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OXIDATIVE STRESS ALLEVIATION THROUGH ENZYMATIC AND NON-ENZYMATIC ANTIOXIDANTS AND OSMOREGULATORS GENERATION IN BARLEY (*HORDEUM VULGARE* L.) UNDER SALT (NACL) STRESS BY ASCORBIC ACID (ASA)

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Abstract

Among crops, *Hordeum vulgare* L. (barley) is one of the most economically important species, considered as an excellent model for studies of agronomy, plant physiology and abiotic stress, with a rapid growth rate and a high adaptability to various habitats. Ascorbic acid (AsA), also referred to as vitamin C, is a major nonenzyme antioxidant in plants and plays an important role in mediating certain oxidative stresses caused by biotic and abiotic stress. Therefore, a pot experiment was conducted to investigate the effect of different levels of AsA (0, 30 and 60 mM) in *H. vulgare* using two specific salinity levels, 0 and 150 mM. The results from the present study showed that the high soil salinity concentration decreased plant growth and biomass and photosynthetic pigments in *H. vulgare* thus elevating the generation of reactive oxygen species (ROS), suggesting that high NaCl concentrations induce oxidative stress in *H. vulgare*. Moreover, the enzymatic activities of superoxidase dismutase (SOD) and peroxidase (POD) came into play to reduce salinity stress and showed that *H. vulgare* could tolerate low levels of salt stress, i.e., 150 mM. It was also noticed that exogenous application of AsA considerably increased plant height, plant leaf area, sheet number, tillers number, plant fresh and dry biomass, photosynthesis pigments, and reduced the oxidative stress (H_2O_2 and MDA) in plants which in turn reduced the enzymatic (SOD and POD) and non-enzymatic antioxidants (GSH and AsA). Research findings, therefore, suggested that AsA application can ameliorate salinity stress in *H. Vulgare* and resulted in improved plant growth and composition under abiotic stress condition, as depicted by reduced the genetaion of ROS.

Key words: Barley (*Hordeum vulgare* L.), Enzymatic antioxidants, Growth, Non-enzymatic antioxidants, Oxidative stress, Plant hormone and Salinity.

Introduction

Salinity is a major stress limiting the increase in the demand for food crops and is the major environmental factor limiting plant growth and productivity (Kamran *et al.*, 2019; Ali *et al.*, 2020; Hassan *et al.*, 2021). Salinity of arable land is an increasing problem of many irrigated, arid and semi-arid areas of the world where rainfall is insufficient to leach salts from the root zone and the physiological responses of a plant to salinity are often complex and multi-faceted, which makes experiments difficult to design and interpret (Parida & Das, 2005; Yadav *et al.*, 2020). Physiological measurements have been revolutionized by new technologies, such as high-throughput phenotyping, bioinformatics and novel analytical methods that have enabled fields such as metabolomics to emerge. Salinity stress involves changes in various physiological and metabolic processes, depending on severity and duration of the stress, and ultimately inhibits crop production (Kazemi *et al.*, 2019; Trabelsi *et al.*, 2019). Increasing level of salt stress reduced the plant fresh and dry weights, germination rate plant length, root dry weight, rate of photosynthesis, lipids and energy production (Farooq *et al.*, 2019; Liu *et al.*, 2020). In addition, stress condition induces toxicity symptoms appear, including reduced yield, poor seed germination, stunted leaf and root growth, and ultrastructural and anatomical alterations leading to the formation of reactive oxygen species (ROS) (Imran *et al.*, 2019; Javed *et al.*, 2020; Parveen *et al.*, 2020; Saleem *et al.*, 2020). Antioxidants such as superoxide dismutase (SOD) and peroxidase (POD) come into play to scavenge ROS (Afzal *et al.*, 2020; Imran *et al.*, 2020; Saleem *et al.*, 2020; Saleem *et al.*, 2020); for example, SOD facilitates the conversion of superoxide radicals (O^{-1}) to hydrogen peroxide (H_2O_2) while POD decomposes H_2O_2 into water (H_2O) and molecular O_2 (Kamran *et al.*, 2020; Nazar *et al.*, 2020; Saleem *et al.*, 2020; Zaheer *et al.*, 2020; Hameed *et al.*, 2021; Mumtaz *et al.*, 2021).

Ascorbic acid (AsA) "commonly known as Vitamin C," is an important non-enzymatic antioxidant play an important role in controlling plant growth and stress production (Fatima *et al.*, 2019;

Hassan *et al.,* 2021). AsA has the ability to promote plant growth and thus give tolerance to the plants against stress environment and also regulate photosynthetic pigments (Huang *et al.,* 2008; Zhou *et al.,* 2016). Many previous studies revealed that the exogenous application of AsA significantly improved plant growth and development in Oryza sativa (Zhou *et al.,* 2016) and Hydrilla verticillata (Xu *et al.,* 2006). AsA is the first line of defense against oxidative stress because it removes many different ROS like H_2O_2 and $^1O_2$. The exogenous application of AsA directly inhibits malondialdehyde (MDA) contents and thus induce antioxidative enzymes in the plant tissues (Farooq *et al.,* 2013).

Barley (*Hordeum vulgare* L.) is an annual crop, member of the grass family (Poaceae), and mostly cultivated in temperate climate globally. *H. vulgare* has been cultivated more than 10,000 years ago and used as animal fodder, beverages, soups, and barley bread (Arab & Ehsanpour, 2006; Aksakal, 2013). Moreover, *H. vulgare* is very tolerant of the salinity when compared with other cereal crops. However, *H. vulgare* has been studied by many researchers against abiotic stress environment (Singh *et al.*, 2008; Shen *et al.*, 2011; Xu *et al.*, 2012; Ullah *et al.*, 2016).

The purpose was to assess the oxidative stress of the present pot experiment, which ultimately enhances the activities of enzymatic and non-enzymatic antioxidants in *H. vulgare* under elevating levels of AsA in saline soil. Although, these characteristics are undefined in *H. vulgare*, but few studies have been conducted on salinity stress in *H. vulgare* (Singh *et al.*, 2007; Srivastava *et al.*, 2008; Hasanuzzaman *et al.*, 2013) but very few studies have been exploring the effect of elevated levels of AsA under saline conditions. The findings will improve our understanding of this study about (i) effect of growth and photosynthetic pigments under elevating levels of AsA in *H. vulgare* under saline condition (ii) exogenous application of AsA induce oxidative stress which enhances the activities of enzymatic and non-enzymatic antioxidants under salinity stress.

Material and Methods

Plant material and experimental treatments: A plant experiment was carried out in the botanical garden of the University of Agriculture, Faisalabad 38040, Pakistan. Barley (*H. vulgare*) genotype seed Fall-87 was obtained from the Ayub Agricultural Research Institute (AARI), Faisalabad, for the pot experiment. The pots (30 cm-total to 40 cm) were filled with 15 kg of salt-free soil which was obtained from the experimental station of the University of Agriculture, Faisalabad, Pakistan. The seeds were washed with 95% ethanol and ten seeds were sown in each pot. Physicochemical properties of soil used for pot experiments were as follows: pH 6.9, EC 0.9 dS/cm, 17 g/kg organic matter, 21 mg/kg convertible K, 0.17 g/kg total P, 16 g/kg total N and 2.3 meq/L total Cl. The soil was artificially supplemented with different levels of sodium chloride (0 and 150 mM) and applied foliar application of AsA (0 mM, 30 mM, 60 mM) exogenously after ten days of sowing (DAS). The pots were put with five replicates of each treatment in a completely randomized system (CRD). After two weeks of germination, thinning was performed and five plants were left in each pot. Weeding and other necessary intercultural operations were carried out when necessary. Moreover, in the winter, plant was placed in the open-air botanical garden, where they received natural light with day/ night temperatures of 25/15 C and day/night humidity of 60/70%.

Sampling and data collection: All plants were collected for different morphological characteristics in late February 2016. The height of the plant was determined by the total shooting and root length than the number of leaves, and the number of tillers. Fresh plant weight was determined using a weighting balance by weighing the total plant weight, and the oven was then dried for dry biomass at 65°C for 72 h. Sampling was carried out on 45 DAS for photosynthetic pigments and antioxidant enzymes. In the early morning (8:00–9:00 A.M.), full functional leaf (fifth from the top) was chosen for each treatment. For further examination, the leaves were washed immediately with distilled water suspended in liquid nitrogen and held at (-80°C).

Chlorophyll contents: Leaves were collected for determination of chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 C in the dark. The absorbance was measured by a spectrophotometer (UV2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6 and 450 nm. Chlorophyll content was calculated by the standard method of (Arnon, 1949).

Oxidative stress and antioxidants: The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) contents. Briefly, 0.1 g of frozen leaves were ground at 4 C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethene pyrrole. The homogenate was centrifuged at 10,000 × g at 4 C for 15 min. The mixtures were heated at 100 C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMarkTM Microplate Absorbance Spectrophotometer; Bio-Rad, United States) at wavelengths of 532, 600 and 450 nm. Lipid peroxidation was expressed as l mol g^{-1} by using the formula: 6.45 (A532-A600)-0.56 A450. Lipid peroxidation was measured by using a method previously published by (Heath and Packer, 1968).

To estimate H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulphate in 20% (v/v) H_2SO_4 and centrifuged at 6000 × g for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}. The contents of H_2O_2 were measured by the method presented by (Jana and Choudhuri, 1981)

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenised in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0) including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at 12,000 × g for 10 min at 4 C, and the supernatant was used for measurement of superoxidase dismutase (SOD) and peroxidase (POD) activities.

SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine and 100 lL enzyme extract. Finally, the sample was measured by using a spectrophotometer (xMarkTM Microplate Absorbance Spectrophotometer; Bio-Rad). Enzyme activity was measured by using a method by (Chen and Pan, 1996) and expressed as U g^{-1} FW.

POD activity in the leaves was estimated by using the method of (Sakharov and Ardila, 1999) by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2 and 0.1 mL of 4% guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme

Plant ethanol extracts were prepared for the determination of non-enzymatic antioxidants and some key osmolytes. For this purpose, 50 mg of plant dry material was homogenized with 10 mL ethanol (80%) and filtered through Whatman No. 41 filter paper. The residue was re-extracted with ethanol and the two extracts were pooled together to a final volume of 20 mL. The determination of ascorbic acid (Azuma *et al.*, 1999) and glutathione (Lewis *et al.*, 1998) was performed from the extracts.

Statistical analysis

All results were presented as arithmetic means with standard deviation except otherwise defined. Data were tested with one-way ANOVA (analysis of variance) followed by HSD test to compare with treatments means using Statistix 8.1. The level of significance was set at (p<0.05 or p<0.01). Graphical analysis using SigmaPlot10 and R Studio tools.

Results

Effect of different levels of salinity with or without the application of ASA on plant growth and biomass: This study studied the effect of different salinity levels (0 mM and 150 mM) and AsA levels (0 mM, 30 mM, and 60 mM) on *H. vulgare*. Table 1 shows growth and biomass in plant height, leaf area, number of leaves, number of tillers, fresh and dry plant biomass. It was found that high soil salinity affected plant growth and biomass, but AsA's foliar application improved plant growth and development. Minimum plant height was observed in plants grown in salinity without AsA foliar application, i.e., 40 cm, while average plant height was observed in plants with 0 mM NaCl in soil while maximum AsA foliar application i.e. 86 cm compared to plants grown without salinity and AsA, was observed. Similarly, the maximum reduction in leaf area, number of leaves, and number of tillers by 36%, 43%, and 57%, respectively, was observed in plants grown in salinity without AsA application compared to plants grown without salinity and exogenous AsA application. Our research results showed that fresh and dry biomass in plants grown in salinity without AsA was reduced by 33% and 46%, respectively, compared to plants grown without AsA salinity and application. In the plant grown without NaCl, maximum plant fresh and dry biomass (19.6 g and 5.6 g respectively) was observed in the soil and maximum foliar application of AsA, i.e., 60 mM.

Effect of different levels of salinity with or without application of ASA on photosynthetic pigments: The photosynthetic pigments in terms of total chlorophyll and carotenoid contents are given in Table 2. These results revealed that high soil concentration of NaCl decreased photosynthetic pigments, while exogenous AsA application increased these contents. The maximum content of total chlorophyll and carotenoids (3.2 and 0.6 mg g^{-1} FW, respectively) was observed in soil-grown plants with 0 mM NaCl concentrations and high AsA concentrations. Compared to plants grown without NaCl and AsA, total chlorophyll and carotenoid content decreased by 42% and 60% respectively in saline soil-grown without AsA. However, due to the exogenous application of AsA i.e. 60 mM, total chlorophyll and carotenoid increased by 21% and 50% under salinity stress.

Effect of different levels of salinity with or without the application of ASA on oxidative stress: The high concentration of malondialdehyde (MDA) in the leaves of *H. vulgare* is the indication of oxidative stress. The results regarding MDA contents and hydrogen peroxide (H_2O_2) are shown in (Fig. 1). The high concentration of NaCl in the soil increased MDA and H_2O_2 in *H. vulgare*. However, elevating levels of exogenous application of plant growth hormone i.e., AsA reduced oxidative stress and decreased the contents of MDA and H_2O_2 in the leaves. The maximum values of MDA and H_2O_2 (89 μ $molesg^{-1}$ FW and 2.3 μ gg^{-1} FW, respectively) were observed in the plants grown under the saline soil condition without the exogenous application of AsA. Increased exogenous application of AsA under saline soil decreased MDA and H_2O_2 by 43% and 56%, respectively.

Table 1. Effect of different levels of salinity with or without the application of ASA on plant height, leaf area, number of leaves, number of tillers, plant fresh weight and plant dry weight in *H. vulgare*.

Treatments	Plant height (cm)	Leaf area (cm^2)	Number of leaves	Number of tillers	Plant fresh weight (g)	Plant dry weight (g)
$S_0 + ASA_0$	69 ± 1.6 c	16.5 ± 0.9 c	10.4 ± 0.6 c	2.3 ± 0.05 ab	16.2 ± 1.6 c	4.8 ± 0.2 b
$S_0 + ASA_1$	76 ± 2 b	18.1 ± 0.8 b	12 ± 1 b	2.6 ± 0.05 a	18.3 ± 1.2 b	5.2 ± 0.3 ab
$S_0 + ASA_2$	86 ± 2.3 a	19.6 ± 0.6 a	13 ± 1.3 a	2.6 ± 0.1 a	19.6 ± 1.3 a	5.6 ± 0.4 a
$S_1 + ASA_0$	40 ± 2.5 f	10.4 ± 0.5 f	6 ± 0.3 f	1 ± 0.05 c	11 ± 0.8 f	2.6 ± 0.05 e
$S_1 + ASA_1$	51 ± 1 e	12 ± 0.6 e	7 ± 0.4 e	1.3 ± 0.1 bc	12.5 ± 0.6 e	3.1 ± 0.2 d
$S_1 + ASA_2$	58 ± 1.6 d	13.2 ± 0.7 d	8.6 ± 0.5 d	1.6 ± 0.05 b	14.3 ± 1 d	3.5 ± 0.1 c

Values in the table is just one harvest. Mean ± SD (n =3). Different letters within a column indicate significant difference between the treatments (p<0.05 or p<0.01). Different lowercase letters on the error bars indicate significant difference between the treatments. Relative radiance of plastic filter used: $S_0 + ASA_0$ (Saline = 0 mM and Ascorbic acid = 0 mM), $S_0 + ASA_1$ (Saline = 0 mM and Ascorbic acid = 30 mM), $S_0 + ASA_2$ (Saline = 0 mM and Ascorbic acid = 60 mM), $S_1 + ASA_0$ (Saline = 150 mM and Ascorbic acid = 0 mM), $S_1 + ASA_1$ (Saline = 150 mM and Ascorbic acid = 30 mM) and $S_1 + ASA_2$ (Saline = 150 mM and Ascorbic acid = 60 mM)

Table 2. Effect of different levels of salinity with or without the application of ASA on chlorophyll A, chlorophyll B, total chlorophyll and carotenoid contents in *H. vulgare*.

Treatments	Chlorophyll A (mg g^{-1} FW)	Chlorophyll B (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
$S_0 + ASA_0$	2.1 ± 0.1 b	0.7 ± 0.03 b	2.9 ± 0.2 b	0.5 ± 0.06 b
$S_0 + ASA_1$	2.3 ± 0.2 ab	0.8 ± 0.02 a	3.1 ± 0.2 ab	0.6 ± 0.03 a
$S_0 + ASA_2$	2.4 ± 0.3 a	0.8 ± 0.03 a	3.2 ± 0.4 a	0.6 ± 0.04 a
$S_1 + ASA_0$	1.4 ± 0.05 d	0.3 ± 0.01 e	1.7 ± 0.3 e	0.2 ± 0.01 d
$S_1 + ASA_1$	1.5 ± 0.05 cd	0.4 ± 0.01 d	1.9 ± 0.2 d	0.3 ± 0.05 c
$S_1 + ASA_2$	1.6 ± 0.1 c	0.5 ± 0.02 c	2.1 ± 0.5 c	0.3 ± 0.01 c

Values in the table is just one harvest. Mean ± SD (n =3). Different letters within a column indicate significant difference between the treatments (p<0.05 or p<0.01). Different lowercase letters on the error bars indicate significant difference between the treatments. Relative radiance of plastic filter used: $S_0 + ASA_0$ (Saline = 0 mM and Ascorbic acid = 0 mM), $S_0 + ASA_1$ (Saline = 0 mM and Ascorbic acid = 30 mM), $S_0 + ASA_2$ (Saline = 0 mM and Ascorbic acid = 60 mM), $S_1 + ASA_0$ (Saline = 150 mM and Ascorbic acid = 0 mM), $S_1 + ASA_1$ (Saline = 150 mM and Ascorbic acid = 30 mM) and $S_1 + ASA_2$ (Saline = 150 mM and Ascorbic acid = 60 mM)

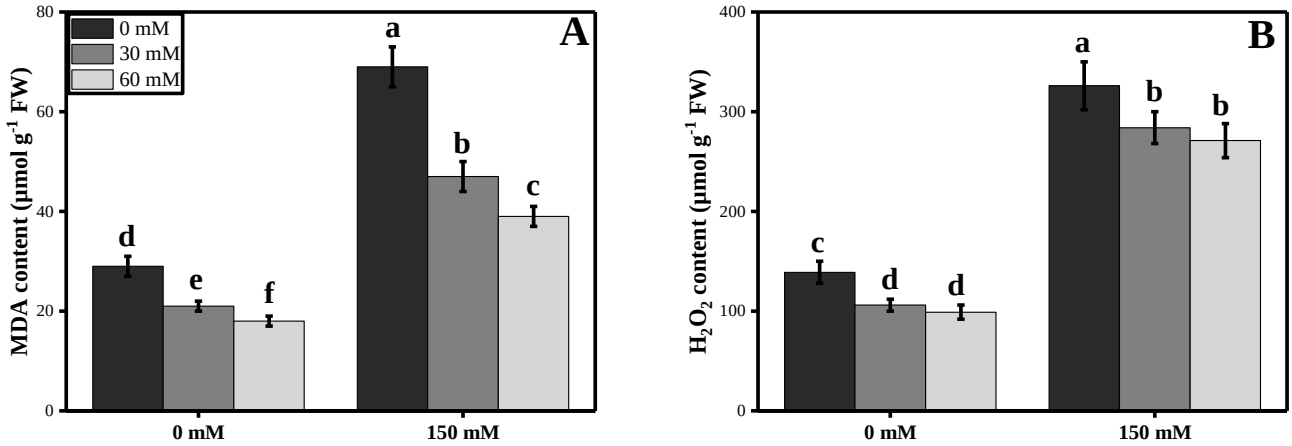

Fig. 1. Effect of different levels of salinity with or without the application of ASA on reactive oxygen species (ROS) in MDA (a) and H_2O_2 (b) in *H. vulgare*. Mean ± SD (n =3). Different letters within a column indicate significant difference between the treatments (p <0.05 or p<0.01). Different lowercase letters on the error bars indicate significant difference between the treatments. Different levels of salinity used in the figure are as follow: 0 mM (Saline = 0 mM) and 150 mM (Saline = 150 mM) and different levels of ASA used in the figure are as follow: 0 mM (ASA 0 mM), 30 mM (ASA 30 mM) and 60 mM (ASA 60 mM).

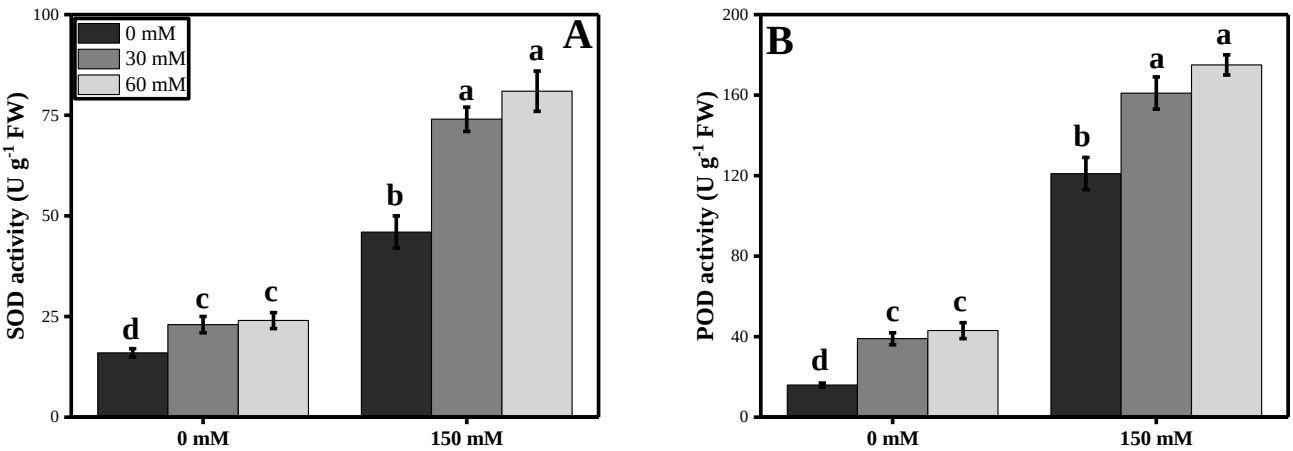

Fig. 2. Effect of different levels of salinity with or without the application of ASA on enzymatic antioxidants in SOD (a) and POD (b) in *H. vulgare*. Mean ± SD (n =3). Different letters within a column indicate significant difference between the treatments (p<0.05 or p<0.01). Different lowercase letters on the error bars indicate significant difference between the treatments. Different levels of salinity used in the figure are as follow: 0 mM (Saline = 0 mM) and 150 mM (Saline = 150 mM) and different levels of ASA used in the figure are as follow: 0 mM (ASA 0 mM), 30 mM (ASA 30 mM) and 60 mM (ASA 60 mM).

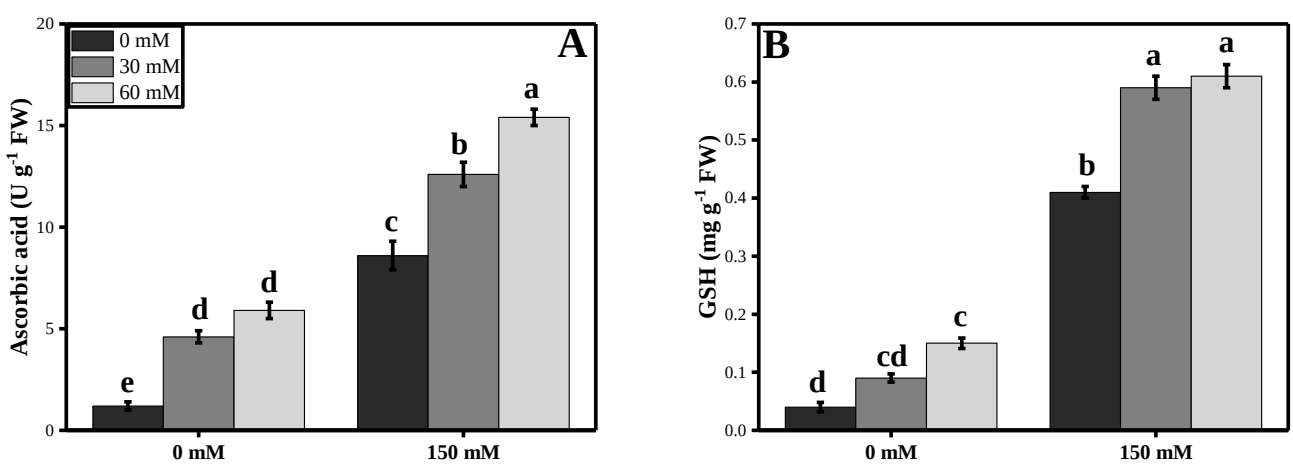

Fig. 3. Effect of different levels of salinity with or without the application of ASA on non-enzymatic antioxidants in ASA (a) and GSH (b) in *H. vulgare*. Mean ± SD (n =3). Different letters within a column indicate significant difference between the treatments (p<0.05 or p<0.01). Different lowercase letters on the error bars indicate significant difference between the treatments. Different levels of salinity used in the figure are as follow: 0 mM (Saline = 0 mM) and 150 mM (Saline = 150 mM) and different levels of ASA used in the figure are as follow: 0 mM (ASA 0 mM), 30 mM (ASA 30 mM) and 60 mM (ASA 60 mM).

Effect of different levels of salinity with or without the application of ASA on enzymatic and non-enzymatic antioxidants: In the present study effect of different levels of salinity (0 mM and 150 mM) and AsA (0 mM, 30 mM and 60 mM) on enzymatic and non-enzymatic antioxidants were also studied. Our results suggested that the high concentration of NaCl in the soil induced of enzymatic (Fig. 2) and non-enzymatic activity (Fig. 3) in the leaves of *H. vulgare*. The maximum value of enzymatic activities of SOD and POD (91 U g^{-1} FW and 156 U g^{-1} FW, respectively) was observed in the plants grown on saline soil without the application of AsA (Fig. 2). However, elevating levels of AsA (30 mM and 60 mM) decreased SOD and POD activities by 19% and 32% in the plants grown on saline soil with the highest concentration of AsA when compared with the plants grown without AsA under salinity. Similarly, changes in the concentration of non-enzymatic antioxidants such as GSH and AsA under salinity stress showed similar trends as enzymatic antioxidants did. The contents of GSH and AsA decreased due to the high concentration of NaCl in the soil while a foliar spray of plant growth hormone i.e., AsA reduced the contents of GSH and AsA in the leaves of *H. vulgare* (Fig. 3). The maximum contents of GSH and AsA (1.9 mg g^{-1} FW and 4.3 mg g^{-1} FW respectively) were observed in the plants grown under salinity without the application of AsA. However, application of AsA reduced the contents of GSH and AsA by 73% and 47% under saline soil.

Correlation between plant morphology and physiology: Pearson's correlation graph was carried out to find a relationship between different morphological and physiological attributes (Fig. 4). Plant height was found positively correlated with other morphological traits while negatively correlated with oxidative stress and antioxidants response. On the other hand, H_2O_2 contents found to be positively correlated with other physiological traits while showed a negative correlation with plant growth and biomass of the plant. The relation between the various growth and physiological attributes of *H. vulgare* showed a close connection.

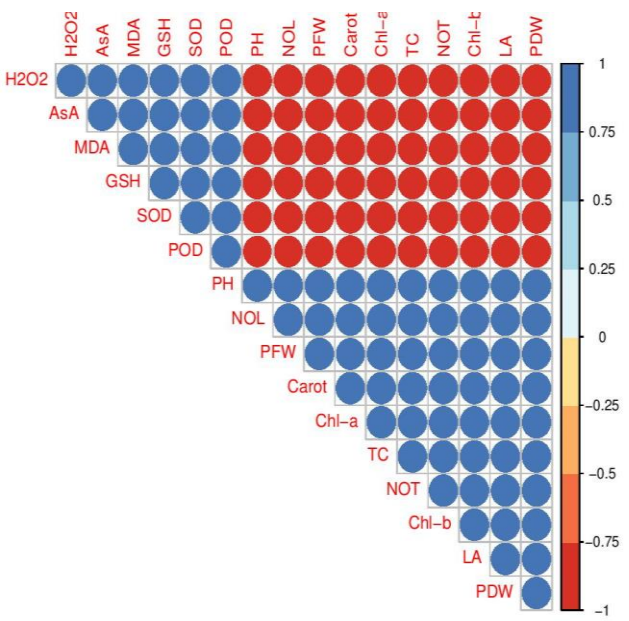

Fig. 4. Correlation between different attributes studied in *H. vulgare*. H_2O_2 (H_2O_2 contents), AsA (ascorbic acid contents), MDA (MDA contents), GSH (GSH contents), SOD (SOD activity), POD (POD activity), PH (plant height), NOL (number of leaves), PFW (plant fresh weight), Carot (Carotenoid contents), Chl-a (Chlorophyll-a contents), TC (total chlorophyll), NOT (number of tillers), Chl-b (Chlorophyll-b contents), LA (leaf area) and PDW (plant dry weight).

Discussions

Initially soil salinity is known to represses plant growth in the form of osmotic stress which is then followed by ion toxicity (Ahmad *et al.*, 2012; Dawood & El-Awadi, 2015). During the initial phases of salinity stress, water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress of high salt (Parihar *et al.*, 2015; Farooq *et al.*, 2019). Salt stress is defined as the negative impact of important nutrients such as Na and Cl on plant growth and development (Ahmad *et al.*, 2012; Dawood & El-Awadi, 2015). One of the major factors of high salinity in the soil is the irrigation of agricultural land, and it is the most crucial factor which directly affects crop yield and productivity. The high concentration of salts in the land is also due to some natural processes like weathering of parental material, which ultimately breaks down many chlorides of calcium and sodium. Moreover, the deposition of seawater to the agricultural land by the wind or other factors causes salinity in the soil (Wang & Li, 2008; Zafar *et al.*, 2019). The high concentration of salt is toxic for the plants as it reduced plant growth and development because salinity reduces the uptake of some important nutrients in the soil. Moreover, if plants accumulate a high concentration of salt in their transpiration stream it causes injury in transpiring leaves and ultimately reduce plant growth and development (Parihar *et al.*, 2015). Previously, studies showed that under salinity conditions, plants could show better growth and development by using some plant growth regulators (PGRs) (Ashraf *et al.*, 2008). Other studies showed that under stress conditions, PGRs overcome the toxicity of excess salt and may mitigate salt-induced injurious effects (Hayat *et al.*, 2010). Exogenous application of AsA is believed to be a reduced toxic effect of salt when plants subjected to abiotic and biotic stress (Wang *et al.*, 2002; Zheng *et al.*, 2006; Wang & Kao, 2007).

Salt stress mainly affects plants in two ways, i.e., osmotic stress, induced by the deposition of large quantities of soluble salts in the soil, which reduces the supply of soil water to plants; and ion toxicity, caused by high salt accumulation within the plant, which disrupts a number of metabolic processes, including the inactivation of certain enzymes. This research also observed reduced plant growth and biomass. Exogenous use of AsA improved plant growth and biomass (Table 1). Similar findings were observed in maize and soybean studies by (Wei *et al.*, 2015) and (Jiang *et al.*, 2016), respectively. The impact of salinity on plants depends on different responses, i.e., nutritional imbalance, which is caused by interference of salinity and reduce uptake of many essential nutrients and water contents. Moreover, nutritional imbalance also causes dehydration in the plant cell, which can cause production in reactive oxygen species (ROS) due to the increase in the concentration of sodium and chloride ions during salinity stress (Mohamed *et al.*, 2020; Saleem *et al.*, 2020; Zaheer *et al.*, 2020). (Barakat, 2003) noticed that foliar application of AsA increased physiological characters in plants leading to the plant tolerance against stress conditions and provide room to uptake more nutrients and water contents. (Hasanuzzaman *et al.*, 2009) studies on *O. sativa* showed that salinity caused a great reduction in plant height and number of tillers. Guan *et al*., (Guan *et al.*, 2011) studied Suaeda salsa and found that a high concentration of NaCl in the soil significantly reduced plant height number of leaves and number of branches. However, Dolatabadian *et al*., (Dolatabadian *et al.*, 2011) studied Glycine max and found that high salinity stress decreased fresh and dry biomass of the plant. While the exogenous application of AsA increased plant growth and development as showed by (Wang & Kao, 2007).

The results of this study also revealed that the photosynthetic pigment of *H. vulgare* was also decreased with the exposure of salinity in the soil (Table 2). However, increase in the exogenous application of AsA causes significantly

increased in the contents of total chlorophyll and carotenoids. In addition, reduction in the growth attributes is strongly linked with photosynthetic pigments while reduction in the chlorophyll contents is due to the high concentration of Na^+ and Cl^- stress (Ahmad *et al.*, 2018). Similar results were found by (Prajuabmon *et al.*, 2009) and (Agami, 2014). AsA is very beneficial to different organelles of the cell under saline conditions as it mitigating oxidative damage. So, due to this reason chlorophyll has high contents of chloroplast in it and thus increased phosphate activity (Farahat *et al.*, 2007; Dolatabadian & Jouneghani, 2009). Exogenous application of AsA with or without the concentration of salt in the soil significantly increased photosynthetic pigments (Woodward & Bennett, 2005; Pattanagul, 2011; Aly *et al.*, 2012).

Many previous studies showed that when plants are subjected to some stress conditions then there is a large amount of generation of reactive oxygen species (ROS) which then ultimately cause peroxidation to many important cellular structures such as lipid, chlorophyll and photosynthetic pigments (Ali *et al.*, 2020; Javed *et al.*, 2020; Parveen *et al.*, 2020; Saleem *et al.*, 2020). Salinity stress is strongly correlated with over production of ROS which are extremely toxic for the plants and ultimately cause oxidative stress (Kamran *et al.*, 2019). ROS generated during salt stress are interconnected with the enzymatic (SOD and POD) and non-enzymatic activities (GSH and AsA) (Hameed *et al.*, 2021; Nawaz *et al.*, 2021; Noor *et al.*, 2021; Perveen *et al.*, 2021). The activities of enzymatic and non-enzymatic antioxidants also enhance under salinity conditions for the scavenging of ROS in the cell. Therefore, under stress conditions enzymatic and non-enzymatic antioxidants plays a crucial part in reducing salt toxicity (Bhantana *et al.*, 2021; Deng *et al.*, 2021; Javed *et al.*, 2021). In the present study, high concentration of NaCl in the soil caused increase in the contents of MDA and H_2O_2 which is the indication of cellular oxidative damage (Fig. 1). However, exogenous application of AsA reduced the contents of MDA and H_2O_2 thus ultimately reducing oxidative stress and improved plant growth and development with or without the salinity in the soil. To overcome the production of ROS in the tissues plants defense system i.e., enzymatic (SOD and POD) (Fig. 2) and non-enzymatic (GSH and AsA) (Fig. 3) comes into play to reduce toxicity (Imran *et al.*, 2019; Rehman *et al.*, 2019; Saleem *et al.*, 2019; Yasmin *et al.*, 2021). The production of ROS is directly linked with antioxidants while foliar application of AsA reduce the activity of both kinds of antioxidants. The plant tolerance under saline conditions by the application of AsA is related to antioxidants as it diminishes H_2O_2, $^1O_2$ and OH which are the types of ROS and may cause toxicity in plants (Plaut *et al.*, 2004; Anjum *et al.*, 2011; Tan *et al.*, 2012; Vaculík *et al.*, 2015; Pereira *et al.*, 2018). In a previous study in *H. vulgare* (Agami, 2014) the foliar application of AsA was used to reduce the oxidative damage by MDA in the plant cells through increasing activities of antioxidants for the scavenging of ROS under the soil containing high concentration of NaCl. These findings suggested that increasing the activities of antioxidants initially protected photosynthetic pigments and increasing plant tolerance mechanism against salinity stress (Ramzan *et al.*, 2014; Zafar *et al.*, 2015).

Conclusion

In this study the effect of various AsA levels was investigated (0 mM, 30 mM and 60 mM) on morphological traits, photosynthetic pigments, oxidative stress and antioxidants under saline or non-saline soil. These results showed that high soil NaCl concentration reduced plant height, leaf area, number of leaves, number of tillers, chlorophyll and carotenoid contents while induced the oxidative stress by generation of large amount of ROS because of large MDA and H_2O_2 content in leaves of *H. Vulgare*. To combat oxidative stress plants, special antioxidant defense mechanism (enzymatic and non-enzymatic) which reduced salt toxicity in *H. vulgare*. The foliar application of AsA also improved plant growth and development significantly by increasing soil absorption of nutrients and water content. Moreover, AsA reduced oxidative stress by decreasing the MDA contents even in saline soil and increased salt tolerance in plants. These results indicated that salt tolerance is increased by exogenous application of AsA.

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