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ORIGINAL ARTICLE

Acclimation to salinity stress through maintaining the redox status by H_2O_2 and arginine application in *Vicia faba*

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Abstract

The generation of reactive oxygen/nitrogen species under either natural or stressful conditions activates antioxidant systems to balance the redox status in plant cells. The effect of seed priming with low concentrations of H_2O_2 and/or arginine (Arg) was examined on redox status of salinity-stressed *Vicia faba* in the present study. Salinity stress produced H_2O_2 and caused oxidative damage to photosynthetic pigments and membrane lipids represented by reduction in chlorophyll contents and carbohydrate production, and high accumulation of malondialdehyde, denoted as oxidative distress. Under saline conditions, Arg and/or H_2O_2 priming increased the activity of antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase), non-enzymatic antioxidants (phenols, flavonoids, anthocyanins and carotenoids), proline and total antioxidant activity (based on the FRAP method) followed by a reduction in the malondialdehyde content and an increase in the chlorophylls and water soluble carbohydrates contents. Altogether, the seed priming with H_2O_2 and Arg could constitute a 'priming memory' in seeds of *V. faba*, which recruited upon a subsequent salinity stress-exposure and induced stress-tolerance of primed beans trough invoking antioxidant systems.

Keywords Antioxidant systems · Nitric oxide · Oxidative stress · Reactive nitrogen species (RNS) · Reactive oxygen species (ROS) · Seed priming

Abbreviations

Anth	Anthocyanins
APX	Ascorbate peroxidase
Arg	Arginine
CAT	Catalase
Chl	Chlorophyll
H_2O_2	Hydrogen peroxide
EDTA	Ethylenediaminetetraacetic acid
Flav	Flavonoids
FRAP	Ferric reducing antioxidant power
MDA	Malondialdehyde
NBT	Nitroblue tetrazolium
NO	Nitric oxide
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
TCA	Trichloroacetic acid
WSC	Water soluble carbohydrates

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Introduction

The redox status of cell is a balance between the production of oxidants, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), and antioxidants (Gallé et al. 2021; Ramakrishnan et al. 2022). In plant species, ROS generation mainly takes place in photosynthesis, mitochondrial electron transport and photorespiration (Brestic et al. 2016; Ramakrishnan et al. 2022; Wei et al. 2022). RNS, a derivative form of nitric oxide (NO) is produced by NO synthase-like, NO reductase and nitrate reductase enzymes (Liu and Liao 2022; Ramakrishnan et al. 2022). Both oxidants regulate important cellular processes, while their excessive production under stress conditions trigger the oxidative stress response to maintain cellular redox status. Exceeding optimum levels of oxidants lead to oxidative distress (Sies 2019; Ramakrishnan et al. 2022).

ROS and RNS at low concentrations act as key signaling molecules to regulate growth, development and defending against abiotic stress, and at high concentrations induce cellular damage (Liu and Liao 2022). H_2O_2, as a major ROS, at 1–10 nM is marked as oxidative eustress, while at concentrations above 100 nM can induce oxidative distress, leading to biomolecules damages. Superoxide dismutase converts superoxide anion radical to endogenous H_2O_2, which involves in physiological oxidative stress denoted as eustress oxidative (Sies 2017). H_2O_2 involves in a number of cellular mechanisms and is utilized by different enzymes as substrate. Catalase and ascorbate peroxidase contribute in removing H_2O_2 to suppress oxidative distress and maintain cellular redox status (Sies 2019; Liu and Liao 2022). NO, as a key RNS, plays a crucial role in regulating cellular mechanisms and modulating the adverse effect of stress in plants (Liu and Liao 2022). Niu and Liao (2016) reported NO generation in response to stress conditions with similar kinetics as H_2O_2. A close interaction exists between H_2O_2 and NO, which may trigger a serious set of biological, physiological and molecular responses in plant cells to stress conditions (Niu and Liao 2016; Gohari et al. 2020).

Salinity stress is a major challenge limiting plants growth and production globally. Increasing the demand for food crops worldwide desires a wide range of researches to improve plant salinity tolerance (Mbarki et al. 2020; Ibrahimova et al. 2021). Chemical priming is one of the easiest and cost-effective techniques to develop salinity tolerance before plant exposure to stress conditions, which have been examined in different crops (Hajihashemi et al. 2022; dos Santos Guaraldo et al. 2023; Hoque et al. 2023; Kaya et al. 2023). Priming induces physicochemical and transcriptional mechanisms upon plant exposure to stress conditions, which can develop faster and more efficient defense mechanism to modulate the adverse effect of stress (Chen and Arora 2013; Hajihashemi et al. 2022). Priming with moleculesinvolved in signaling events can induce stress-tolerance through 'priming memory', which is established during priming practice (Chen and Arora 2013; Carvalho and Silveira 2020). Previous studies have shown the improvement of plants defense mechanisms against salinity stress after H_2O_2 and an NO donor priming (Ibrahim 2016; Hajihashemi et al. 2020).

The accumulation of N-containing compounds, such as arginine, proline, glutamine, asparagine and ornithine, has been observed in plants subjected to salinity stress (Zeid 2009). Arginine (Arg) is an amino acid containing a high nitrogen/carbon ratio, with numerous functions in higher plants physiological processes (Silveira et al. 2021). The Arg catabolism is linked to the proline synthesis in plant cell through ornithine (Silveira et al. 2021). There are reports showing NO accumulation after the exogenous application of Arg (Astier et al. 2018; Hasanuzzaman et al. 2018). The sodium nitroprusside (SNP), an NO donor, priming alleviated the salinity enduring damages in several plant species including quinoa, cotton, basil and wheat (Ali et al. 2017; Gohari et al. 2020; Hajihashemi et al. 2020; dos Santos Guaraldo et al. 2023).

However, priming potentially improves stress tolerance in plants, the mechanisms behind the plant endurance are still challenging to date. The cross-action of NO and H_2O_2priming may robust stress-tolerance and improve crop productivity under stress condition. Although various studies have reported a positive correlation between the NO donor SNP and H_2O_2 priming on robust salinity tolerance of plants (Gohari et al. 2020; Hajihashemi et al. 2020), the interaction of NO sourced from L-Arg with H_2O_2 may advance the physiochemical cues contributed to role of signaling molecules in stress tolerance. *Vicia faba* (faba bean or fava bean) is an economically important crop and a good source of protein for human, and an eco-friendly plant with the excellent ability of fixing nitrogen. Regardless of increasing globally demand for faba bean, salinity stress has been limiting its growth and yield (Kumar et al. 2022). Faba bean is a salt sensitive crop (Sagervanshi et al. 2021; Kumar et al. 2022), so the present study aimed to improve its salinity tolerance of with the rapid and eco-friendly practice of seed priming with Arg and H_2O_2.

Materials and methods

The *V. faba* seeds were received from Pakan Bazr, Isfahan, Iran. Plant culture was done in field under natural environmental conditions. The day and night temperatures during the experimentation were 37 ± 4 ºC and 23 ± 2 ºC, respectively. The seeds were surface sterilized with 70% (v/v) ethanol for 1 min, and 10% (v/v) sodium hypochlorite solution for 10 min, then washed thrice with distilled water. Subsequently, the seeds were soaked in distilled water (control), Arg (0.2 mM) and H_2O_2 (0.2 mM), and kept in dark for 12 h at 20 ºC ± 2. After soaking, the seeds were sown in rows with twenty centimeters distance and 5 cm depth consisted on 18th of April 2022. The pH of soil was about 6.5 and with about 5% of organic matter. The soil contained 1.3% nitrogen according to the micro-Kjeldahl assay (Bremner 1960). The seedlings of the similar size were selected and the experiment was continued with uniform plants. The irrigation was done daily with tap water at 80% field capacity (Hajihashemi 2020). Salinity stress was started with irrigation at 0 (distilled water) and 100 mM NaCl on 2^{ed} of May 2022. Salinity stress continued for one month until 3^{ed} of June. Then, the plant samples were harvested and used for further physiochemical analysis as described in follow.

Photosynthetic pigments

One hundred mg of fresh leaves was extracted using 10 ml acetone (80% v/v). The samples were centrifuged and the absorbance of supernatants were recorded spectrophotometrically at 663, 646 and 470. The chlorophyll a (Chl a), Chl b and carotenoids contents were calculated according to the Wellburn and Lichtenthaler (1984) formulae.

Water soluble carbohydrates

The water soluble carbohydrates (WSC) were extracted from one hundred mg of fresh leaves in 5 ml ethanol (96% v/v). Then, the samples were diluted to 10 ml using distilled water, and centrifuged. The supernatants were used to determine WSC content using the phenol-sulfuric acid assay (DuBois et al. 1956). The WSC content was calculated using glucose as a standard.

Total phenols

In order to extract phenols from fresh leaves, one hundred mg of samples were homogenized in 5 mL of ethanol (96% v/v) and kept in the dark over one night. Then, the samples were centrifuged and aliquots were used to analyse phenols using Folin's reagent as described by Singleton and Rossi (1965). The total phenolic compounds were quantified based on the gallic acid standard curve.

Total flavonoids

The fresh leaves (0.1 g) were homogenized with 2 ml methanol: HCl (99:1 v/v) solution and centrifuged. The assay for detection flavonoids was performed on supernatants according to Zhishen et al. (1999) method. Rutin was used as reference standard for calculation.

Anthocyanins

Wagner (1979) protocol was followed to measure the anthocyanins contents of samples. Extraction mixture was the mixture of methanol:HCl in the ratio of 99:1 (v/v). The fresh leaves (0.1 g) were homogenized in 2 mL of extraction solution. The samples were incubated in dark over one night and then centrifuged. The absorbance of the supernatant was recorded at 530 nm wavelength. The anthocyanins content was calculated using extinction coefficient of 33,000 mol^{-1} cm^{-1}.

Proline

Proline content of fresh leaves were measured as already described by Bates et al. (1973). Samples (0.1 g) were homogenized in 2 mL of sulphosalicylic acid (3% w/v). After centrifugation, the supernatant was used to detect proline using ninhydrin solution and extraction in toluene. The absorbance of toluene phase containing proline was recorded at 520 nm against toluene and proline was used as reference standard.

Hydrogen peroxide (H_2O_2)

H_2O_2 content was determined by following the method of Velikova et al. (2000). The homogenization of fresh leaves (100 mg) was done in 0.1% (w/v) of trichloroacetic acid (TCA) and centrifuged. Then, supernatants were mixed with potassium phosphate buffer and potassium iodide. The sample absorbances were measured at 390 nm and the calculation was done based on the H_2O_2 standard curve.

Lipid peroxidation

Lipid peroxidation was determined by measuring malondialdehyde (MDA) content (Heath and Packer 1968). The fresh leaves (0.1 g) were extracted in 2 mL of TCA (1% w/v) and centrifuged. The solution of 0.5% (w/v) thiobarbituric acid in 20% (v/v) TCA was added to supernatant. The mixtures were heated in boiling water for 30 min and cooled in an ice bath. The absorbance of the samples was recorded at 532 and 600 nm. The MDA content was determined using extinction coefficient of 155 mM^{-1} cm^{-1}.

Total antioxidants power (FRAP)

Fresh leaves (1 g) were extracted using 100 mM potassium phosphate buffer (pH 7.6) including 0.1 mM ethylenediaminetetraacetic acid (EDTA). The samples were centrifuged and the supernatants were used to assay ferric reducing antioxidant power (FRAP), denoted as "antioxidant power", according to Szôllôsi and Varga (2002) procedure. The working solution mixture was consisted of 25 ml acetate buffer (300 mmol l^{-1}, pH 3.6), 2.5 ml of 2,4,6-tripyridyl-s-triazine (10 mmol l^{-1}) in hydrochloric acid (40 mmol l^{-1}) and 2.5 ml of ferric chloride (20 mmol l^{-1}). The supernatant (0.5 ml) was added to the mixture solution (1.5 ml). The absorbance of samples was recorded at 593 nm and calculated based on the L-ascorbic acid as a standard curve.

Total soluble proteins

The frozen leaves samples (1 g) were homogenized in 3 ml of 50 mM potassium phosphate buffer (pH 6.8) containing 1.0 mM EDTA and 2% (w/v) polyvinyl polypyrrolidone. The homogenates were centrifuged at 4 °C. The supernatants were used for protein analysis and enzyme activity assays. The total soluble proteins contents were analysed according

to the Bradford (1976) assay and calculated using bovine serum albumin standard curve.

Activity of antioxidant enzymes

Superoxide dismutase (SOD) activity

The SOD activity was assayed based on the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) (Beauchamp and Fridovich 1971). The reaction mixture included potassium phosphate buffer (50 mM) pH 7.8, NBT (33 mM), methionine (10 mM), riboflavin (1.17 µM) and 100 µl of protein extract. One unit of SOD activity was measured under the assay conditions and calculated as the enzyme quantity produce a 50% inhibition of NBT reduction and expressed per milligram protein.

Catalase (CAT) activity

The activity of CAT was assayed in the reaction mixture of 100 mM potassium phosphate buffer (pH 6.8), H_2O_2 (150 mM) and 50 µL protein extract (Aebi 1984). The decrease in the sample absorbance based on the H_2O_2 disappearance at 240 nm was monitored for 180 s. The extinction coefficient of 43.6 M^{-1} cm^{-1} was used to calculate enzyme activity per milligram protein.

Ascorbate peroxidase (APX) activity

The protein extraction should be done with buffer containing ascorbate (1mM) to inhibit inactivation of APX. The reaction mixture was consisted of 100 mM potassium phosphate buffer (pH 7.0), 5 mM ascorbate, 0.5 mM H_2O_2 and 50 µL protein extract. The reduction in absorbance was recorded at 290 nm for 3 min. (Asada 1992). The extinction coefficient of 2.8 mM^{-1} cm^{-1} was applied to calculate enzyme activity per milligram proteins.

Statistical analyses

The seeds were sown in three detached plots per treatments. Every ten plants per each plot was counted as a replication for every treatment. The data analysis was accomplished using one-way analysis of variance (ANOVA) by SPSS statistical (version 24) package. The Tukey's test ($P \leq 0.05$) was used to characterize the significant differences from control. The values per every column in the figures represent the mean of three biological replicates. The letters on the columns in the figures are the outcome of statistical analysis and the error bars represent standard deviation.

Results

The results of present study showed a significant increse in the H_2O_2 content in response to salinity stress by 33% higher than that in controls (Fig. 1A). H_2O_2 application alone or in combination with Arg significantly incresed the H_2O_2 content by 17% and 23%, respectively, while Arg decreased that by 40%, as compared with controls (Fig. 1A). İn salinity-stressed plants, the H_2O_2 content in the Arg, H_2O_2 and Arg + H_2O_2-treated plants decreased by 14%, 15% and 12%, repectively, less than that in salinity stress

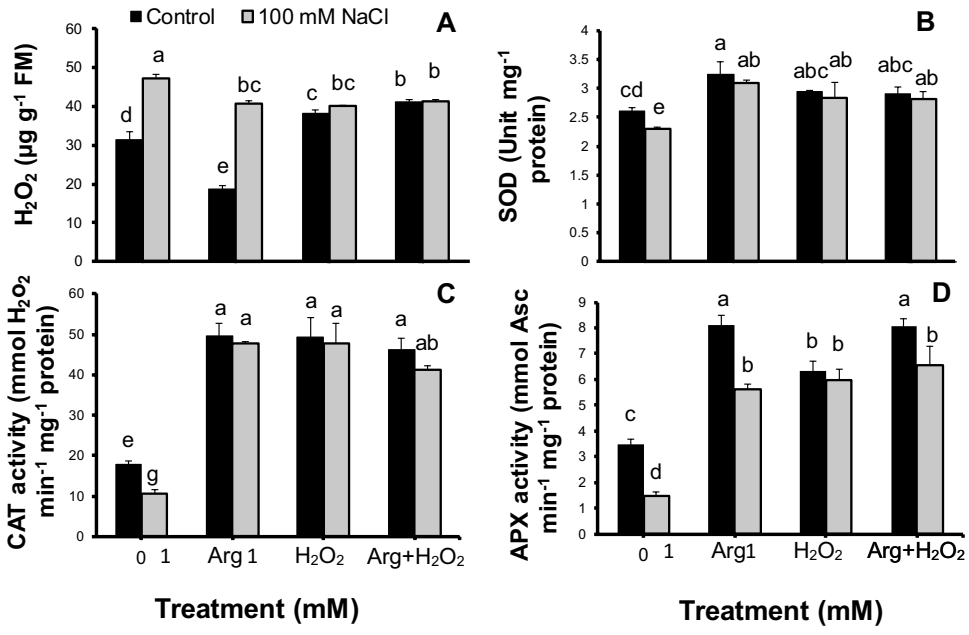

Fig. 1 (**A**) hydrogen peroxide (H_2O_2), (**B**) superoxide dismutase (SOD) activity, (**C**) catalase (CAT) activity and (**D**) ascorbate peroxidase (APX) activity in *Vicia faba* under priming biomolecules of H_2O_2 and arginine (Arg), and salinity stress. Different letters on columns represent significantly different at $p < 0.05$

alone (Fig. 1A). Salinity stress (12%) decreased the SOD activity, while the exogenous Arg (19%), H_2O_2 (11%) and Arg + H_2O_2 (10%) application incresed that higher than in controls (Fig. 1B). Under saline conditions, the Arg, H_2O_2, Arg and H_2O_2 + Arg treatment incresed the SOD activity, by 25%, 19% and 18%, respectively, relative to salinity stress alone (Fig. 1B). The activity of CAT decreased in salinity-stressed plants (40%), while it incresed in the Arg (64%), H_2O_2 (64%) and Arg + H_2O_2 (61%) treatment, as compared with controls (Fig. 1C). Exogenous application of Arg, H_2O_2, and Arg + H_2O_2 in salinity-stressed plants increased by 78%, 78% and 74%, respectively, higher than that in salinity stress alone (Fig. 1C). In response to salinity stress, the APX activity was by 57% less than in controls (Fig. 1D). The APX activity in Arg, H_2O_2 and Arg + H_2O_2-treated plants increased by 87%, 84% and 87%, repectively, higher than its value in controls (Fig. 1D). Arg, H_2O_2 and Arg + H_2O_2 with NaCl incresed the APX acitivity by 73%, 75% and 77%, respectively, more than that in NaCl alone (Fig. 1D).

The phenols content in the salinity-stressed plants decreased by 28% less than that in controls (Fig. 2A). Exogenous Arg significantly incresed the phenols accumulation by 15%, while H_2O_2 and H_2O_2 + Arg induced no significant changes in its value (Fig. 2A). Arg and Arg + H_2O_2 significantly incresed the phenols value by 32% and 21%, respectively, higher than that in untreated salinity-stressed plants (Fig. 2A). Exogenous H_2O_2 application in the NaCl-irrigated plants caused no significant changes in the phenols content (Fig. 2A). The salinity-stressed plants had a lower portion of anthocyanins (14%) than that in controls (Fig. 2B). The antocyanins content in Arg, H_2O_2 and Arg + H_2O_2-treated plants exhibited an increase by 42%, 13% and 26%, respectively, greater than that in controls (Fig. 2B). The anthocyanins analysis showed that the Arg, H_2O_2 and Arg + H_2O_2 priming increased that in salinity-stressed plants by 43%, 40% and 47%, respectively, higher than that in salinity stress alone (Fig. 2B). As illustrated in Fig. 2C, NaCl irrigation had no significant effect on the flavonoids accumulation, while the exogenous Arg, H_2O_2 and Arg + H_2O_2 enlarged the flavonoids accumulation (40%, 21% and 31%, respectively). The flavonoids content increased in response to Arg, H_2O_2 and Arg + H_2O_2 with NaCl (25%, 31% and 53%, respectively), larger than in untreated NaCl-irrigated plants (Fig. 2C). The FRAP value showed no significant changes in response to NaCl irrigation, while it slightly increased after Arg, H_2O_2 and Arg + H_2O_2 priming, with or without salt stress (Fig. 2D). The greatest increase in the FRAP value was observed in the Arg priming with or without NaCl, by 10% larger than that in controls (Fig. 2D).

Salinity stress and the seed priming with H_2O_2 and Arg + H_2O_2 induced no significant changes in the proteins content, while a slight increase was observed in its value in response to Arg (11%), relative to controls (Fig. 3A). The Arg, H_2O_2 and Arg + H_2O_2 priming in salinity-stressed plants slightly increased the level of proteins (7%, 8%, 8%, respectively) higher than that in salinity stress alone (Fig. 3A). Salinity stress and the exogenous H_2O_2 decreased the proline content (11% and 12%, respectively), whereas the Arg treatment incresed that (26%), as compared to controls (Fig. 3B). Under salinity stress, the H_2O_2 and Arg + H_2O_2 application increased the proline content (23% and 18%, respectively), greater than that in salinity stress alone, while

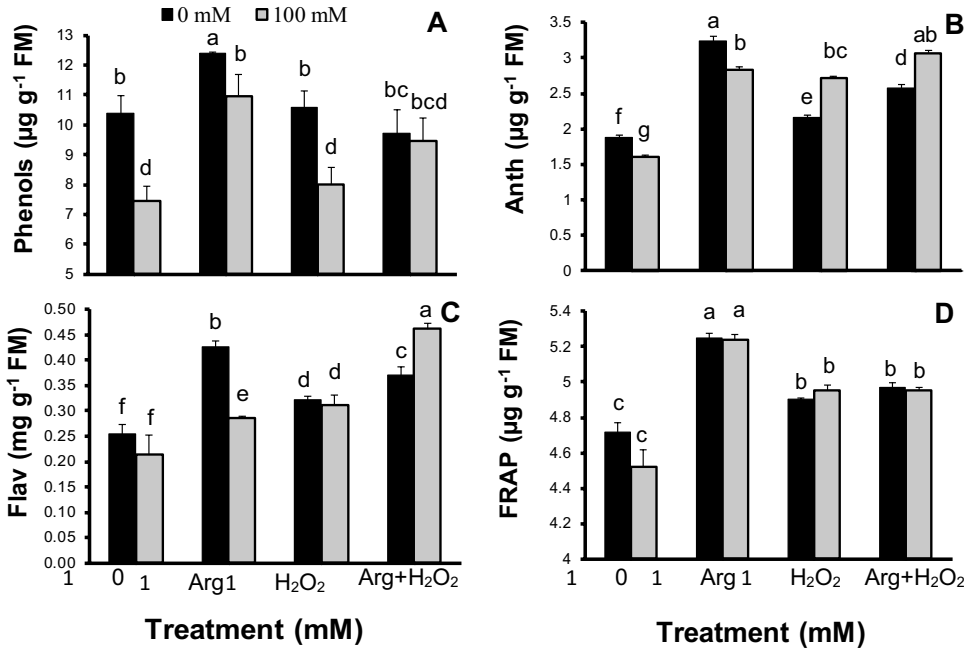

Fig. 2 (**A**) phenols, (**B**) anthocyanins (Anth), (**C**) flavonoids (Flav) and (**D**) total antioxidant power (FRAP) in *Vicia faba* under priming biomolecules of hydrogen peroxide (H_2O_2) and arginine (Arg), and salinity stress. Different letters on columns represent significantly different at $p < 0.05$

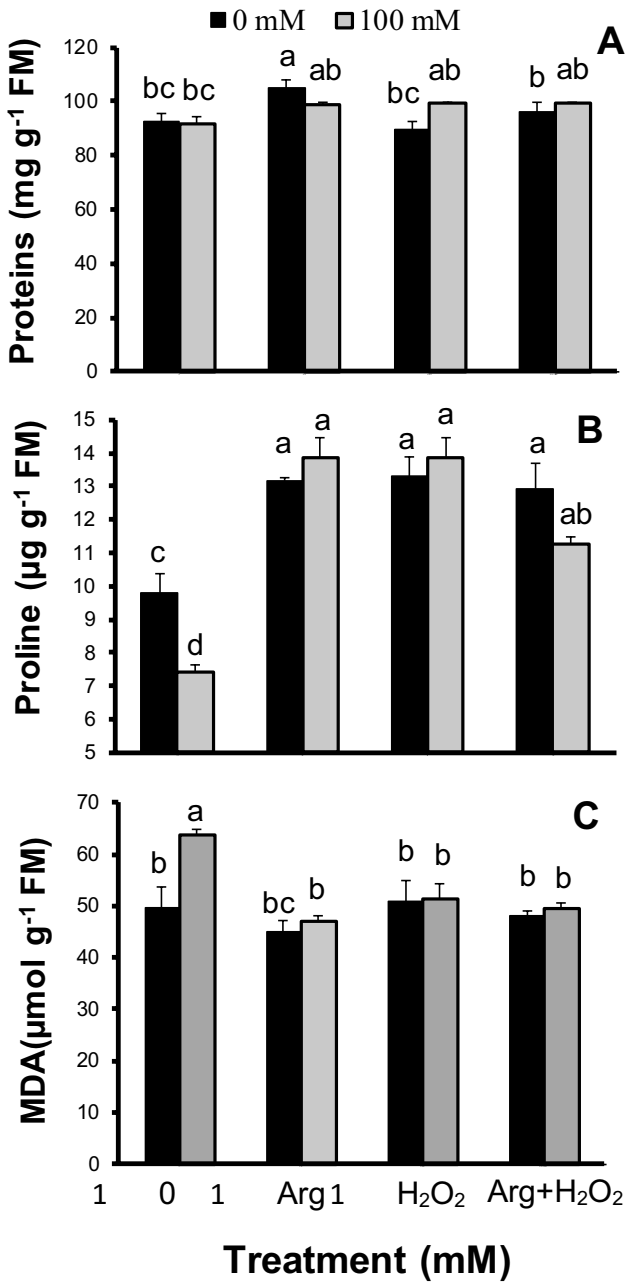

Fig. 3 (**A**) proteins, (**B**) proline and (**C**) malondialdehyde (MDA) in *Vicia faba* under priming biomolecules of hydrogen peroxide (H_2O_2) and arginine (Arg), and salinity stress. Different letters on columns represent significantly different at $p < 0.05$

Arg had no significant effect on that (Fig. 3B). The MDA content in the salinity-stressed plants significantly incresed by 22% compared to controls. The exogenous H_2O_2 and Arg + H_2O_2 had no significant effect on the MDA content (Fig. 3C). The application of Arg, H_2O_2 and Arg + H_2O_2 in salinity-stressed plants decreased the MDA content (26%, 20% and 22%, respectively), lower than that in salinity stress alone (Fig. 3C).

Salinity stress significantly decreased Chl a and b by 15% and 17%, respectively, as compared to control plants, while no significant changes were observed in the carotenoids content (Fig. 4A–C). The Chl a content significantly increased in response to the Arg and Arg + H_2O_2 by 20% and 16%, respectively, while the seed prining in un-stressed plants induced no sigignificant changes in the amount of Chl b, relative to control (Fig. 4A and B). At H_2O_2 and Arg + H_2O_2, the carotenoids content increased by 16% and 15%, respectively, above their values in control plants (Fig. 4C). In salinity-stressed plants, the value of Chl a was significantly incresed in Arg, H_2O_2 and Arg + H_2O_2-treated plants by 15%, 10% and 24%, respectively, higher than them in salinity stress alone (Fig. 4A). The Arg, H_2O_2 and Arg + H_2O_2 treatments, in presence of NaCl, promoted an increase in Chl b by 20%, 20% and 27%, respectively, relative to NaCl alone (Fig. 4B). The carotenoids value in the plants treated with Arg, H_2O_2 and Arg + H_2O_2, in salt-stressed plants, were incresed by 10%, 9% and 13%, respectively, higher than them in salinity stress alone (Fig. 4C). The adverse effect of salinity stress on photosynthetic pigments was followed by a significant reduction in the WSC contents by 20%, less than that in control plants (Fig. 4D). The application of Arg, H_2O_2 and Arg + H_2O_2 significantly incresed the WSC accumulation by 20%, 18% and 14%, respectively, higher than that in controls (Fig. 4D). The exogenous Arg, H_2O_2 and Arg + H_2O_2 incresed WSC by 30%, 28% and 38%, respectively, higher than them in salinity stress alone (Fig. 4D).

Discussion

Salinity stress-induced ROS accumulation and associated oxidative damages to macromolecules and cellular compartment have been already reported in crops (Wang et al. 2018). Antioxidant system is a protection mechanism in balancing oxidants generation and scavenging to maintain plant tolerance to abiotic stresses (Hajihashemi and Ehsanpour 2014). Existence of signaling networks between H_2O_2 and NO is marked as key directions in managing imbalances in metabolic mechanisms due to stressors with the aim of reaching tolerance (Lu et al. 2009; Gohari et al. 2020; Hajihashemi et al. 2020). The results of present study demonstrated the potential of seed priming with H_2O_2 and Arg in protecting fava bean against salinity stress. The present data showed an increase in the endogenous H_2O_2 production in response to the H_2O_2 priming alone or in combination with Arg, while the application of Arg alone inhibited the H_2O_2 accumulation in fava bean. Uchida et al. (2002) claimed that, in a coarse time experiment, pretreatment of rice with low concentration of H_2O_2 induced a rapid increase in the endogenous H_2O_2 level at early stage of salinity stress to acclimate plant to stress.

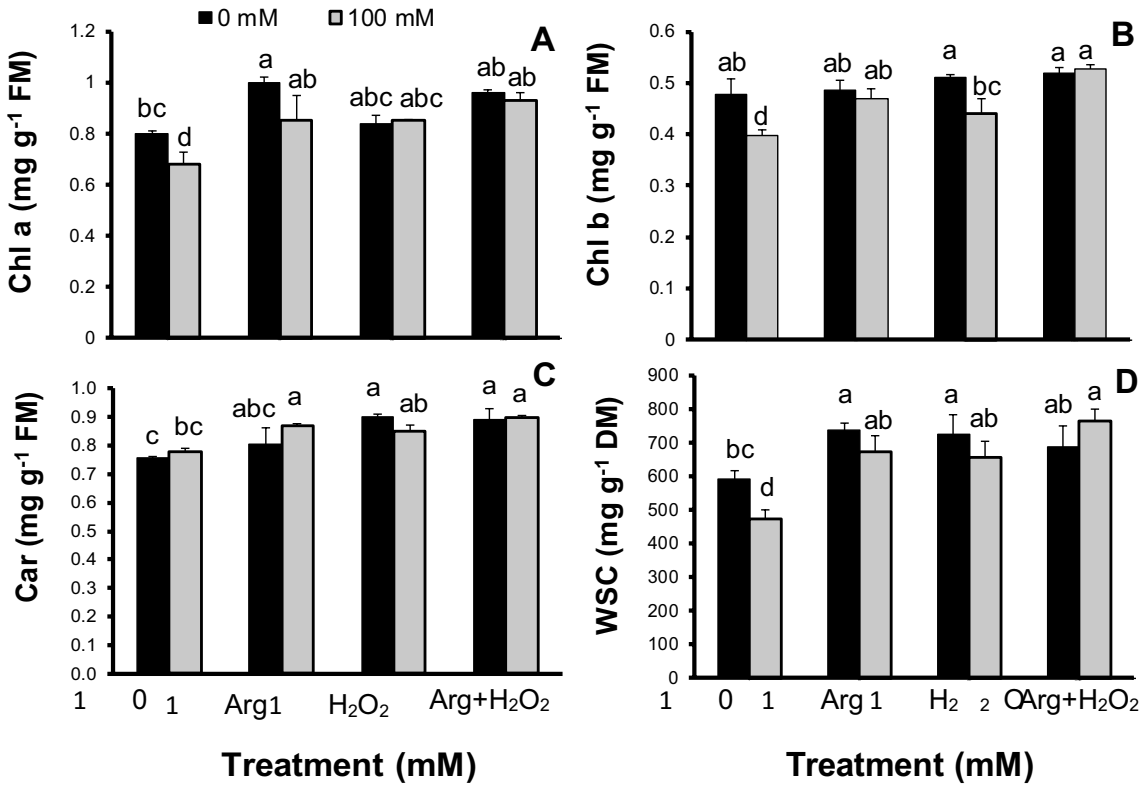

Fig. 4 (**A**) chlorophyll a (Chl a), (**B**) Chl b, (**C**) carotenoids and (**D**) water soluble carbohydrate (WSC) in *Vicia faba* under priming biomolecules of hydrogen peroxide (H_2O_2) and arginine (Arg), and salinity stress. Different letters on columns represent significantly different at $p < 0.05$

Despite the oxidative damages of H_2O_2 at high concentration under stressful conditions, H_2O_2 priming at low concentration induced a rapid activation of enzymatic antioxidative defense against salinity stress (Uchida et al. 2002; Brestic et al. 2016; Gohari et al. 2020). Contrary to salinity stress alone, a concomitant increase was observed in the activity of antioxidant enzymes such as SOD, CAT and APX by the exogenous Arg, H_2O_2 and Arg + H_2O_2 in salinity-stressed beans. In rice plants-treated with H_2O_2 and an NO donor (SNP), a mild oxidative stress (eustress) through activation of SOD, Cat, APX and POX was responsible to protect plants against salinity stress, which is in parallel to the results of present study. Lu et al. (2009), through a study on application of an NO scavenger (2-phenyl- 4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide), an inhibitor of NO synthase (N^w-nitro-L-arginine), and an inhibitor of nitrite reductase ($NaVO_3$) in H_2O_2-treated bermudagrass, indicated that the enhancing effects of H_2O_2 on SOD and CAT activity were dependent upon NO value. In contrary, the SOD and CAT activities-induced by SNP (an NO donor) were not inhibited by a H_2O_2 scavenger (dimethylthiourea) and an NADPH oxidase inhibitor (pyridine), revealing that NO-induced enzymes activities were independent of H_2O_2 (Lu et al. 2009). The results of present study are consistent with previous reports on the cross-talk between H_2O_2 and NO signaling pathways in mediating cellular response to stressors, whereas H_2O_2 acts upstream of NO (Lu et al. 2009; Gohari et al. 2020; Hajihashemi et al. 2020). Altogether, the observed increase in the activity of SOD, CAT and APX were responsible in balancing the H_2O_2 value in salinity-stressed fava bean.

The redox homeostasis in response to stressor involves different enzymatic and non-enzymatic antioxidants to mitigate damages by stressors (Hajihashemi et al. 2022; Sohag et al. 2020). The phenolic compounds, anthocyanins and flavonoids are secondary metabolites with antioxidant properties (Akhtar et al. 2010; Mohamed et al. 2016). Flavonoids include anthocyanins, flavones, flavonols, flavanones and isoflavones, which accumulate in response to environmental stresses (Akhtar et al. 2010). Anthocyanins act either as inhibitor of free radicals production and their scavengers (Gohari et al. 2020). In opposite to salinity stress alone, the exogenous H_2O_2 and/or Arg significantly increased the values of flavonoids and anthocyanins in stressed beans, which was more pronounced in presences of Arg than in H_2O_2 alone. Flavonoids can protect plants against ROS by scavenging singlet oxygen, superoxide anion and H_2O_2 (Akhtar et al. 2010). Akhtar et al. (2010) reported that exogenous H_2O_2 induced flavonoid synthesis, which is in according to the results of present study. Additionally, Li et al. (2019)

revealed that exogenous NO increased the flavonoids accumulation in tea plant. The present data showed that the phenolic compounds level increased in response to the Arg application, while no significant changes was achieved in response to the exogenous H_2O_2. Mohamed et al. (2016) demonstrated that NO significantly increased the accumulation of phenolic compounds in the shoots of *Vicia faba*, which confirms the results of present study. Further, higher value of the FRAP in response to the application of H_2O_2 and/or Arg in salt-stressed beans than in the salinity-stressed plants without treatment can represent the importance of seed priming with low concentrations of signaling ROS and RNS molecules in turning oxidative distress to oxidative eustress.

An important parameter to represent the oxidative damage of plant cells due to environmental stress is the MDA level, indicator of lipid peroxidation (Mohamed et al. 2016; Gohari et al. 2020). The MDA content in both H_2O_2 and Arg-treated plants under salinity stress was consistently less than that in salinity stress alone, indicating that treatments alleviated oxidative injury induced by stressor. NO reportedly prevents membrane injury by inhibiting lipid peroxidation through scavenging the radicals of lipid peroxyl and preventing peroxidation enzymes (Mohamed et al. 2016). Data showed no significant variation in the total soluble proteins in stressed and non-stressed beans in response to either H_2O_2 or Arg priming. Proline accumulation has been known as one of the key metabolic responses to salinity stress (Hajihashemi et al. 2021; Silveira et al. 2021). Seed priming with Arg under non-stressed conditions induced a significant increase in the proline content in un-stressed beans, which can be due to the Arg-dependent proline synthesis pathway through ornithine (Silveira et al. 2021). Yang et al. (2009) reported that exogenous H_2O_2 increased the proline content in maize seedlings through increasing the activity of the Δ^1-pyrroline-5-carboxylate synthetase and glutamate dehydrogenase, and decreasing of the activity of proline dehydrogenase, while beans showed no increase in response to the H_2O_2 priming without stress. In contrary to stress conditions, the H_2O_2 priming increased the proline content in stressed beans, while the Arg priming did not change that. There are different reports on variation of proline response to stressors, which might be contributed to the plant sensitivity to stressor (Guo et al. 2018; Rasel et al. 2021; Silveira et al. 2021). Under salinity stress, NO-releasing substances application counteracted redox homeostasis by influencing antioxidant enzyme activities along with promoting proline accumulation, which is proved by retardation of ROS overproduction along with a decrease in H_2O_2, thiobarbituric acid reactive substances, and lipid peroxidation (Singhal et al. 2021).

In follow, the response of photosynthetic pigments was evaluated in response to H_2O_2/Arg priming and salinity stress. Damages of the photosynthetic pigments has been considered as one of the most critical factors inducing reduction of photosynthesis, carbohydrates synthesis, plant growth and yield in plants exposed to salinity stress (Ibrahimova et al. 2021; Hajihashemi et al. 2021). In non-stressed plants, the photosynthetic pigments did not show remarkable increase in response to seed priming, with some exception, while the H_2O_2/Arg supply induced a concomitant increase in Chl a, Chl b and carotenoids under stress conditions. The ROS production occurs in chloroplast during electron transport, CO_2 fixation where the oxygen is an electron acceptor (Wei et al. 2022). According to Uchida et al. (2002), the H_2O_2 or NO pretreatment on salinity-stressed rice prevented a drastic decrease in the activity of PS II. It might be hypothesized that the preservation of photosynthetic pigments against salinity stress in the H_2O_2/Arg-treated bean might be connected to the reduction of damages to ultrastructure of chloroplast by ROS connected to the activation of antioxidant systems. The exogenous application of NO in salinity-stressed plants resulted in a balanced photosynthetic pigments and rate and re-establishment of vegetative growth in *Lactuca sativa* (Campos et al. 2019). Following the observed increase in photosynthates, the WSC content increased in salinity-stressed plants subjected to H_2O_2/Arg priming. In line with the present data, the exogenous H_2O_2 and NO inhibited the adverse effect of drought stress on carbohydrate biosynthesis in the marigold (Liao et al. 2012).

Conclusions

The seed priming is a rapid alternative to improve stress tolerance of plants with eco-friendly molecules. Seed priming with low concentrations of H_2O_2 and NO, with free radical properties, provided opportunities for salinity stress tolerance in *V. faba* through developing the redox status of the cells under stress conditions. In the primed plants, the plants owned a strong antioxidant systems followed by reducing stress damages to photosynthetic system and lipid membranes. Further molecular analysis may support the importance of seed priming with free radicals to improve the knowledge behind priming memory in establishing redox balance.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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