcium, leaf potassium, leaf nitrate and leaf phosphate (26%, 53%, 40% and 33%) in V1 and (32%, 59%, 44% and 29%) in V2 at T2 while increased for leaf sodium were (40% and 40%). Plant growth regulators ascorbic acid overcome this decrease in leaf ions significantly for leaf sodium , leaf calcium, leaf potassium, leaf nitrate and leaf phosphate (40%, 26%, 53%, 40% and 33%) in V1 and (49%, 32%, 59%, 44% and 29%) in V2 while reduce for leaf sodium were (35% and 40%).at T3 as compared to control conditions.

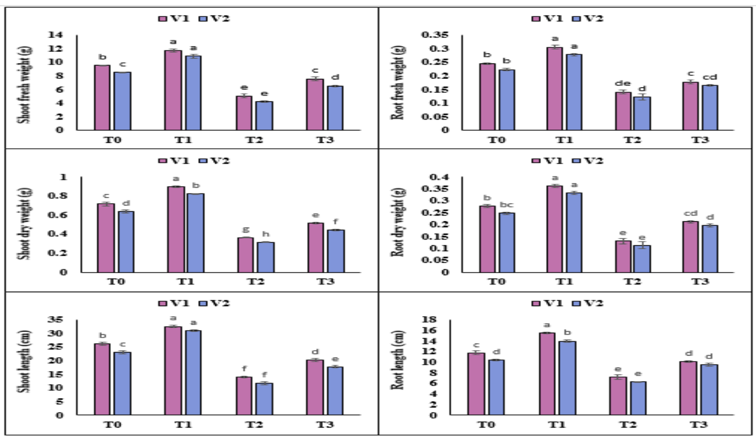


Fig: Shoot fresh weight, root fresh weight, Shoot dry weight root dry weight, shoot length and root length of Mustard (*Brassica juncea* L.) plants when ascorbic acid were foliarly applied under salt stress conditions.

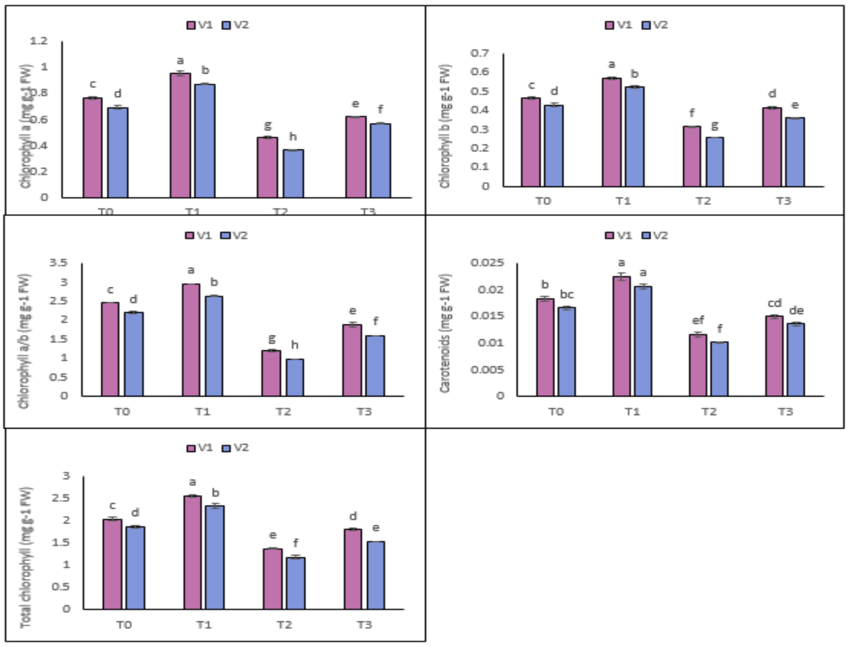


Fig: Chlorophyll *a*, chlorophyll *b*, chlorophyll *a/b*, carotenoids and total chlorophyll of Mustard (*Brassica juncea* L.) plants when ascorbic acid were foliarly applied under salt stress conditions.

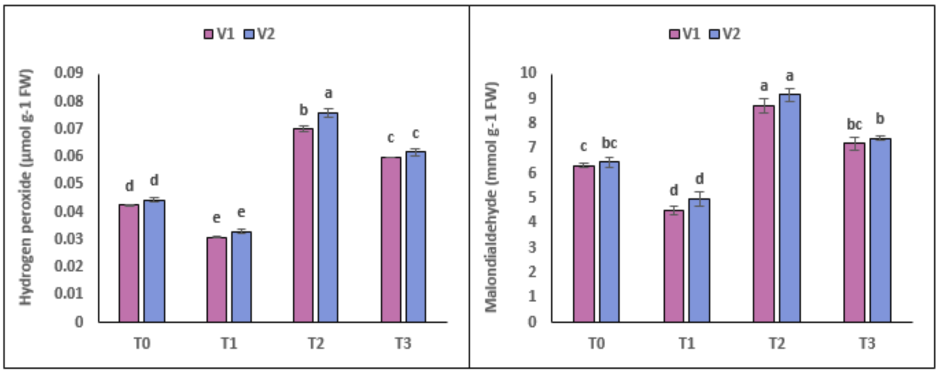


Fig: Hydrogen peroxide and malondialdehyde of Mustard (*Brassica juncea* L.) plants when ascorbic acid were foliarly applied under salt stress conditions.

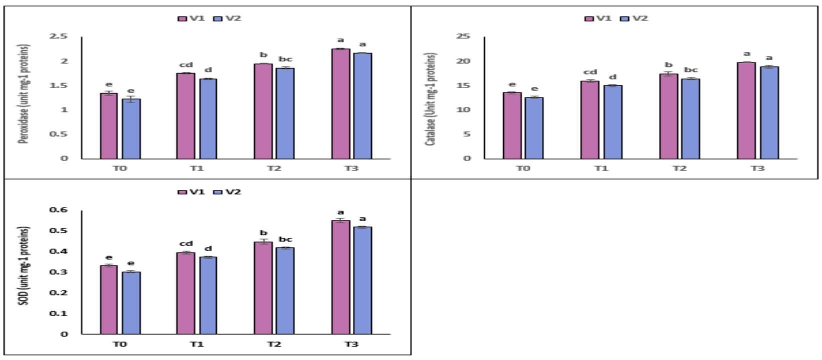


Fig: Activity of peroxidase, catalase super oxidase of Mustard (*Brassica juncea* L.) plants when ascorbic acid were foliarly applied under salt conditions.

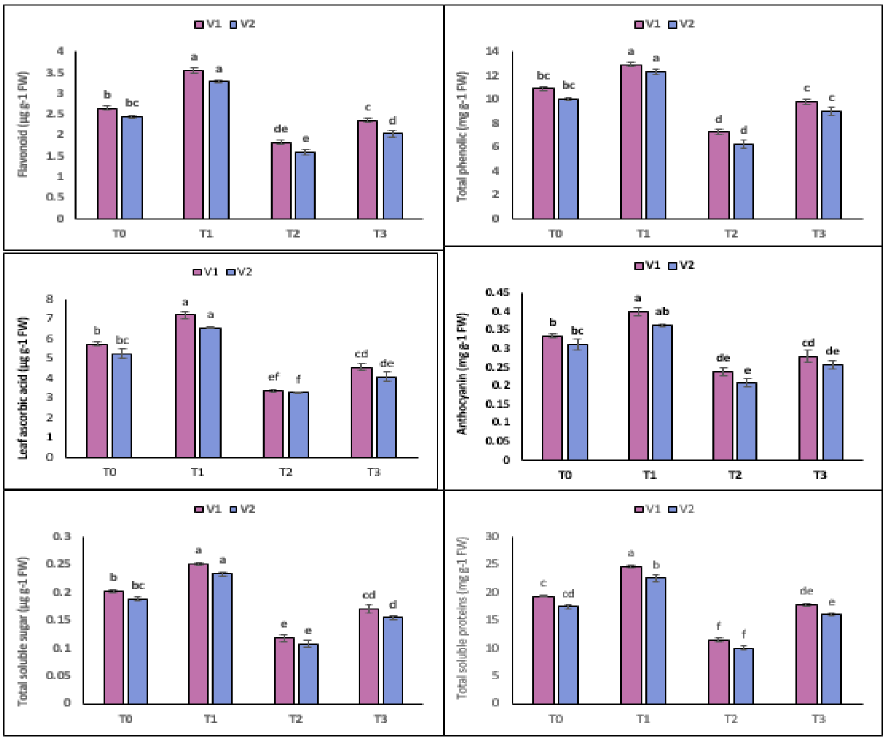
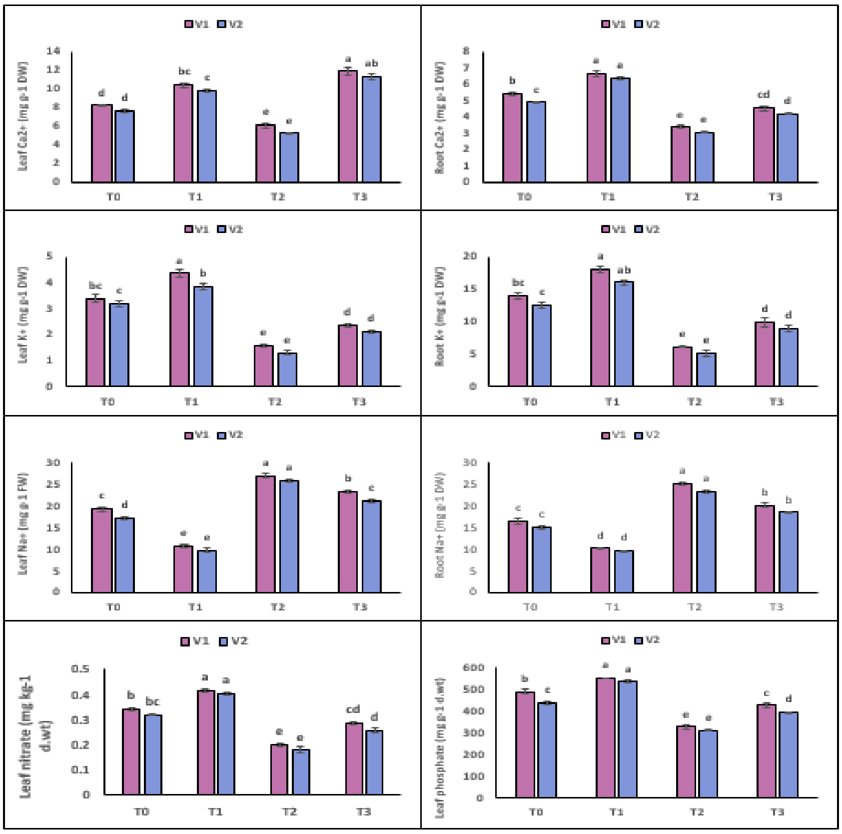


Fig: Flavonoids, total phenolics, leaf ascorbic acid, anthocyanin, total soluble sugar and total soluble proteins of Mustard (*Brassica juncea* L.) plants when ascorbic acid were foliarly applied under salt conditions.



Discussion

Current studies showed that application of AsA has positive impacts on plant morphological, biochemical and physiological attributes under salinity. The study comprised two *Brassica juncea* L. cultivars, Super Raya (V1) and Faisalabad Mustard (V2), being treated with 0 mM and 100 mM salt stress with control and foliar application of AsA 0 mM and 10 mM.

Morphological attributes decreased under salinity in both cultivars of mustard (V1 and V2) while decline was much prominent in Faisalabad Mustard (V2). The study suggested that decrease in vegetative biomass was due to inhibitory effects resulted ionic imbalance, stomatal closure and ultimately loss in growth (Liu *et al*., 2020). Use of AsA reduce negative effects of salt stress in mustard (Singh and Sengar, 2019). Our results were similar to Zeeshan *et al*. (2020) on wheat and barly and Noreen *et al*. (2021) on barley under salt stress conditions plant morphological attributes reduced due to disturbance in membrane permeability and ionic disparity lead to restrict plant growth and development.

In current experiment, salt stress caused significant decrease in content of chlorophyll *a*, *b*, carotenoid and total chlorophyll in both cultivars. Same results were shown by (Wani *et al*., 2013) in two cultivar of mustard *Brassica juncea* L. Ascorbic acid application increased photosynthetic pigment in millet plant (Hussein *et al*., 2014). Chloroplast is the key source of reactive oxygen species (ROS) formation in plants but catalase that can scavenge the harmful effects of ROS is absent, therefore AsA act as substrate to ascorbate peroxidase (APX) that can scavenge ROS production (Davey *et al*., 2000).

Analysis of this work suggested that salt stress caused a raise in malondialdehyde and H_2O_2 content. Our results were similar to Ebrahimian and Bybordi (2012) in sunflower and (Liu, and Baird, 2003) in sweet sorghum and sunflower respectively. Change in structure and composition of cell membrane lipids content occurred in response to high MDA level under salinity (Krupa-Małkiewicz *et al*., 2019). Under salinity production of H_2O_2 increased that can damage important cell components (Gill and Tuteja, 2010). When hydrogen peroxide increased in concentration then it causes reduction in photosynthetic pigment and photosystem II activities (Miller *et al*., 2010). AsA application reduces effects of salinity by enhancing plant defense mechanism. In past research AsA reduced harmful effect of salinity by reducing MDA and H_2O_2 in maize (Ahmad *et al*., 2013).

Enzymatic antioxidants like catalase, super oxide dismutase and peroxidase showed significant results in present study in both cultivar of mustard. Our results were related to previous study of salinity increased activity of antioxidant enzymes SOD, CAT and POD in canola (Ashraf and Ali, 2008), Proso Millet (Sabir *et al*., 2011), sunflower (Noreen and Ashraf, 2009), wheat (Ashraf *et al*., 2010), mustard (Ahmad, 2012), and chickpea (Ahmad *et al*., 2016). A key enzyme in cellular defense known as superoxide dismutase (SOD) stimulates reduction of superoxide radicals into H_2O_2 and O_2 (Foyer and Noctor, 2000). Ascorbic acid act as signaling molecule in plant under stress (Billah *et al*., 2017).

In present study, total soluble protein contents enhanced during application of ascorbic acid under salinity. While exposure of salinity increase TSP in wheat (Ishaq *et al*., 2021). Protein synthesis pathway is damaged because of stress (Abdelsayed *et al*., 2009). While rise in salt stress increase the amount of total soluble proteins in wheat (Ishaq *et al*., 2021).

According to our findings ascorbic acid application increased anthocyanin content could be associated with upregulation of antioxidant enzymes (Kovinich *et al*., 2018). In our study, under salinity total soluble sugar content increased. Our results were similar to (Siringam *et al*., 2012) found under salt stress increased total soluble sugar concentration in rice. It is supposed under salinity sugars and other compatible solutes have role in osmotic adjustment help plant to increase storage of reserves to improve plant metabolism under stress (Ozturk *et al*., 2021).

The result displayed that flavonoid content increased under the salt stress. Same results shown by Azizi *et al*. (2021) in marigold. Flavonoids act as antioxidant compound that enhance ability of plant to cope with reactive oxygen species production under stress. Ascorbic acid (AsA) content increased under application of ascorbic acid in mustard. The results suggested that higher level of AsA might be due to its relative more tolerance to salinity same results were observed by (Hassanein *et al*., 2009) in maize.

Results of current study reveal that micronutrients such as shoot and root potassium and calcium ions showed decrease and showed increase in mustard under salinity. While application of ascorbic acid causes significant decrease in concentrations of sodium ions in both cultivars. These results were similar to (Gul and Hamayun, 2015) in cluster bean. In previous study salinity stress decreased the shoot potassium ion contents in radish (Akram *et al*., 2015) and quinoa (Elewa *et al*., 2017).

Conclusion

The findings of this study concluded the salt stress considerably reduced shoot and root fresh dry weight and length in both cultivars of mustard. However, ascorbic acid implementation improved above mentioned parameters. Physiological parameters were also declined by NaCl treatment while these were enhanced when subjected to exogenous ascorbic acid treatment. Further, it was noted that imposition of salinity led to higher production of oxidants that were positively scavenged by the synthesis of antioxidants like CAT, SOD and POD by ascorbic acid in response to ascorbic acid under salt stress. In similar way, the accumulation of flavonoid contents, anthocyanin, leaf ascorbic acid, leaf phenolics, TSS and TSP were statistical higher in response to interactive application of ascorbic and salt stress. Moreover, leaf and root mineral ions (Ca^{2+} and K^+) were reduced while leaf and root Na^+, leaf phosphorus and nitrate seemed to be increased in mustard plants associated to stress. Overall result showed that Super Raya cultivar performed better under stress and foliar application of ascorbic acid.

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