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Research Article

Alleviation of salinity triggered oxidative damage and photoinhibition in *Vigna radiata* by individual and combined treatments of selenium and jasmonic acid

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Abstract

Experiments were performed to evaluate the effect of selenium (Se) and methyl jasmonate (Me-JA) in alleviating the salinity (100 mM NaCl) stress triggered decline in growth and photosynthetic in *Vigna radiata*. Salinity stress significantly reduced the growth measured in terms of plant height and dry mass which was significantly alleviated by Se and /or Me-JA. Treatment of Se and Me-JA increased the synthesis of prototoporphyrin IX (Proto IX), Mg-prototoporphyrin IX (Mg-Proto IX), chlorophylls and carotenoids, increased photosynthesis and PSII activity. Besides, the supplementation of Se and Me-JA significantly declined the reactive oxygen species (H_2O_2 and O_2^-) levels causing increased membrane stability. Treatment of Se and MeJA up-regulated the antioxidant system by increasing the activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase, and the content of ascorbate and glutathione in both control and salt stress treated plants. The Se and Me-JA treatment significantly increased the synthesis of total phenols and flavonoids reflecting in increased total antioxidant activity. Accumulation of proline, sugars, glycine betaine and the activity of γ-glutamyl kinase was significantly improved in Se and Me-JA treatments affecting the tissue water content. Decline in nitrate reductase activity due to salinity stress was alleviated significantly due to Se and Me-JA treatment. *Vigna radiata* plants treated with Se and Me-JA accumulated less Na and more K and N compared to salt stressed plants.

Keywords: antioxidants; compatible osmolytes; *Vigna radiata;* photosynthesis; salinity stress

Introduction

Salinity stress is a devastating stress factor resulting in remarkable decline in crop yield globally. Excess use of saline water for irrigation is continuously results in conversion of productive lands into unproductive lands. Excess sodium has been considered as the main contributor of high salinity. Salinity stress results in oxidative and ionic stress affecting the growth and productivity significantly. The damaging consequences of salt stress include the over-production of toxic reactive oxygen species (ROS) leading to impaired growth and development by hampering photosynthesis, enzyme functioning, uptake and assimilation of mineral ions, metabolite production and gene expression (Soliman *et al.*, 2020; Qin *et al.*, 2021; El-Taher, 2022). Salinity mediated alteration in the chlorophyll synthesis, redox homeostasis and membrane functioning affects the

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growth and yield performance of plants considerably (Ahanger *et al.*, 2019a, b). To avert the salinity induced toxic effects, plants up-regulate the tolerance mechanisms which include: (a) antioxidant system, (b) compatible osmolyte accumulation and (c) ion compartmentalisation (Fatma *et al.*, 2016; Elkelish *et al.*, 2019; Ahanger *et al.*, 2019a). Up-regulation of these mechanisms leads to growth protection and increased yield (Ahmad *et al.*, 2016; El-Taher *et al.*, 2022). There have been considerable evidences wherein it has been proved that phytohormones and certain non-essential elements can potentially prevent the salinity mediated damage by actively triggering the tolerance mechanisms (Ryu and Cho, 2015; Zhao *et al.*, 2021; Trifunovic-Momcilov *et al.*, 2021).

Jasmonic acid (JA) and it conjugates like methyl jasmonate (MeJA) and jasmonoyl-isoleucine (JA-Ile) are an important plant hormone that have active role in plant development (Per *et al.*, 2018; Delgado *et al.*, 2021). Jasmonates can suppress or improve the plat response (Agrawal et al. 2003). Active involvement of JA and its conjugates in improving the physiological and biochemical responses of plants under stresses has been reported (Per *et al.*, 2016; Ahmad *et al.*, 2016; Ali *et al.*, 2022). Plants treated with exogenous JA exhibit increased tolerance to stresses by strengthening of the key tolerance mechanisms like antioxidant system, osmolyte production and gene expression (Sirhindi *et al.*, 2016; Ahmad *et al.*, 2016). However, contradictory reports are also available for example, inhibition of growth and photosynthesis due to JA has been reported (Anjum *et al.*, 2011). It has been reported that JA interact with other phytohormones and mineral elements to strengthen the tolerance mechanisms for better alleviation of the stress (Per *et al.*, 2016).

Selenium (Se) is considered as non-essential nutrient for plant growth however recent research reports have showed its beneficial role in alleviation of stress induced growth and developmental alterations. Exogenous treatment of Se alleviates the salinity (Elkelish *et al.*, 2019), cadmium (Alyemeni *et al.*, 2018), drought (Hasanuzzaman and Fujita, 2011) and osmotic stress (Galic *et al.*, 2021). However, at higher concentrations leads to toxicity and oxidative damage leading to interference in protein structure and their functioning (Gupta and Gupta, 2017). Selenium is similar to sulphur therefore is taken by plants through the sulphur transporters (Gupta and Gupta, 2017). Application Se mitigates the adverse effects of cadmium stress comparable to sulphur treatments in wheat (Khan *et al.*, 2015). Besides this Se supplementation potentially reduces the accumulation of toxic ions like Na, Cd etc in different crop species (Elkelish *et al.*, 2019; Alyemeni *et al.*, 2018). Supplementation of Se up-regulates the functioning of antioxidants, glyoxylase system and the accumulation redox components thereby resulting in increased potential to withstand the deleterious effects of stresses (Hasanuzzaman and Fujita, 2011; Khan *et al.*, 2015). However, the interaction of Se with phytohormones in mitigating the damaging effects of stresses has not been worked. Therefore, in present the interactive effects of Se and JA was studied in relation to the salinity stress.

Vigna radiata is an important legume plant known as mung bean. Grown worldwide for its high nutritious value which is attributed to greater presence of carbohydrates, proteins, fats and nutrients. It requires warm environment for better growth and increased soil salinity adversely affects its normal growth by affecting the root growth, nitrogen fixation potential and ion stability (Rao *et al.*, 2016). Latest research advancement involving the integration of efficient management techniques like exogenous supplementation of elements and phytohormones for improving indigenous tolerance mechanisms for coping the damaging effects of stresses can be better exploited for its growth and yield protection. In this backdrop we hypothesise that combined application of Se and JA can protect the *Vigna radiata* seedlings against the negative effects of increased salinity on growth, chlorophyll synthesis and photosynthesis by up-regulation of osmolyte accumulation, antioxidant system and secondary metabolites.

Materials and Methods

Experimental design and treatment

Seeds of *Vigna radiata* were sterilized by washing in 0.001% $HgCl_2$ for five minutes. Sterilised seeds were thoroughly washed with distilled water for five times and blot dried with sterilised filter paper. Sterilised seeds were sown in pots filled with acid washed sand and all pots were saturated by irrigating with 200 mL full strength Hoagland nutrient solution. After germination, the number of plants per pot was thinned to three per pot and were irrigated with normal Hoagland nutrient solution for ten days. After ten days of seedling growth pots were divided into two groups in which one group was irrigated with normal Hoagland solution while other group was irrigated with modified Hoagland nutrient solution containing 100 mM NaCl. Selenium (Se) in the form of sodium selenate (Na_2SeO_4) was given at 5 µM concentration along with the Hoagland solution while as 1 µM JA (Methyl jasmonate – Sigma Aldrich) was foliarly sprayed onto the foliage. Treatment of NaCl, Se and JA continued for another fifteen days and were given on every alternate day. Therefore, the detailed treatments included: (a) control (Hoagland solution), (b) 100 mM NaCl (c) 5 µM Se (d) 1 µM Me-JA (e) Se + Me-JA (f) 100 mM NaCl + 5 µM Se, (g) 100 mM NaCl + 1 µM Me-JA and (h) 100 NaCl + 5 µM Se + 1 µM Me-JA. Pots were arranged in complete randomized block design in green house. Composition of nutrient solution used was 3 mM KNO_3, 2 mM $Ca(NO_3)_2$, 2 mM $MgSO_4$, 1 mM $NH_4H_3PO_4$, 50 µM KCl, 25 µM H_3BO_4, 2 µM $MnCl_2$, 20 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM $(NH_4)6Mo_7O_{24}$, and 20 µM Na_2Fe-EDTA. Twenty-five days old plants (fifteen days after NaCl, Se and Me-JA treatment) were carefully up-rooted, washed and analysed for different parameters like chlorophyll synthesis, oxidative damage, osmolytes and antioxidant components using standard methods. Plant height was measured annually using tape while as plant dry weight was recorded after oven drying the whole plant at 70 °C 48 hours.

Estimation of prototoporphyrin IX (Proto IX), Mg-prototoporphyrin IX (Mg-Proto IX), total chlorophylls and carotenoids

For the estimation of Proto IX and Mg-Proto IX content in fresh leaves method of Hodgins and Van Huystee (1986) was adopted. Fresh 300 mg leaves were extracted in 5 mL of alkaline acetone (80%). Thereafter volume of homogenate was made up to 25 mL by 80% alkaline acetone. After bleaching the extract by incubating under dark, centrifugation was done for 10 min at 1500 g. The absorbance of supernatant was measured at 575 nm, 590 nm and 628 nm. For determination of chlorophylls and carotenoids, fresh 100 mg leaf tissue was homogenised in 80% acetone. After centrifuging the extract at 3000g for 20 minutes volume of supernatant was made to 10 mL using 80% acetone and absorbance was taken at 480, 645 and 663 nm (Arnon, 1949).

Photosynthetic activity and chlorophyll fluorescence (PSII activity)

Net photosynthetic efficiency was measured in fully expanded leaf using infrared gas analyzer (CID-340, Photosynthesis System, Bio-Science, Washington, USA). For measuring the activity of PSII (Chlorophyll fluorescence) Chlorophyll Fluorometer (PAM 2500; Walz, Germany) was used and measurement was carried after dark adapting leaves for 30 minutes.

Estimation of glycine betaine, soluble sugar and relative water content

For measuring the content of glycine betaine extraction of powdered plant material was done in distilled water. Thereafter sulphuric acid and cold potassium iodide reagent were added to extract and periodide crystals formed were dissolved in 1, 2-dichloroethane and read at 365 nm (Grieve and Grattan, 1983). Content of soluble sugar was extracted in 80% ethanol. After centrifuging the extract at 5000g for 10 minutes, anthrone reagent was added to supernatant. Absorbance was taken at 585 nm (Shields and Burnet, 1960).

Relative water content (RWC) in fresh leaves was measured according to method of Smart and Bingham (1974). Leaf discs were punched fresh weight (FW) was determined. Thereafter same leaf discs were floated on

distilled water to get turgid weight (TW). After oven drying the discs dry weight (DW) was determined and following formula was employed for calculation.

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Where: FW = fresh weight, DW = dry weight, and TW = turgid weight.

Measurement of proline and activity of γ-glutamyl kinase

Content of proline was extracted by homogenising tissue in 3% sulphosalicylic acid and centrifuging the homogenate at 3000g for 20 minutes. Thereafter supernatant was reacted with ninhydrin reagent and glacial acetic acid. Proline was separated using toluene and absorbance was read at 520 nm (Bates *et al.*, 1973). For determination of γ-Glutamyl kinase (GK, EC 2.7.2.11) activity, 500 mg fresh leaf tissue was extracted in Tris buffer (pH 7.5). Homogenate was centrifuged at 30,000 g for 30 minutes and activity of γ-GK was determined in pellet according to Hayzer and Leisinger (1980). Assay mixture contained 50 mM Tris buffer (pH 7.0), 50 mM L-glutamate, 20 mM $MgCl_2$, 10 mM ATP, 100 mM hydroxamate–HCl and the enzyme extract. Reaction was terminated using stop buffer ($FeCl_3$ and TCA) and optical density was recorded at 535 nm. Activity of γ-GK was measured as μg of γ-glutamyl hydroxamate formed and expressed as U mg^{-1} protein min^{-1}.

Estimation of hydrogen peroxide, superoxide, lipid peroxidation and membrane stability index

For measurement of hydrogen peroxide, fresh 500 mg leaf tissue was macerated in 0.1% TCA and homogenate was centrifuged for 15 minutes at 12,000g. Thereafter, to 500 μL supernatant was added potassium phosphate buffer (500 μL; pH 7.0) and potassium iodide (1 mL). Optimal density of the mixture was taken at 390 nm (Velikova *et al.*, 2000). Superoxide content in fresh 100 mg tissue was determined by homogenizing the tissue in potassium phosphate buffer (65 mM; pH 7.8) followed by centrifugation for 10 minutes at 5000 g. Thereafter supernatant was mixed with hydroxylamine hydrochloride and after 20 minutes sulfanilamide and naphthylamine were added. Absorbance was recorded at 530 nm and calculation were carried using standard graph of $NaNO_2$ (Yang *et al.*, 2011). For measuring the lipid peroxidation method of Heath and Packer (1968) was followed and malonaldehyde (MDA) content formed was measured. Briefly, fresh 100 mg leaf tissue was macerated in TCA (1%) and homogenate was centrifuged at 10,000g for 5 min. Thereafter 1.0 mL supernatant was mixed with 4 mL of 0.5% thiobarbituric acid and mixture was incubated for 30 minutes at 95 °C. After cooling the samples on ice bath centrifugation was again carried at 5000g for 5 min and absorbance was taken at 532 and 600 nm. For measuring the membrane stability index (MSI), method described by Sairam *et al.* (1997) was used and calculations were done according to formula as:

MSI (%) = {1-(C1/C2)} ×100

Where; C1 = electrical conductivity at 25 °C and C2 = electrical conductivity at 120 °C.

Assay of nitrate reductase activity

Activity of nitrate reductase (E.C. 1.6.6.1.) was determined by cutting 500 mg leaf tissue into vials containing 2.5 mL phosphate buffer (pH 7.5) supplemented with potassium nitrate (20 mM) and isopropanol (5%). After two hours of incubation in dark, 0.4 mL aliquot was taken and mixed with 0.3 mL sulphanilamide (1%) and naphthylethylene diamine hydrochloride (0.02%). After 20 minutes of incubation at room temperature absorbance was read at 540 nm (Jaworski, 1971).

Assay of antioxidant enzymes

Antioxidant enzymes were extracted by macerating fresh 500 mg leaf tissue in cold sodium phosphate buffer (50 mM, pH 7.0) having polyvinyl pyrolidine (1%) and EDTA (1 mM) using chilled pestle and mortar. After centrifuging the homogenate at 15,000g at 4 °C supernatant was collected and used for assaying enzyme activity. For determining the activity of superoxide dismutase (SOD, EC 1.15.1.1) method of Bayer and Fridovich (1987) was used. Inhibition of the photochemical reduction of nitroblue tetrazolium chloride

(NBT) by enzyme was recorded at 560 nm. Activity of ascorbate peroxidase (APX, EC 1.11.1.11) was measured following method of Nakano and Asada (1981) and change in absorbance was recorded at 290 nm for 3 minutes. Foyer and Halliwell's (1976) method were used for assaying the activity of glutathione reductase (GR; EC 1.6.4.2). Glutathione dependent oxidation of NADPH was measured as change in optical density at 340 nm for 2 min. Activities of antioxidant enzymes are expressed as U mg^{-1} protein. Content of protein was determined by Lowry *et al.* (1951).

Determination of ascorbate and reduced glutathione

The content of ascorbate (AsA) was estimated according to Mukherjee and Choudhuri (1983). Briefly, fresh plant material was extracted in 6% TCA and centrifuged at 1000g for 10 minutes. To supernatant was added 2% dinitrophenylhydrazine and 10% thiourea. After incubating the mixture in water bath for 15 min ice cooled 80% H_2SO_4 was added. Absorbance was taken at 530 and content was calculated using standard curve of ascorbate. For estimation of reduced glutathione (GSH), method of Ellman (1959) was used and supernatant was reacted with 5, 5-dithiobis-2-nitrobenzoic acid and read at 412 nm. Standard curve of GSH was used for calculation (Ellman, 1959).

Determination of phenols, flavonoids and total antioxidant activity

Total phenols were extracted by macerating the dry powdered material in methanol and reacting the supernatant with Folin-Ciocalteu reagent. Absorbance was taken at 765 nm and gallic acid was used as standard (Singleton and Rossi, 1965). For measuring the flavonoid content, method of Zhishen *et al.* (1999) was followed and catechin was used as standard. Briefly methanolic extract was reacted with $NaNO_2$ and $AlCl_3$ followed by addition of 2 mL NaOH. Total antioxidant activity was determined by extracting the leaf tissue in methanol containing 0.1% HCl. After centrifuging the extract at 10, 000g, supernatant was reacted with 1, 1-dipheny l-2-picrylhydrazyl (DPPH, 0.1 mM). After incubation of 10 minutes the absorbance was recorded at 517 nm (Shimada *et al.*, 1992).

Estimation of nitrogen, sodium and potassium

Content of nitrogen was quantified following the method of Subbiah and Asija (1956). Dried leaf (1.0 g) tissue was acid digested in $HClO_4$ and HNO_3 until solution turned clear. The digested materials were made to 100 mL by distilled water and were directly read on flame photometer for estimation of Na and K.

Statistical analysis

Data presented is mean of three (±SE) replicates and data was analysed statistically using ANOVA and least significant was calculated using Duncan's Multiple Range Test at $p < 0.05$.

Results

Results showing the influence of salinity stress with and without the supplementation of Se and JA on plant height and plant dry weight in *Vigna radiata* plants are given in Table 1. As compared to control, salinity stress declined the plant height (22.46%) and plant dry mass (33.33%) significantly however, Se as well JA treatment resulted in considerable enhancement of plant height and dry weight with maximal increase of 37.31% and 28.88% due to Se + JA. Alleviation of salinity induced decline was observed in both Se as well as JA treated seedlings. Relative to NaCl treated plants alleviation of 26.98% and 47.50% in plant height and dry weight was observed in NaCl + Se + JA treated plants (Table 1).

Treatment of NaCl significantly declined (40.53%) the activity of nitrate reductase over control and application of Se (23.40%), JA (45.14%) and Se + JA (59.33%) significantly increased the activity. Decline

induced due to NaCl was mitigated to considerable extent due to Se and/or JA with maximal alleviation of 53.99% in NaCl + Se + JA treated plants (Table 1). Application of Se and/or JA improved the content of nitrogen (N) over control and alleviated the decline caused due to NaCl stress. Relative to control, N was declined by 38.86% due to NaCl while as Se, JA and Se + JA treatment resulted in improvement of 17.27%, 38.35% and 54.37% respectively. Application of Se and/or JA to NaCl stressed plants resulted in alleviation of 16.44% (NaCl + Se), 33.45% (NaCl + JA) and 55.20% (NaCl + Se + JA) compared to NaCl stressed plants (Table 1). Salinity stress resulted in accumulation of Na (211.5%) with concomitant decline in K (37.50%) over the control however, Se as well as JA treatment in declined Na and increased K uptake. Relative to control, decline of 51.70% in Na and increase of 65.39% in K was observed in seedlings treated with Se + JA. Application of Se (14.92%), JA (28.41%) and Se + JA (38.47%) declined the accumulation of Na respectively over the NaCl treated plants. Decline in K was alleviated by the treatment of Se and JA with maximal alleviation of 59.66% in NaCl + Se + JA over NaCl stressed counterparts (Table 1).

Table 1. Effect of salinity stress on plant height (cm), plant dry weight (g plant^{-1}), nitrogen (mg g^{-1} DW), sodium (mg g^{-1} DW), potassium (mg g^{-1} DW), activity of nitrate reductase (nmol NO_2^- hr^{-1} g^{-1} FW), relative water content (percent) and total antioxidant activity (percent) in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA)

Parameter	Control	Se	JA	Se + JA	NaCl	NaCl + Se	NaCl + JA	NaCl + Se + JA
Plant height (cm)	21.41 ±1.01	23.7 ± 1.22	26.4 ± 1.27	29.4 ± 2.18	16.6 ± 1.01	17.28± 1.06	19.03± 1.12	21.08 ± 1.18
Plant dry weight (g plant^{-1})	1.80 ± 0.78	1.91 ± 0.81	2.17± 0.922	2.32± 0.998	1.20 ± 0.33	1.39± 0.342	1.51± 0.459	1.77 ± 0.578
Na (mg g^{-1} DW)	2.00± 0.45e	1.566± 0.05f	1.296± 0.20g	0.966± 0.05h	6.23± 0.15a	5.30± 0.20b	4.46± 0.15c	3.833 ± 0.25d
K (mg g^{-1} DW)	14.16± 0.70d	17.49± 0.69c	19.9± 1.05b	23.42± 0.85a	8.85± 0.49g	9.60± 0.45f	11.46± 0.54e	14.13 ± 0.35d
N (mg g^{-1} DW)	17.6± 0.81d	20.64± 1.6c	24.35± 1.06b	27.17± 1.6a	10.76± 0.67g	12.53± 0.50f	14.36± 0.54e	16.70 ± 0.50d
Nitrate reductase activity (nmol NO_2^- hr^{-1} g^{-1} FW)	221.3± 9.01d	273.1± 10.0c	321.2± 12.01b	352.6± 12.0a	131.6± 5.5h	162.00± 6.2g	179.66± 6.9f	202.66 ± 7.5e
RWC (Percent)	82.18± 3.7c	83.73± 2.19c	86.48± 3.2b	92.11± 2.9a	56.16± 2.8g	60.67± 3.6f	65.99± 3.5e	71.40 ± 2.8d
Total antioxidant activity (Percent DPPH radical scavenging)	50.03± 3.9g	59.03± 3.15e	64.75± 3.7d	68.23± 3.8c	55.61± 2.8f	67.10± 2.9c	75.60± 3.7b	83.53 ± 4.6a

Data is mean (SE) of three replicates and different letters denote significant difference at $P<0.05$.

Total antioxidant activity measured as DPPH radical scavenging activity was increased due to application of Se and JA individually as well as combinedly. Relative to control total antioxidant activity increased by 17.98% in Se, 29.42% in JA and 36.37% in Se + JA treated plants. Maximal increase of 66.95% was observed in NaCl + Se + JA treated plants over the control (Table 1). Application of Se and /or JA increased RWC imparting maximal increase of 12.08% due to their combined application. NaCl reduced RWC by 31.66% over control. When applied to NaCl treated plants Se and JA resulted in alleviation of NaCl mediated decline with maximal alleviation of 27.13% in NaCl + Se + JA treated plants over the NaCl stressed counterparts (Table 1).

It was observed that application of NaCl resulted in significant decline in the synthesis of chlorophylls and carotenoids resulting in declined photosynthesis and PSII functioning. Relative to control, NaCl stress significantly declined Proto-IX, Mg-Proto-IX, total chlorophylls and carotenoids while as Se and/or JA resulted in considerable improvement under normal conditions and alleviation under NaCl treatments (Figure 1). NaCl treatment declined Proto-IX, Mg-Proto-IX, total chlorophylls and carotenoids by 50.50%, 48.24%, 46.47% and 42.31% respectively over the control. Under normal conditions content of Proto-IX, Mg-Proto-

IX, total chlorophylls and carotenoids maximally increased by 30.17%, 34.72%, 61.27% and 30.25% respectively due to combined application of Se + JA. Application of Se and /or JA to NaCl stressed plants alleviated the decline considerably with maximal amelioration of 64.67%, 38.20%, 67.16% and 28.82% in Proto-IX, Mg-Proto-IX, total chlorophylls and carotenoids respectively over the NaCl treated counterparts (Figure 1A-D). Reduced synthesis of pigments due to NaCl was correlated with the photosynthesis rate and PSII functioning. Relative to control, net photosynthesis (Pn) and PSII activity (Fv/Fm) declined by 34.12% and 21.13% due to NaCl over control (Figure 2). Application of Se and/or JA increased Pn and PSII activity significantly attaining maximal increase of 57.93% and 27.68% respectively due to combined application of Se + JA. Application of Se and/or JA affectively alleviated the decline in Pn and PSII activity with maximal mitigation of 33.13% and 21.17% in NaCl + Se + JA treated plants over the NaCl stressed counterparts (Figure 2).

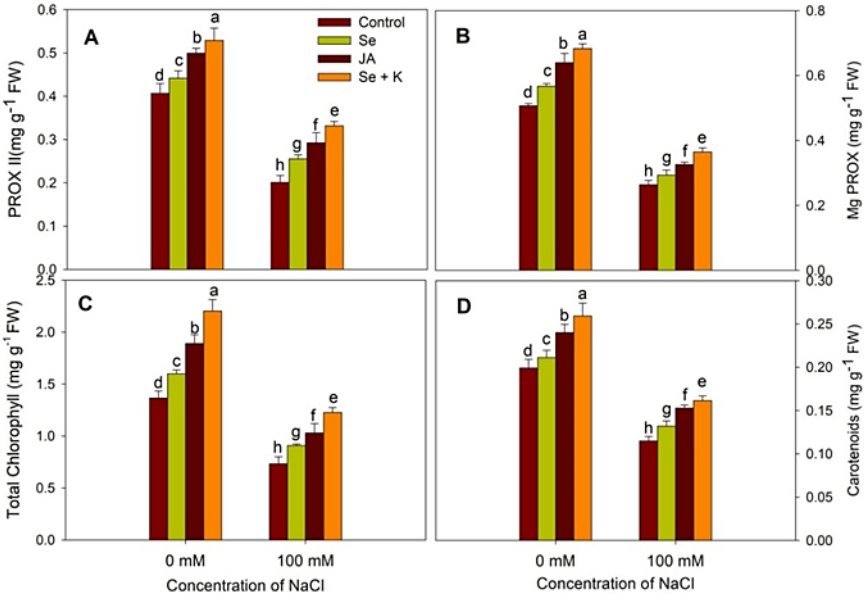

Figure 1. Effect of salinity stress on (A) prototoporphyrin IX (Proto IX), (B) Mg-prototoporphyrin IX (Mg-Proto IX), (C) total chlorophylls and (D) carotenoids in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA)

Data is mean (SE) of three replicates and different letters denote significant difference at $P<0.05$.

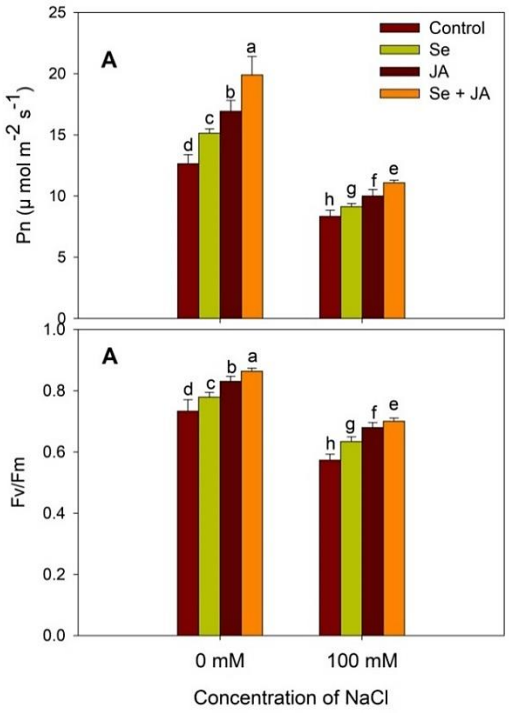

Figure 2. Effect of salinity stress on (A) net photosynthesis (Pn) and (B) maximal photochemical efficiency (Fv/Fm) in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA).
Data is mean (SE) of three replicates and different letters denote significant difference at P<0.05.

Application of Se and/ or JA individually as well jointly enhanced the content of proline, glycine betaine, sugars and activity of γ-glutamyl kinase (γ-GK) under normal as well NaCl stressed conditions (Figure 3). Relatively to control, proline, glycine betaine and sugars increased maximally by 150.52%, 177.29% and 79.04% due to combined application of Se + JA. Plants treated with NaCl exhibited an increase of 62.03%, 22.64% and 119.16% in proline, glycine betaine and sugars over the control plants. Application of Se and/or JA to NaCl treated plants further improved the content of proline, glycine betaine and sugars attaining maximal values in NaCl + Se + JA treated plants. Compared to control, maximal increase in proline, glycine betaine and sugars was 186.59%, 203.56% and 195.38% respectively in NaCl + Se + JA (Figure 3A, C and D). The activity of γ-GK increased by 19.31% in NaCl treated plants while as increased by 15.22%, 34.60% and 46.49% due to Se, JA and Se + JA treatments. Maximal increase of 58.25% in the activity of γ-GK was observed in NaCl + Se + JA treated plants (Figure 3B).

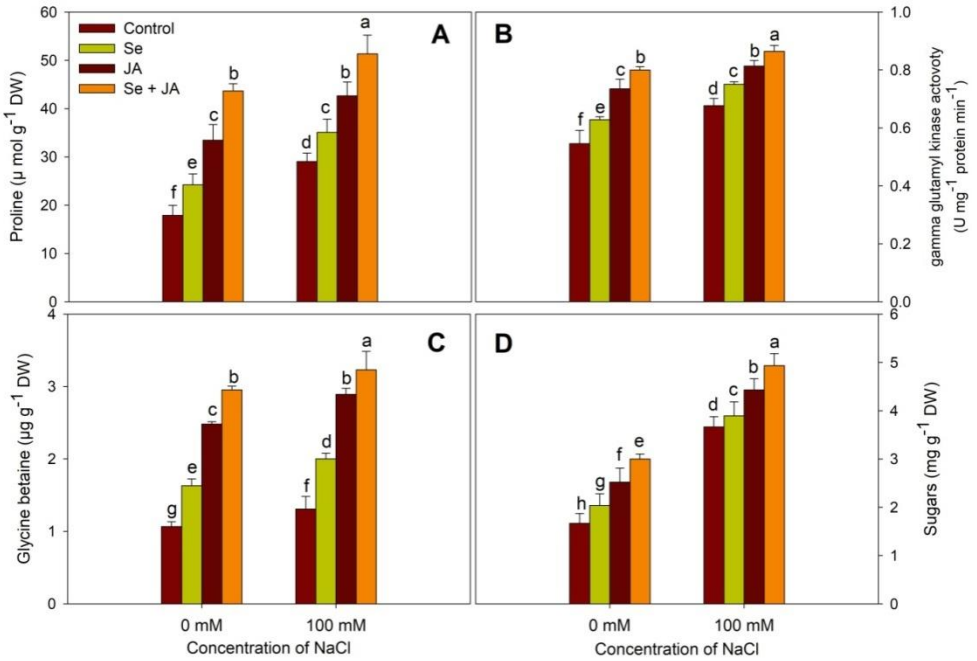

Figure 3. Effect of salinity stress on (A) proline, (B) γ-glutamyl kinase activity, (C) glycine betaine, and (D) sugars in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA) Data is mean (SE) of three replicates and different letters denote significant difference at $P<0.05$.

Vigna radiata plants exposed to NaCl stress exhibited significant increase in the accumulation of toxic reactive oxygen species like H_2O_2 and O_2^- over the control. Application of Se and/ or JA decreased the accumulation of ROS individually as well as combinedly (Figure 4). Relative to control plants H_2O_2, O_2^- and lipid peroxidation increased by 108.22%, 48.02% and 99.87% respectively in NaCl stressed plants resulting in decline of % in membrane stability index. Decline in H_2O_2, O_2^- and lipid peroxidation due to Se was 15.48%, 14.38% and 23.94%, due to JA was 26.38%, 25.93% and 37.58% and due to Se + JA was 46.94%, 38.43% and 50.97% over the control. Both Se and JA treatment increased the membrane stability index attaining maximal increase of 14.50% due to combined application of Se and JA. Salinity induced increase in H_2O_2, O_2^- and lipid peroxidation was mitigated by application of Se and/or JA with maximal alleviation of 49.17%, 47.61% and 48.74% respectively observed in NaCl + Se + JA treated plants (Figure 4A-C). In addition to this, the reduction in membrane stability index due to salinity was alleviated by application of Se (9.92%), JA (17.04%) and Se + JA (28.43%) over NaCl treated plants (Figure 4D).

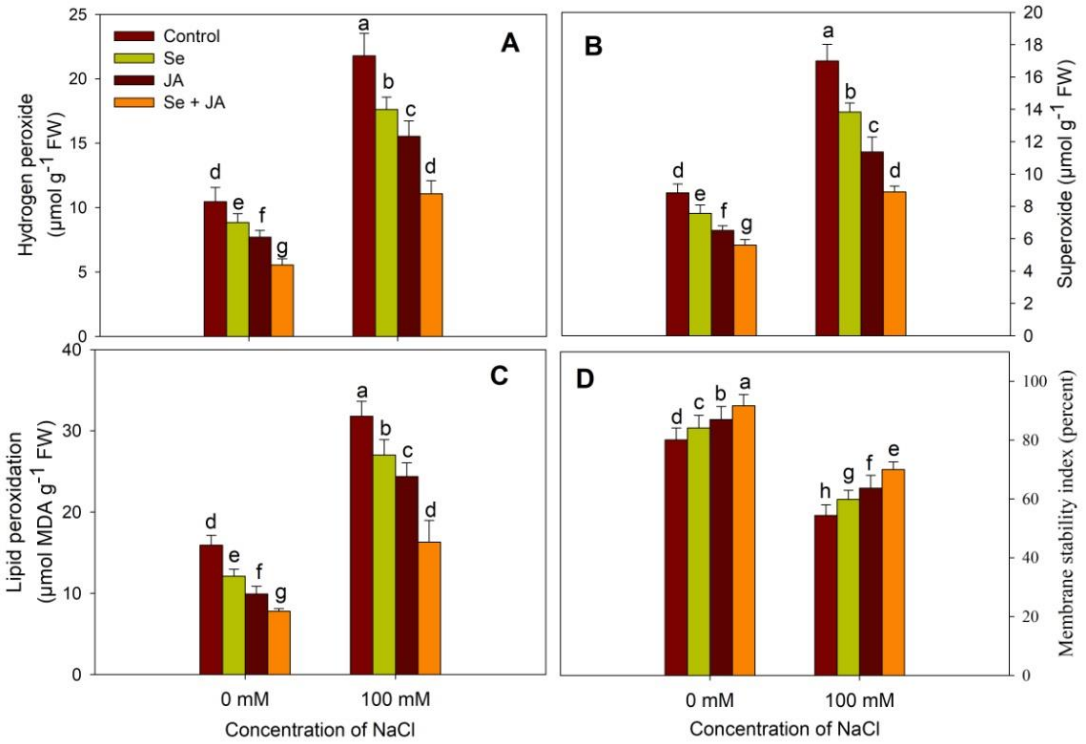

Figure 4. Effect of salinity stress on (A) hydrogen peroxide, (B) superoxide, (C) lipid peroxidation, and (D) membrane stability index in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA)

Data is mean (SE) of three replicates and different letters denote significant difference at P<0.05.

Application of Se and/or JA up-regulated the activity of antioxidant system by increasing the activity of antioxidant enzymes and contents of non-enzymatic antioxidants (Figure 5 and 6). Relative to control, activity of SOD, APX and GR increased by 28.41%, 11.60% and 8.09% due to NaCl treatment. Under normal conditions activity of SOD increased by 45.10%, APX by 19.64% and GR by 20.77% due to Se application while as an increase of 66.18% (SOD), 41.07% (APX) and 29.19% (GR) was observed due to JA application. Activity of SOD (84.93%), APX (50.89%) and GR (37.24%) increased maximally in plants treated with Se + JA over the control. However, application of Se and/or JA further increased the activities of antioxidant enzymes under NaCl treated conditions attaining maximal increase of 97.80% for SOD, 60.08% for APX and 47.84% for GR (Figure 5). Relative to control, GSH (11.85%) increased while as AsA (18.31%) decreased due to salinity stress (Figure 6). Increase in GSH and AsA was maximum of 37.18% and 41.55% in Se + JA treated plants. Under NaCl conditions application of Se + JA increased GSH by 49.29% maximally over control while as mitigated the decline in AsA by 33.70% over NaCl stressed plants (Figure 6).

Increase in total flavonoids and phenols due to NaCl stress was 6.31% and 11.91% respectively over control. However, increase due to Se, JA and Se + JA was 26.31%, 35.78% and 43.47% for total flavonoids and 19.47%, 31.00% and 39.89% for phenols under normal conditions. Application of Se and/or JA further increased the flavonoids and phenols over the NaCl treated plants attaining a maximal increase of 54.73% and 62.17% in NaCl + Se + JA treated plants over control plants (Figure 7A and B).

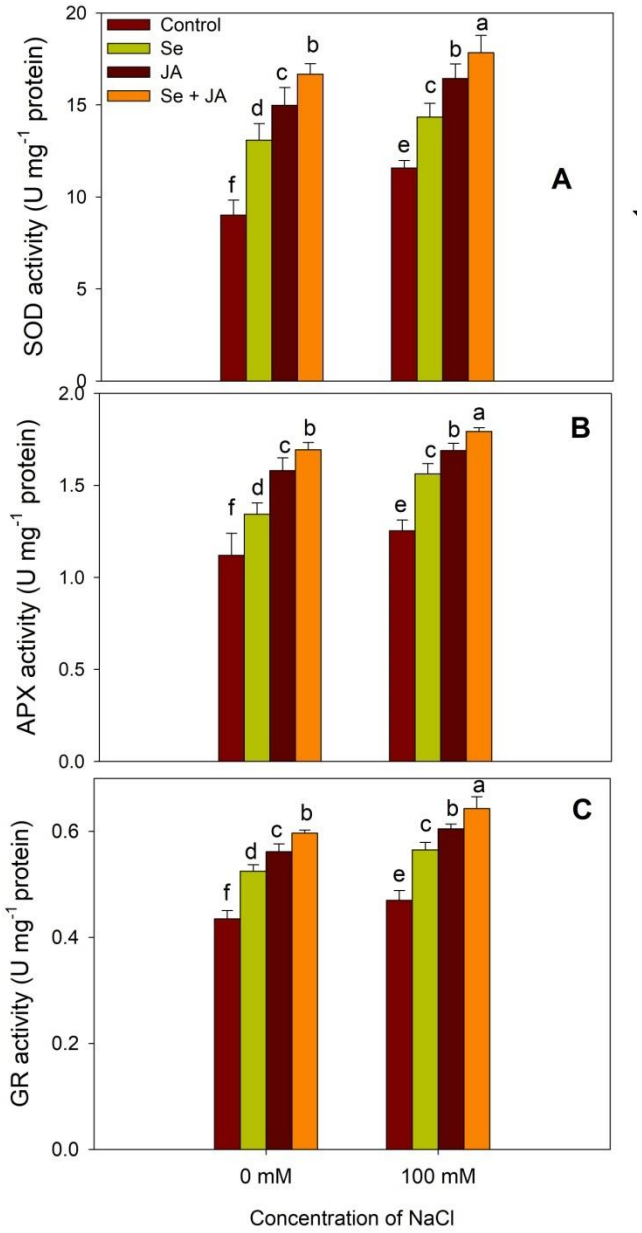

Figure 5. Effect of salinity stress on the activity of (A) superoxide dismutase (SOD), (B) ascorbate peroxidase (APX), and glutathione reductase (GR) in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA)

Data is mean (SE) of three replicates and different letters denote significant difference at $P<0.05$.

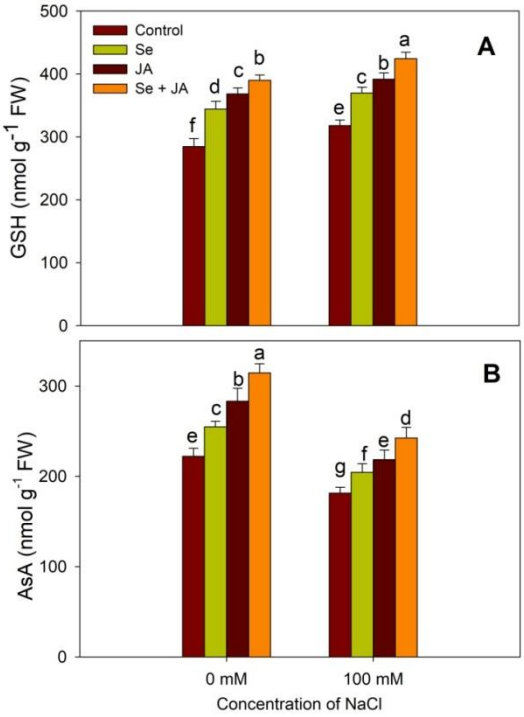

Figure 6. Effect of salinity stress on (A) ascorbate and (B) reduced glutathione content in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA)
Data is mean (SE) of three replicates and different letters denote significant difference at P<0.05.

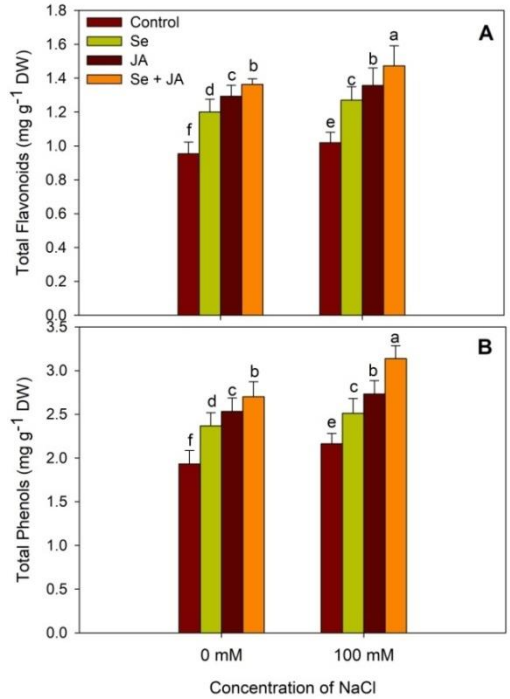

Figure 7. Effect of salinity stress on (A) total flavonoids and (B) total phenols in *Vigna radiata* L. with and without the supplementation of selenium (Se) and jasmonic acid (JA)
Data is mean (SE) of three replicates and different letters denote significant difference at P<0.05.

Discussion

In contemporary era plants are adversely affected by different stresses and global climate change has aggravated the situation. In this context different management techniques have been introduced and tested for their potential to reduce the devastating effects of stresses to ensure sustainable crop growth and production. In present study, exogenously applied Se and JA was tested for their affectivity to alleviate the negative effects of high salinity in *Vigna radiata*. Supplementation of Se and the application of JA considerably enhanced growth and biomass production with effect being much obvious with their combined application. Salinity adversely affects the plant growth and development by hampering the cell division and proliferation (Qi and Zhang, 2019). Different workers have reported reduced height and biomass accumulation due to salinity (Ahmad *et al*., 2018; Soliman *et al*., 2020; Ahanger *et al*., 2019a, b). Increased growth and biomass accumulation due to the Se (Elkelish *et al*., 2019; Alyemeni *et al*., 2018) and JA (Ahmad *et al*., 2018; Ali e*t al*., 2022) treatment has been reported, but the influence of their combined treatment has not been reported. Increased growth and salt stress tolerance in Se and JA treated plants can be due to significant decline in accumulation of Na ions, maintenance of tissue water potential and reduced ROS production thereby resulting declining the osmotic and oxidative effects of salinity. Besides, salinity stress reduces the root hydraulic conductivity and the expression of key genes coding for plasma membrane and tonoplast intrinsic proteins thereby affecting growth and metabolism seriously (Boursiac *et al*., 2005). In present study application of Se and JA significantly mitigated the salinity triggered reduction in RWC which could have also contributed to growth maintenance. Earlier it has been reported that declined RWC in salinity stressed plants leads to reduction in growth, photoinhibition and hampered enzyme functioning (Negrao *et al*., 2017; Khan *et al*., 2021). Application of JA (Ahmad *et al*., 2018) and Se (Elkelish *et al*., 2019) improved the RWC in *Solanum lycopersicum* and *Triticum aestivum* respectively which was also reflected as maintained membrane functioning and enzyme activity. Increased growth due to Se and JA treatment can have resulted from the declined accumulation of Na and increased uptake of beneficial elements like N and K observed in Se and JA treated seedlings. Both N (Iqbal *et al*., 2015) and K (Ahanger and Agarwal, 2017) regulate growth by activity the enzyme activity and restricting excess accumulation of Na. Both JA and Se treatment may have regulated the expression of transport protein mediating Na uptake at root plasma membrane as well as tonoplast so that concentrations can be maintained at non-toxic levels. In salinity stressed *Zea mays*, application of Se regulated the compartmentalisation of Na ions by improving the expression of NHX1 (Jiang *et al*., 2017). Salinity stress potentially alters the expression and functioning of membrane transporters leading to deficient uptake and accumulation of beneficial elements including K and N (Keisham *et al*., 2018). Maintaining increased K content and reduced Na content significantly improves the growth and photosynthesis under saline environment (Balti *et al*., 2021). Earlier Se supplementation (Astaneh *et al*., 2018) and JA application (Al-harthi *et al*., 2021) has been reported to reduce uptake of Na concomitant with increased uptake of beneficial elements. Recently, El-Taher (2022) has demonstrated significant decline in N, P and K in *Vigna unguiculata* due to salinity stress. Reports discussing the interactive influence of Se and JA on the uptake of Na, K and N are not available however, it could be inferred that Se and JA may have fine regulated the activity of membrane transporters leading to reduced uptake of Na and efficient sequestration and compartmentalisation of Na into vacuoles.

Salinity mediated decline in chlorophyll synthesis and photosynthesis was ameliorated considerably due to individual as well as combined application of Se and JA. Content of Proto-IX and Mg-Proto-IX, the intermediates of chlorophyll biosynthesis were significantly enhanced in the Se, JA and Se + JA treated seedlings reflecting in increased chlorophyll synthesis in them. Increased Proto-IX, Mg-Proto-IX and chlorophyll synthesis in Se and JA treated plants can be attributed to up-regulation of the activity of enzymes chlorophyll biosynthesis cycle. Stresses including salinity adversely affects the functioning of key enzymes of chlorophyll biosynthesis (Dalal and Tripathy, 2012) and Se as well as JA individually and combinedly may have

increased their activity. Earlier Qin *et al.* (2020) has reported that salinity stress significantly declines the synthesis of Proto-IX and Mg-Proto-IX as well the expression of chlorophyll metabolism genes thereby causing considerable decline in chlorophyll, photosynthesis and growth. Elkelish *et al.* (2019) has also demonstrated significant enhancement in chlorophyll synthesis and photosynthesis due to Se supplementation. In salt stressed *Brassica napus* (Ahmadi *et al.*, 2018) and sorghum (Ali *et al.*, 2022) significant increase in chlorophyll and photosynthesis due to exogenous JA application has been reported. Combined application proved much beneficial in preventing chlorophyll degradation and photosynthetic inhibition under saline conditions. Salinity triggers chlorophyll degradation and damages the D1 protein of PSII thereby causing significant decline in photosynthetic performance (Hu *et al.*, 2016). Applying JA maintains the functioning of stomatal and non-stomatal attributes of photosynthesis by maintaining the redox homeostasis, low levels of ROS and protecting the chloroplast structure (Per *et al.*, 2016). Increased photosynthesis due to Se and JA can be due to up-regulation of antioxidant system thereby maintaining lesser concentration of ROS within the chloroplast. Besides Se has also a beneficial role n protecting chloroplast structure (Yu *et al.*, 2003). In present study combined application of Se and JA was much affective in alleviating the damaging effect of salinity.

Supplementation of Se and the foliar application of JA significantly ameliorated the oxidative effects of salinity by reducing the generation of ROS including H_2O_2 and O_2^-. Salinity stress resulted in significant increase in the H_2O_2 and O_2^- concentration hampering the membrane functioning. Salinity mediated decline in membrane stability has been attributed exclusively ROS production and lipid peroxidation (Ahanger *et al.*, 2019a, 2020; Soliman *et al.*, 2020). In corroboration to our results salinity induced increase in ROS and lipid peroxidation has been reported in several crop plants like chickpea (Ahmad *et al.*, 2016), tomato and wheat (Ahanger *et al.*, 2019a, b), *Brassica juncea* (Fatma *et al.*, 2016), soybean (Soliman *et al.*, 2020) and *Nicotiana benthamiana* (Qin *et al.*, 2021). Stress induced accumulation of ROS trigger lipid peroxidation and up-regulate lipoxygenase activity to impede the structural and functional stability of membranes (Nahar *et al.*, 2016; Ahanger *et al.*, 2019a, 2020; Begum *et al.*, 2020). In addition, increased ROS affect photosynthetic functioning by deteriorating the chloroplast membrane stability (Li and Kim, 2022). Plants treated with Se and JA exhibited a significant decline in ROS production and lipid peroxidation reflecting in increased membrane stability in them. Reduction in ROS accumulation and lipid peroxidation due to Se (Jóźwiak and Politycka, 2019) and JA (Kamran *et al.*, 2021) has been reported however their interactive effect has not been worked.

Reduced lipid peroxidation and ROS accumulation in Se and JA treated plants can be attributed to significant up-regulation of antioxidant system. Both supplementation of Se and JA application up-regulated the activity of antioxidant enzymes (SOD, APX and GR) and increased the synthesis of non-enzymatic antioxidants (ascorbic acid and reduced glutathione). Salinity stress triggers the activity of antioxidant enzymes to counter the damaging effects by promoting ROS neutralisation (Ahanger *et al.*, 2017; Ahmad *et al.*, 2016; Soliman *et al.*, 2020). Superoxide dismutase is unique and neutralises superoxide radical while as APX, GR, AsA and GSH are key components of ascorbate-glutathione cycle resulting in scavenging of H_2O_2 (Soliman *et al.*, 2019; Hasanuzzaman *et al.*, 2020). Increased functioning of ascorbate-glutathione cycle affects the plant functioning by preventing the peroxidation of membrane proteins and lipids in addition of the maintenance of redox homeostasis reflecting in smooth functioning of photosynthetic electron transport (Jan *et al.*, 2018; Per *et al.*, 2016). Up-regulation of antioxidant system due to Se (Elkelish *et al.*, 2019; Alyemeni *et al.*, 2018) and JA (Per *et al.*, 2016; Ahmad *et al.*, 2017; Mir *et al.*, 2018) has been reported to prevent the stress induced oxidative damage to structural and functional integrity of plants. Ascorbate and reduced glutathione are key non-enzymatic antioxidants and redox buffers contributing significantly to stress tolerance (Zur *et al.*, 2021).

The antioxidant system was further strengthened by the significant enhancement in the accumulation of secondary metabolites including phenols and flavonoids which was also obvious as increased antioxidant activity measured as DPPH radical scavenging. Salinity stress results in increased synthesis of secondary metabolites including phenols and flavonoids (Ahanger *et al.*, 2019a, b; Soliman *et al.*, 2020). Both phenols and

flavonoids have been reported to show ROS scavenging properties thereby leading to improved structural and functional stability of organelles and cells (Sharma *et al.*, 2019; Samec *et al.*, 2021). Secondary metabolites synthesis significantly influences the plant potential to tolerance stress (Begum *et al.*, 2021). Recently, Kiani *et al.* (2021) has demonstrated that salinity stress exposure significantly increased the accumulation of phenolic and flavonoid compounds reflecting in increased antioxidant activity. Exposure to moderate salinity induces the accumulation of phenols and volatile compounds in *Salvia mirzayanii* reflecting in increased total antioxidant activity (Valifard *et al.*, 2014). In present study, Se and JA application significantly increased total phenols, total flavonoids and total antioxidant activity. Increase in flavonoid content due to application of JA has been reported by Ahmad *et al.* (2018). Increased accumulation of secondary compounds results due to increased activity of enzymes involved in their synthesis (Ahanger *et al.*, 2019a, b). Application of JA has been reported to improve the expression of genes coding for enzymes of phenolic compound biosynthetic pathway (Thiruvengadam *et al.* (2016). Besides increased accumulation of secondary compounds due to Se application has been reported earlier by Skrypnik *et al.* (2021) in *Valerianella locusta*.

In addition to this the accumulation of osmolytes was stimulated significantly due to Se and JA application maintaining the effects under salt stress as well. Significant accumulation of proline, glycine betaine and sugars protect plants from the damaging effects of stresses by mediating the maintenance of tissue water potential, savaging the ROS, maintaining redox state and the enzyme activity (Ahanger *et al.*, 2014; Jogawat, 2019). Plants accumulating significant osmolytes better perform under stressful conditions through maintenance of photosynthetic functioning and enzyme activity (Khan *et al.*, 2015; Ahanger *et al.*, 2019a, b). Accumulation of osmolytes act as signals for better elicitation of tolerance response in plants (Dutta *et al.*, 2019). Increased accumulation of osmolytes results directly from the modulation in their biosynthesis pathway obvious as increased activity of enzymes catalysing their synthesis (Khan *et al.*, 2015). Increased proline resulting from up-regulated γ-glutamyl kinase activity has been earlier reported by others as well (Khan *et al.*, 2015; Elkelish *et al.*, 2019). However, Se and JA mediated regulation of proline and activity of γ-glutamyl kinase has not been reported. Therefore, further studies are required to unravel the actual mechanisms.

Conclusions

Conclusively salinity stress adversely affected the growth of *Vigna radiata* by increasing the production of toxic ROS which led to oxidative effects on key processes including photosynthesis and enzyme activity. However, Se and JA, individual as well as combined, application proved affective in mitigating the harmful effects of salinity stress which can be attributed to: (a) up-regulation of antioxidant system, (b) increased osmolyte accumulation, (c) selective absorption of Na and (d) secondary metabolite accumulation.

Authors' Contributions

The author read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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