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Research article

Selenium protects wheat seedlings against salt stress-mediated oxidative damage by up-regulating antioxidants and osmolytes metabolism

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ABSTRACT

Salinity stress hampers the growth of most crop plants and reduces yield considerably. Therefore, experiments were conducted on wheat (*Triticum aestivum* L.) plants for studying the role of selenium (5 and 10 μM Se) supplementation in strengthening the salinity stress tolerance. Exposure to salinity (100 mM NaCl) reduced growth in terms of length, fresh and dry biomass yield. Se was affective in ameliorating the deleterious effects of NaCl stress to significant levels when supplied at 5 μM concentrations compared to 10 μM. Application of Se at 5 μM concentration did not show significant impacts on the physiological and biochemical parameters studied. Plants supplemented with 5 μM Se exhibited the highest RWC, chlorophyll synthesis, and photosynthesis. Se supplementation reduced the NaCl-mediated oxidative damage by up-regulating the activity of enzymatic components of the antioxidant system and the accumulation of ascorbate and glutathione. Furthermore, 5 μM Se proved beneficial in enhancing proline and sugar accumulation in normal and NaCl-stressed seedlings providing extra osmolarity to maintain RWC and protect photosynthesis. Se also affected proline metabolism by modulating the activities of the γ-glutamyl kinase (γ-GK) and proline oxidase (PROX) leading to its greater synthesis and lesser degradation. Moreover, it was observed that Se declined the Na/K ratio and also improved nitrogen and Ca uptake. Conclusively, Se at low concentration can be beneficial in preventing salinity-mediated damage and further studies are required to unravel underlying mechanisms.

1. Introduction

Salinity is one of the damaging stress factors which affects the growth of plants by altering the metabolism and hence declines yield considerably (Ahanger and Agarwal, 2017; Ahanger et al., 2018; El-Esawi et al., 2018a,b,c,d, 2019). Salinity stress is a multicomponent stress factor and is controlled by multitude of genes and gene networks (Assaha et al., 2017). After exposure to salinity, plants experience osmotic stress which is the first impact of high salinity and usually arises from the reduction in the solute potential of the soil solution. This directly influences the hydraulic conductivity often observed as a reduction in water and solute uptake (Munns and Tester, 2008). Excess salinity mediates ion toxicity resulting from the excess accumulation of toxic ions like Na^+ and hence affecting growth and development. Excess accumulation of Na^+ induces efflux of cytosolic K^+ and Ca^{2+} consequently leading to imbalance in their cellular homeostasis, nutrient deficiency, oxidative stress, retarded growth and cell death (Craig Plett and Moller, 2010; Cabot et al., 2014; Ahanger et al., 2017). It is believed that salinity-mediated hindrances in metabolism are the resultant effects on specific assimilatory pathways like nitrogen or carbon metabolism (Tejera et al., 2004). Agricultural malpractices have further aggravated these problems.

Other main reasons for the salinity-mediated growth restrictions are attributed to the excess accumulation of the reactive oxygen species (ROS) that cause oxidative damage to key molecules (Ahanger et al., 2017; Foyer, 2018). Salinity-induced ROS accumulation inhibits photosynthesis, nitrogen and carbohydrate metabolism (Ahanger and Agarwal, 2017) and affects the expression of genes. Multifaceted and elaborated mechanisms are up-regulated to avert the high salinity-mediated damage in growth and productivity. These include efficient partitioning of toxic ions, through channel proteins and up-regulation of ROS scavenging system as well as accumulation of osmolytes like

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sugars and amino acids (Bolouri-Moghaddam et al., 2010). Besides their roles in maintaining tissue water content, osmolytes protect enzyme structure and functioning by mediating the ROS scavenging and stress signalling (Suzuki and Mittler, 2006; Couee et al., 2006). Therefore, strengthening the tolerance mechanisms and understanding their implications on the overall growth performance are necessary for achieving sustainable productivity.

Selenium (Se) is an important element for animals and reportedly imparts several beneficial effects in plants as well, particularly under stressed conditions. Increased cold tolerance in *Cucumis sativus* due to Se has been reported by Hawrylak-Nowak et al. (2010). Se treatment enhanced production and weight of seeds of *Brassica rapa* (Lyons et al., 2008). Response to Se depends on the concentration applied (Terry et al., 2000), and higher concentrations can be toxic (Hermosillo-Cereceres et al., 2013). Se has been demonstrated to regulate plant growth by strengthening the stress tolerance mechanisms like antioxidant and secondary metabolite metabolism (Filek et al., 2008; Ahmad et al., 2015; Jiang et al., 2017). Hasanuzzaman et al. (2012) have demonstrated increased cadmium tolerance of *Brassica napus* due to Se application through enhanced enzymatic and non-enzymatic antioxidant systems. Therefore, we conducted certain experiments to investigate the possible role of Se in improving salt tolerance in wheat seedlings. We hypothesized that application of Se could protect the metabolism and cellular functioning of wheat by up-regulating the ROS neutralizing pathways and the osmoregulatory mechanisms.

2. Materials and methods

2.1. Experimental design and treatment

Sterilized wheat (*Triticum aestivum* L.) seeds were sown in earthen pots filled with reconstituted soil containing peat, compost, and sand in the ratio of 4:1:1. Before sowing, the soil in each pot was wetted by applying 250 mL of full strength Hoagland's solution (Hoagland and Arnon, 1950). After seed germination and seedling growth for seven days, the number of seedlings per pot was thinned to five. Ten days after germination, pots were supplemented with (a) full strength Hoagland solution (control), (b) 100 mM NaCl, (c) 5 μM Se, (d) 10 μM Se, (e) 100 NaCl + 5 μM Se and (f) 100 NaCl + 10 μM Se for another fifteen days. All treatments were supplemented in the form of a modified Hoagland solution. Se was given in the form of sodium selenate (Na_2SeO_4). Pots were maintained under greenhouse conditions and laid in a complete randomized block design with four replicates for each treatment. Twenty-five days old seedlings were harvested and analysed for photosynthetic parameters, oxidative stress attributes, antioxidants, and metabolites.

2.2. Measurement of photosynthetic pigments, stomatal conductance, and photosynthetic efficiency

For estimation of photosynthetic pigments (chlorophylls and carotenoids), 100 mg fresh leaf tissue was extracted in dimethyl sulfoxide (DMSO), and the optical density of supernatant was taken spectrophotometrically at 480, 510, 645, 663 nm against DMSO (Hiscox and Israelstam, 1979).

The photosynthetic efficiency and stomatal conductance in fully expanded upper leaf was recorded using the infrared gas analyzer (CID-340, Photosynthesis System, Bio-Science, USA).

2.3. Determination of leaf water content, soluble sugars, proline and activity of γ-glutamyl kinase and proline oxidase

For determination of relative water content (RWC), twenty leaf discs were punched, and their fresh weight was determined. The same leaf discs from both normal and treated plants were kept in petri dishes containing distilled water for 1 h to record the turgid weight and after that discs were oven dried at 80 °C for 24 h to record the dry weight (Smart and Bihgham, 1974). Calculation was done by the following formula:

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \text{ x } 100$$

Method of Schields and Burnett (1960) was adopted for the estimation of sugar content. 500 mg oven dried plant samples were extracted in ethanol (80%) followed by centrifugation for 20 min at 5000*g*. The content of sugar in the supernatant was determined by reacting known volume with anthrone reagent and measuring the optical density at 585 nm.

Proline was extracted by crushing 0.5 gm of leaf sample in 3% sulphosalicylic acid and the homogenate was then centrifuged at 3000*g* for 20 min. Thereafter, 2 mL supernatant was boiled with 2 mL of each glacial acetic acid and ninhydrin reagent for 1 h. The reaction was terminated on ice bath, and proline was separated using toluene. Absorbance was read spectrophotometrically at 520 nm (Sadasivam and Manickam, 1996).

For assaying γ-Glutamyl kinase (GK, EC 2.7.2.11) and proline oxidase (PROX, EC 1.4.3.1) activities, 500 mg fresh tissue was homogenised in Tris buffer (pH 7.5), and the homogenate was centrifuged at 30,000*g* for 30 min. Activity of γ-GK and PROX was determined in pellet by following the method of Hayzer and Leisinger (1980) and Huang and Cavelieri (1979) respectively. For γ-GK, reaction mixture contained 50 mM Tris buffer (pH 7.0), L-glutamate, 20 mM $MgCl_2$, ATP, hydroxamate–HCl and the enzyme extract in a final volume of 1.0 mL. Stop buffer ($FeCl_3$ and TCA) was then added to terminate the reaction and absorbance was taken 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}. For PROX assay, reaction mixture contained 50 mM Tris buffer (pH 8.5), 1 mL $MgCl_2$, 100 μL of each NADP, KCN, phenazine methanosulphate, 2, 6 - dichlorophenol indophenol (DCPIP) and proline. Change in absorbance was monitored for 3 min at 600 nm, and activity was considered as mmol DCPIP reduced and expressed as U mg^{-1} protein min^{-1}.

2.4. Membrane stability index, lipid peroxidation, and hydrogen peroxide estimation

For determining the membrane stability index (MSI), 0.5 gm fresh leaf tissue was cut into pieces and immersed into test tubes containing 10 mL distilled water and boiled at 40 °C for recording the electric conductivity (EC_1). The same tubes were again boiled at 100 °C to record EC_2 (Sairam et al., 1997), and MSI was calculated using the formula:

$$(\text{MSI}) = [1 - (EC_1/EC_2)] \times 100$$

Lipid peroxidation, measured in terms of malonaldehyde (MDA) content formation, was determined by homogenizing fresh leaves (100 mg) in 1% trichloro acetic acid followed by centrifugation at 10,000*g* for 5 min. 0.5% thiobarbituric acid was then added, and mixture was boiled at 95 °C for half an hour. Tubes were then kept on ice bath followed by centrifugation for 5 min at 5000*g* for clarification, and optical density was read at 532 and 600 nm (Heath and Packer, 1968).

Concentration of hydrogen peroxide (H_2O_2) was estimated by extracting 200 mg fresh leaf sample in 0.1% TCA using pestle and mortar. After centrifugation at 12000*g* for 15 min, known volume (0.5 mL) of supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 M potassium iodide (1 mL). Subsequently, the optical density of mixture was taken at 390 nm (Sergiev et al., 1997), and computation was done using a standard curve of H_2O_2.

2.5. Assay of antioxidant enzymes

Extraction of antioxidant enzymes was done by homogenizing 1.0 gm fresh leaves in chilled pestle and mortar using 50 mM sodium phosphate buffer (pH 7.0) containing 1% polyvinyl pyrolidine. Resulting homogenate was used as enzyme source after centrifugation at 15,000*g* for 20 min at 4 °C, and the protein content was determined as reported by Lowry et al. (1951).

The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by adopting the method of Bayer and Fridovich (1987). Briefly, assay mixture containing 100 mM phosphate buffer (pH 7.4), 13 mM methionine, 75 μM NBT, 0.1 mM EDTA, 2 μM riboflavin and 100 μL enzyme extract was incubated under light for 15 min, and the reaction was stopped by turning the light off. Photoreduction of nitroblue tetrazolium was recorded at 560 nm against the dark incubated samples and activity of SOD was expressed as enzyme unit (EU) mg^{-1} protein. For assaying activity of catalase (CAT, EC 1.11.1.6) Luck's (1974) method was adopted and the change in optical density was recorded at 240 nm. 3 mL reaction mixture contained phosphate buffer (50 mM, pH 7.0), 10 mM H_2O_2, 100 μL of the enzyme extract and extinction co-efficient of 36×10^3 mM^{-1} cm^{-1} was used for calculation and the activity was expressed as EU mg^{-1} protein. For determination of APX activity, 0.1 mL enzyme was added to 1 mL potassium phosphate buffer (100 mM, pH 7.0), 0.1 mM EDTA, 0.5 mM ascorbate and 0.1 mM H_2O_2. Disappearance of H_2O_2 was observed as change in absorbance at 290 nm (Nakano and Asada, 1981).

For determination of glutathione reductase (GR, EC 1.6.4.2) activity, change in absorbance was recorded at 340 nm for 3 min following Carlberg and Mannervik (1985).

GST (EC: 2.5.1.18) activity was assayed spectrophotometrically by monitoring change in absorbance at 340 nm for 2 min following Hossain et al. (2006) with some modifications. Assay mixture contained 100 mM Tris-HCl buffer (pH 6.5), GSH (1.5 mM), 1 mM 1-chloro-2, 4-dinitrobenzene and enzyme in a final volume of 1 mL. An extinction coefficient of 9.6 $mM^{-1}cm^{-1}$ was used for calculation.

2.6. Determination of ascorbate and reduced glutathione

Ascorbic acid was extracted from 500 mg fresh leaf tissues by macerating them in 5% TCA and the extract was mixed with dinitrophenylhydrazine (2%) and thiourea (10%). Tubes were then kept in boiling water bath for 15 min followed by cooling and centrifugation at 1000*g* for 10 min. Pellet was dissolved by adding 80% H_2SO_4, and optical density was taken at 530 nm (Mukherjee and Choudhuri, 1983). A standard curve of ascorbic acid was used for calculation.

For estimation of reduced glutathione (GSH), 500 mg fresh leaf tissues were homogenised in phosphate buffer (pH 8.0) and the extract was centrifuged at 3000*g* for 15 min. 500 μL supernatant was mixed with 5, 5-dithiobis-2-nitrobenzoic acid and left for 10 min. Absorbance was taken at 412 nm (Ellman, 1959). A standard curve of GSH was used for calculation.

2.7. Estimation of ions

1.0 gm dry plant tissue was digested in a mixture of $HClO_4$ and HNO_3. After digestion, solutions were diluted to 100 mL with distilled water and read on flame photometer for Na, K and Ca (Wolf, 1982). For nitrogen estimation, method of Subbaiah and Asija (1956) was followed. Briefly, dry plant samples were digested in H_2SO_4 and were distilled for an hour. The distilled samples were then titrated with NaOH.

2.8. Statistical analysis

Data is mean (± SE) of three replicates. For testing significance of the data, Duncan's Multiple Range Test was performed using One Way ANOVA and least significant difference (LSD) was calculated at $p < 0.05$.

Table 1
Effect of selenium supplementation (5 and 10 μM) on length (cm), fresh (gm) and dry weight (gm/plant) of shoot in wheat (*Triticum aestivum* L) seedlings treated with 100 mM NaCl. Data presented is mean (± SE) of three replicates and different letters indicate significant difference at $P < 0.05$.

	S Length	S Fresh Weight	S Dry Weight
Control	19.03 ± 0.960b	4.363 ± 0.239b	1.136 ± 0.050bc
NaCl (100 mM)	13.09 ± 0.106d	2.409 ± 0.196d	0.7290 ± 0.043e
5 μM Se	22.91 ± 0.945a	5.366 ± 0.263a	1.525 ± 0.066a
10 μM Se	19.30 ± 0.585b	4.096 ± 0.110b	1.206 ± 0.011b
NaCl + 5 μM Se	16.68 ± 0.523c	3.079 ± 0.053c	0.9106 ± 0.016d
NaCl + 10 μM Se	12.57 ± 1.241d	2.179 ± 0.135d	0.7884 ± 0.070e

3. Results

3.1. Se improves length and biomass accumulation under salinity stress

Table 1 shows the results of influence of NaCl (100 mM) and Se supplementation on the growth parameters like length, fresh and dry biomass of shoot. Se supplementation at 5 μM proved much beneficial under control conditions and caused an increase of 16.93, 18.69 and 25.50% in length, fresh and dry biomass over the control plants. Upon supplementation of NaCl-treated plants with 5 μM Se (100 mM NaCl + 5 μM Se), amelioration of 21.52, 21.76 and 19.94% was observed over the NaCl counterparts. However, supplementation of Se at 10 μM does not show any positive impact either under control conditions or NaCl stressed conditions (Table 1).

3.2. Se supplementation improves photosynthetic attributes by increasing chlorophyll synthesis

A significant enhancement in the synthesis of chlorophyll *a* (Chl a), chlorophyll *b* (Chl b), carotenoids and total pigments was observed in the wheat seedlings treated with 5 μM Se. However, at slightly higher concentration (10 μM Se) almost no such effect was observed. Compared to controls, 5 μM Se increased Chl a content by 9.29%, Chl b content by 6.76%, carotenoids content by 8.01% and total pigments by 13.23%. NaCl stress reduced Chl a, Chl b, carotenoids and total pigments by 39.27, 38.27, 25.98 and 42.37%, respectively, over the control plants. When Se (5 μM) was supplied to NaCl stressed plants, an increase of 16.11% for Chl a, 4.97% for Chl b, 9.87% carotenoids and 13.93% for total pigments was observed over the NaCl stressed counterparts (Fig. 1A–D). Furthermore, application of Se at 5 μM (100 mM NaCl + 5 μM Se) proved much affective in ameliorating the negative effect of NaCl on stomatal conductance and photosynthetic efficiency. Relative to control, 5 μM Se improved photosynthetic rate by 22.77% and stomatal conductance by 18.54% (Fig. 1E and F). However, 10 μM concentration of Se was not affective compared to the control plants.

3.3. Application of Se improves RWC through modulation of proline metabolism and accumulation of sugars

Results showing the effect of NaCl and Se on the synthesis of proline, sugars, and RWC are shown in Fig. 2A–E. Se (5 μM) application enhanced the synthesis of proline and sugars by 30.24 and 7.60% over the control plants. In NaCl stressed plants, accumulation of proline and sugars increased by 20.12 and 36.60% over the respective controls while RWC exhibited a decline of 30.81% (Fig. 2C–E). Se application at 5 μM improved RWC when applied to NaCl stressed plants. Unlike 10 μM Se, treatment of 5 μM Se proved much beneficial in improving the synthesis of proline as well as sugar content under normal and NaCl stress conditions. Relative to control, Se (5 μM) application also

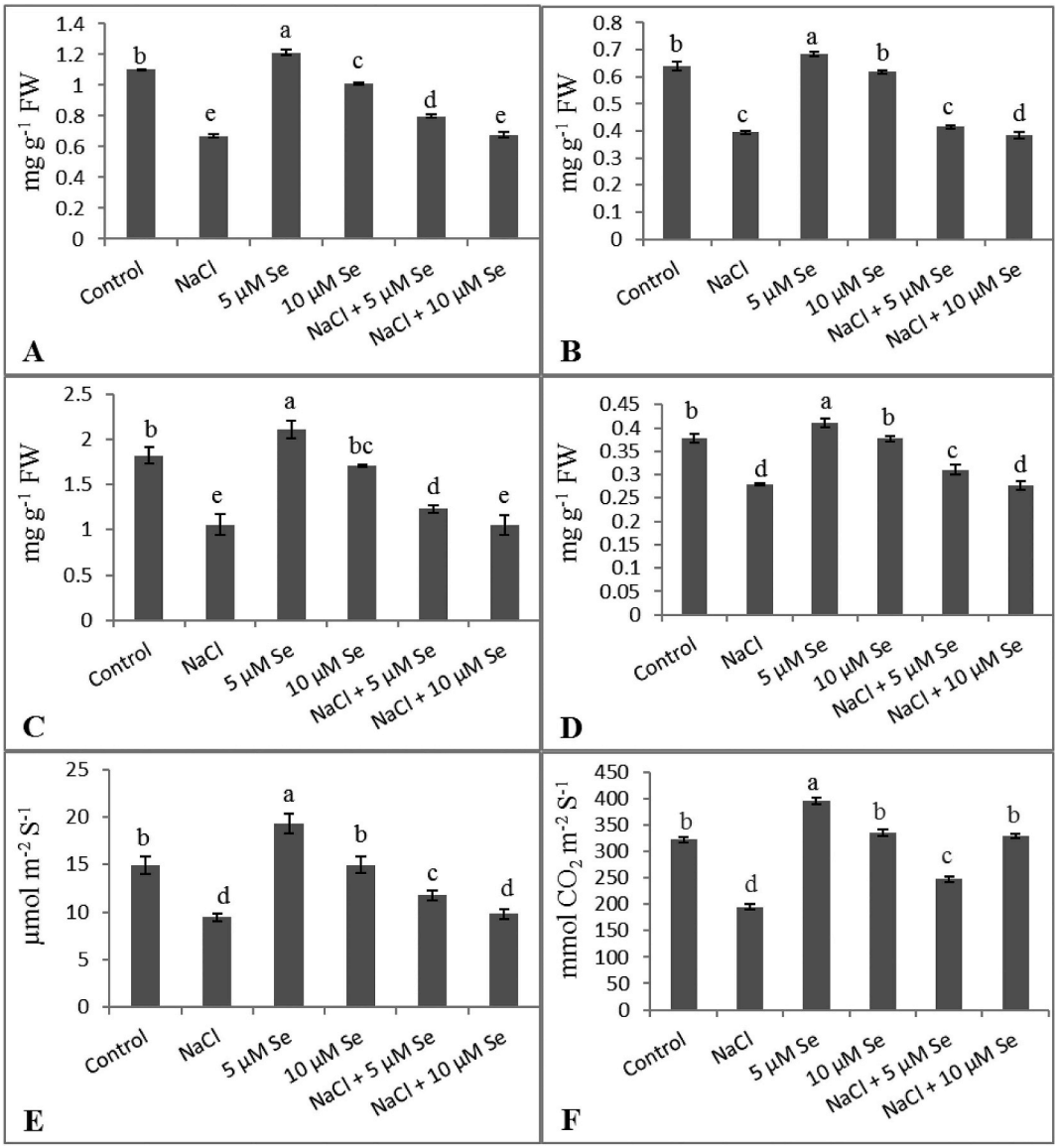

Fig. 1. Effect of salinity stress (100 mM NaCl) on (A) Chlorophyll *a*, (B) chlorophyll *b*, (C) total chlorophyll, (D) carotenoids, (E) photosynthetic rate and (F) stomatal conductance in wheat (*Triticum aestivum* L) treated with selenium. Data presented is mean (± SE) of three replicates and bars with different letters denote significant difference at $P \leq 0.05$.

increased activity of γ-GK by 45.31% and reduced PROX by 48.62%. NaCl stressed plants exhibited an increase and decrease of 21.28% and 21.71% in activity of γ-GK and PROX, respectively. Se application maintained its effect when supplied to NaCl stressed plants (Fig. 2A–E).

3.4. Supplementation of Se protects membranes by reducing the formation of hydrogen peroxide

Se supplementation significantly reduced the generation of hydrogen peroxide (H_2O_2) therefore preventing lipid peroxidation and giving enough stability to membranes (Fig. 3A–C). Treatment of NaCl increased H_2O_2 by 55.72% over control plants. However, application of 5 µM Se reduced the H_2O_2 generation by 17.11% over the NaCl stressed plants (Fig. 3A). Relative to control, H_2O_2 generation declined by 28.70 and 3.15% respectively in 5 and 10 µM Se treated wheat plants. The reduced generation of H_2O_2 due to Se (5 µM) supplementation caused a reduction of 16.92% in lipid peroxidation, resulting in enhancement of 3.04% in membrane stability index (MSI). However, NaCl stress decreased MSI by 34.60% and triggered lipid peroxidation by 22.50% over the control seedlings (Fig. 3B and C). However maximal amelioration of NaCl-mediated H_2O_2 (17.11%) and lipid peroxidation (13.11%) was observed in seedlings treated with NaCl and 5 µM Se over the NaCl stressed plants (Fig. 3A–C).

3.5. Se supplementation up-regulates antioxidant systems in NaCl stressed wheat

Supplementation of Se at 5 µM significantly improved the antioxidant defence systems in wheat seedlings (Figs. 4 and 5). Relative to control, Se at 5 µM enhanced the activity of SOD by 17.02%, CAT by 14.97%, GST by 16.31%, APX by 31.91% and GR by 25.75%, however 10 µM Se does not prove any significant impact on their activities. NaCl stressed seedlings exhibited an increase of 26.08, 25.24, 22.42, 57.65 and 36.09% in SOD, CAT, GST, APX and GR activities over the controls. Upon supplementation of salt stressed plants with Se (NaCl + 5 µM Se), further enhancement of 16.16% in SOD, 10.14% in CAT, 16.22% in GST, 10.63% in APX and 22.10% in GR was observed over the NaCl stressed plants. However, 10 µM Se application showed no significant effects (Figs. 4 and 5). Furthermore, salt-treated wheat seedlings exhibited a decline in AsA (20.37%) and increase in GSH (28.55%)

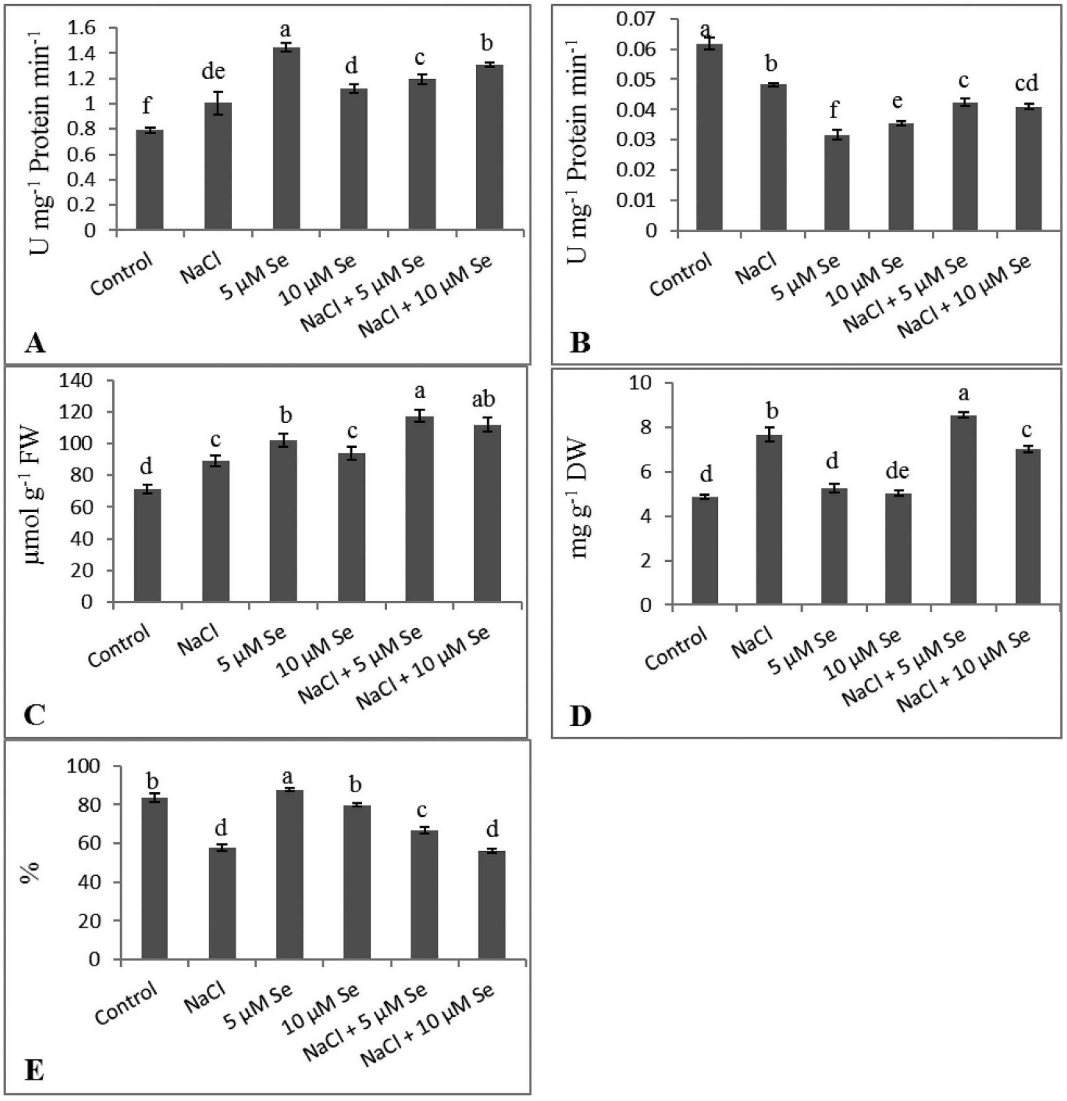

Fig. 2. Effect of salinity stress (100 mM NaCl) on activity of (A) γ-glutamyl kinase, (B) proline oxidase, (C) proline, (D) soluble sugars and (E) RWC in wheat (*Triticum aestivum* L) treated with selenium. Data presented is mean (± SE) of three replicates and bars with different letters denote significant difference at $P \leq 0.05$.

content over the control (Fig. 5C and D). Supplementation of 5 µM Se increased the accumulation of AsA and GSH by 9.22 and 29.47% respectively over the control. Supplementation of 5 µM Se increased the accumulation of AsA and GSH in salt stressed plants.

3.6. Se prevents Na toxicity by reducing its uptake and reduces Na/K ratio

Supplementation of Se reduced the uptake of Na in the upper parts (leaf) and significantly improved the uptake of other elements such as N, K and Ca (Table 2). Compared to control plants, N, K and Ca decreased by 31.60, 33.37 and 39.77% in leaf and 41.96, 29.09 and 39.18% in root tissues, respectively, due to NaCl treatment, however an increase of 60.54% (leaf) and 53.40% (root) was observed in the accumulation of Na. Relative to control plants, Se at 5 µM increased N (14.41%), K (11.45%) and Ca (15.99%) in leaves, and also resulted in amelioration of 14.29% for N, 13.92% for K and 1.36% for Ca in NaCl + 5 µM Se treated plants. In control as well as NaCl treated seedlings, 5 µM Se reduced Na/K ratio significantly. However, 10 µM Se application does not impart any significant positive effects on the mineral uptake (Table 2).

4. Discussion

Salinity has the potential to hamper the growth and development of plants. Increasing salt levels in agricultural soils mainly result from the agricultural malpractices including the increasing use of saline water for irrigations leading to the failure of most crops to survive and reducing the potential yield, therefore rendering threat on world food security. In the current study, we analysed the efficiency of Se supplementation in enhancing wheat growth through its involvement in the regulation of key physiological and biochemical attributes. Salinity restricts growth by hampering the cellular division and the cellular elongation through direct influences on the activity of transport proteins such as H^+-ATPase and H^+-PPase (Shi et al., 2007). Plants which have potential to efficiently transport the available Se from soil solution have greater tolerance potential to environmental extremes including pathogens, herbivores, and allelopathy (Lima et al., 2018). Application of Se at 5 µM proved beneficial in enhancing growth and photosynthesis in salinity-stressed wheat seedlings compared to the higher Se level (10 µM) Se which imparted no significant effect on growth and salt stress tolerance.

Khalid (2017) have demonstrated greater *synthesis of* chlorophyll pigments *Allium schoenoprasum* varieties due to the application of Se (6 mg^{-1} L) resulting in enhanced growth and biomass accumulation.

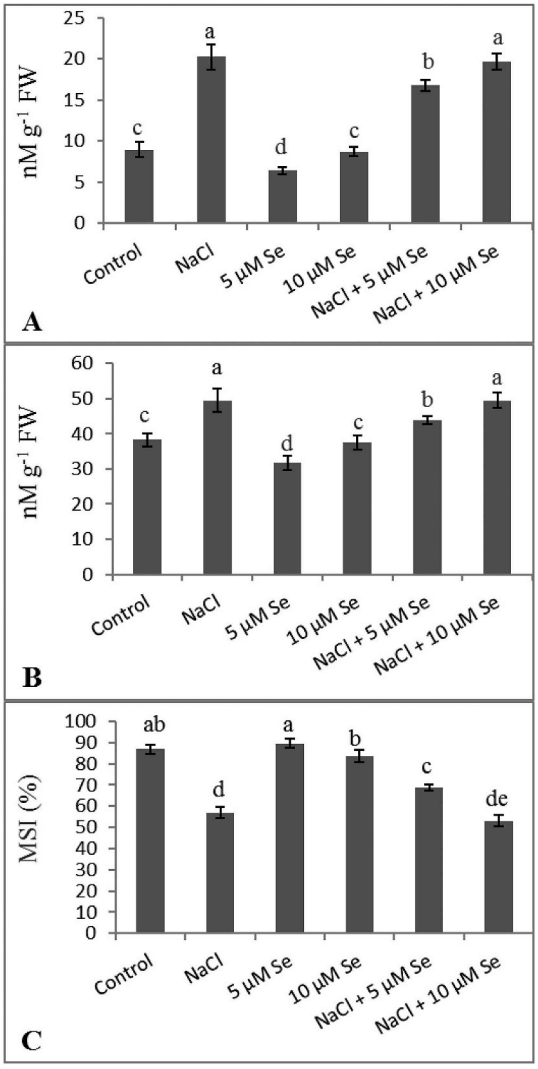

Fig. 3. Effect of salinity stress (100 mM NaCl) on (A) hydrogen peroxide (B) lipid peroxidation (MDA) and (C) membrane stability index (MSI) in wheat (*Triticum aestivum* L) treated with selenium. Data presented is mean (± SE) of three replicates and bars with different letters denote significant difference at $P \leq 0.05$.

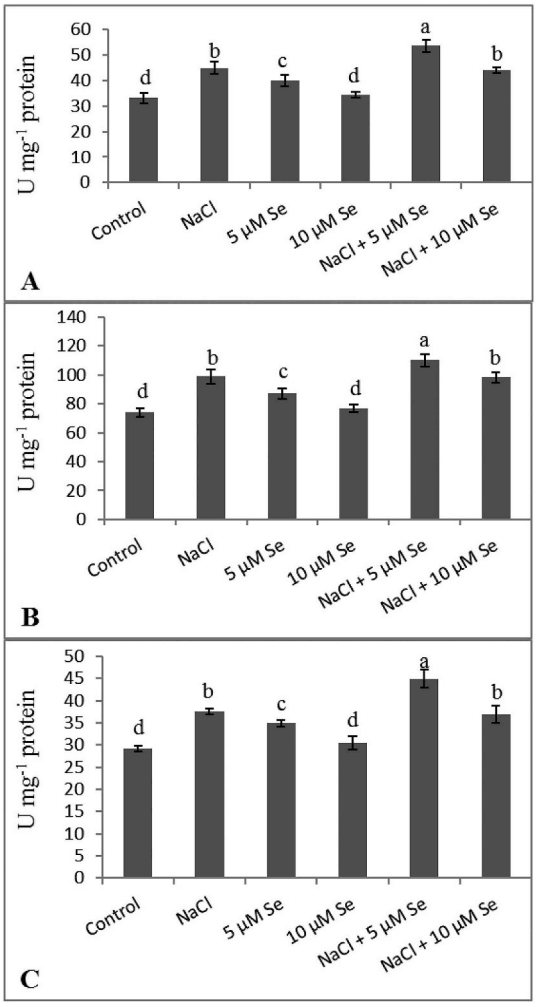

Fig. 4. Effect of salinity stress (100 mM NaCl) on activity of (A) superoxide dismutase (B) catalase and (C) glutathione S-transferase in wheat (*Triticum aestivum* L) treated with selenium. Data presented is mean (± SE) of three replicates and bars with different letters denote significant difference at $P \leq 0.05$.

However, at higher concentration (10 µM) Se showed slightly effects by imparting declines in growth and synthesis of chlorophyll pigments. Similar to our studies, Padmaja et al. (1989) have reported declined synthesis of chlorophyll due to inhibition of chlorophyll biosynthesizing enzymes and the synthesis of 5-aminolevulinic acid and protochlorophyllide. Application of Se at lower concentrations imparted beneficial impacts on the growth of plants by enhancing the photosynthetic attributes such as stomatal conductance and internal CO_2 concentration as well as its assimilation compared to the higher concentrations (4 and 6 mg L^{-1}) as previously demonstrated in cucumber (Haghighi et al., 2016). Wheat growth and chlorophyll synthesis were declined due to the treatment of NaCl and thus reflecting a significant decline in the photosynthetic rate. These results are in corroboration with the results of Ahanger and Agarwal (2017). Khan et al. (2014) have observed photosynthetic inhibition due to salinity stress in mungbean. It is believed that Se-mediated amelioration of photosynthetic inhibition is a cumulative effect on the defence mechanisms, resulting in quick elimination of ROS, uptake, and accumulation of key mineral nutrients (Alyemeni et al., 2018). Se (5 µM) application was advantageous in enhancing the chlorophyll and carotenoid pigments imparting a positive impact on the photosynthetic efficiency and stomatal conductance. Plant exposure to salinity stress restricts the synthesis of chlorophyll pigments and triggers alterations in the structure and function of pigment protein complex (Levitt, 1980). Excess of salt accumulation reduces the synthesis of pigments by reducing the activity of enzymes such as 5-aminolevulinic acid dehydratase, porphobilinogen deaminase, coproporphyrinogen III oxidase, porphyrinogen IX oxidase, Mg chelatase and protochlorophyllide oxidoreductase (Pattanayak and Tripathy, 2011), which might be accompanied by increased chlorophyllase activity (Santos 2004), reduced leaf water potential, nitrogen uptake and allocation for synthesis of Rubisco, hence reflecting reduced photosynthetic efficiency (Ahanger et al., 2017; Khan et al., 2014). The mechanisms underlying the Se-mediated regulation are largely unknown. Optimal supplementation of Se modulates photosynthetic functioning by enhancing the CO_2 assimilation, photosynthetic rate and chlorophyll fluorescence characteristics under normal and stressful conditions (Alyemeni et al., 2018).

Increased growth in Se (5 µM) supplemented seedlings was observed and exhibit a correlation with the restricted accumulation of toxic Na ion in the upper plant parts such as leaves, thereby preventing the intensity of NaCl-mediated generation of oxidative stress (Table 2). It may be postulated that appropriate Se supplementation may have improved the expression of tonoplast H^+ ATPase and Na^+/H^+ antiport at the root membranes restricting the partitioning of Na to upper tissues,

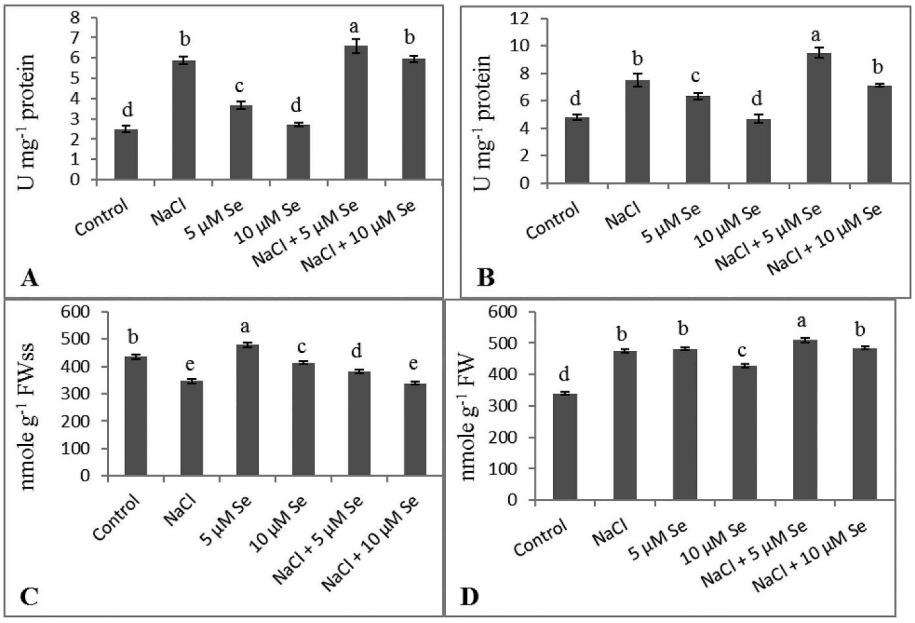

Fig. 5. Effect of salinity stress (100 mM NaCl) on activity of (A) ascorbate peroxidase, (B) glutathione reductase, and content of (C) ascorbic acid and (E) reduced glutathione in wheat (*Triticum aestivum* L) treated with selenium. Data presented is mean (± SE) of three replicates and bars with different letters denote significant difference at $P \leq 0.05$.

hence reducing its toxic effects (Zhang et al., 2006). Drahonovsky et al. (2016) have demonstrated differential increases in the uptake of K, Mg, Ca, S and Zn in different tree species treated with Se. Ions like N, K and Ca are involved in growth regulation through their influence on the key metabolic pathways such as nitrogen assimilation, antioxidant metabolism and cellular stress signalling (Ahanger et al., 2015, 2017; Ahmad et al., 2015). In the present study, Se supplementation enhanced N, K and Ca uptake which may have lead to greater production of amino acids, metabolites and stress signalling for better salt tolerance in wheat. Recently, it has been demonstrated that supplementation of Se (0, 4, 8 and 16 mg L^{-1}) improved K and reduced Na uptake significantly in *Allium sativum* (Astaneh et al., 2018). Se reduced Na/K ratio under NaCl stress leading to the maintenance of osmotic balance and protection of vital processes (Gupta and Gupta, 2016). Sodium opposes the active uptake of K at the membrane transport level, and Se supplementation may have influenced the expression of Na^+ transporters and H^+ pumps. Efficient partitioning and compartmentations of toxic ions including Na into vacuole have been considered as beneficial tolerance strategy for salt tolerance (Shabala and Pottosin, 2014). Se (5 µM) maintained lower Na/K ratio leading to better plant functioning under NaCl stress.

In the present study, NaCl stress has been reported to reduce the tissue water content and improve proline accumulation. These results are in consistent with Ahmad and Jhon (2005), Ahanger and Agarwal (2017) and Ma et al. (2016). However, it was interesting to observe that RWC increased with the treatment of Se (5 µM) compared to the control. Application of Se at 5 µM concentration regulated proline accumulation by enhancing the activity of γ-GK enzyme leading to the enhanced synthesis of proline with subsequent declines in its degradation via slowing down the activity of PROX. Recently, Asgher et al. (2018) have shown that improved proline accumulation due to ethylene treatment resulted from enhanced and reduced activities of γ-GK and PROX enzymes, respectively, under chromium stress. Increased RWC by application of Se was further strengthened by the increased accumulation of sugars content. Sufficient RWC prevents photosynthetic arrest, desiccation and the protein structure, reflecting greater plant productivity under extreme conditions (Munns and Tester, 2008). In halophytic grasses, Guo et al. (2015) have demonstrated that increased accumulation of proline leads to enhanced photosynthetic efficiency and ATP production, resulting in greater water use efficiency. Reports discussing roles of Se in osmolyte metabolism are very few. In the present study, application of Se at 5 µM effectively prevented photosynthetic arrest by maintaining proline, sugar and nitrogen contents which ultimately enhance protection of photosynthetic apparatus through maintenance of RWC and allocating significant nitrogen for synthesis of Rubisco. Osmolytes potentially protect the carboxylase activity of Rubisco (Sivakumar et al., 2000). Se-mediated increments in proline and sugar accumulation resulted in greater RWC and photosynthetic efficiency in wheat under saline stress. In addition, proline and sugars have been suggested to mediate ROS scavenging (Ahanger et al., 2017). Similar to our observations, Se-mediated enhancement in proline accumulation has been reported due to the higher and lower activities of γ-GK and PROX under cadmium stress (Khan et al., 2015).

NaCl stress increased the production of ROS such as H_2O_2, resulting in greater peroxidation of lipids and declining the membrane stability significantly, however supplementation of Se (5 µM) reduced H_2O_2 generation in NaCl treated seedlings. Recently, Ahanger et al. (2018) have reported salinity stress-mediated declines in membrane stability due to H_2O_2-mediated membrane peroxidation. Salinity stress up-regulates lipoxygenase activity (Heidari and Tafazoli, 2005) and reduces the poly unsaturated fatty acids significantly, leading to greater membrane instability (Alqarawi et al., 2014). Appropriate concentration of Se may have restricted the over-expression of lipoxygenase for maintaining the fatty acid composition in addition to the reduced ROS generation which was accompanied by up-regulation of antioxidant systems in the present study. Filek et al. (2008) and Hawrylak-Nowak (2013) have also demonstrated reduced generation of H_2O_2 due to Se supplementation. Recently, Alyemeni et al. (2018) have demonstrated a significant amelioration of cadmium-mediated lipid peroxidation and electrolyte leakage in tomato plants due to Se supplementation. Se potentially activated the antioxidant systems leading to reduced oxidative damage by eliminating ROS. Under salt stress, Se reduces oxidative damage to membranes by up-regulating the antioxidant system (KeLing et al., 2013). It was observed that application of Se at 5 µM up-regulated the antioxidant defence systems by increasing the activity of SOD, CAT, APX, GR and GST with significant improvements in the content of GSH and AsA. For avoiding the negative effects of ROS-induced oxidative damage, plants up-regulate the antioxidant defence system (Ahanger et al., 2018). Increases in the activities of SOD, CAT, GST, APX and GR due to salinity stress have been earlier reported in several crop plants such as wheat (Ahanger and Agarwal, 2017), *Brassica juncea* (Ahmad et al., 2015) and *Solanum lycopersicum* (Ahanger et al., 2018). The increased activity of SOD due to the application of Se (5 µM) resulted in fast conversions of the superoxide radicals generated

Table 2
Effect of selenium supplementation (5 and 10 μM) on nitrogen (N), sodium (Na), potassium (K), calcium (Ca) and Na/K ratio in leaf and root of wheat (*Triticum aestivum* L) seedlings treated with 100 mM NaCl. Data presented is mean (± SE) of three replicates and different letters indicate significant difference at $P < 0.05$.

	N		Na		K		Ca		Na/K	
	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root
Control	29.46 ± 2.30b	15.25 ± 1.10b	4.79 ± 0.66c	7.11 ± 0.48c	33.77 ± 1.43c	21.79 ± 1.22b	4.78 ± 0.49b	3.93 ± 0.25b	0.1418	0.3262
NaCl (100 mM)	20.15 ± 1.05e	8.854 ± 0.93d	12.14 ± 0.99a	15.26 ± 1.17a	22.50 ± 1.57e	15.45 ± 0.87d	2.88 ± 0.18d	2.39 ± 0.20c	0.5395	0.9877
5 μM Se	34.42 ± 1.22a	18.07 ± 1.01a	3.55 ± 0.436d	5.94 ± 0.31d	38.14 ± 1.52a	23.04 ± 1.18a	5.69 ± 0.49a	5.08 ± 0.41a	0.0930	0.2578
10 μM Se	25.51 ± 0.57c	13.10 ± 1.01c	4.33 ± 0.35c	6.50 ± 0.52c	35.61 ± 1.26 ab	21.56 ± 2.00b	3.96 ± 0.38c	3.75 ± 0.25b	0.1215	0.3014
NaCl + 5 μM Se	23.51 ± 1.05cd	9.77 ± 0.48d	9.32 ± 0.49b	13.75 ± 0.95b	26.14 ± 0.72d	17.92 ± 0.99c	2.92 ± 0.22d	2.38 ± 0.20c	0.3565	0.7672
NaCl + 10 μM Se	17.08 ± 0.55e	7.24 ± 0.31e	12.04 ± 0.87a	14.83 ± 0.75a	21.67 ± 1.61e	13.81 ± 0.98de	2.25 ± 0.28e	2.03 ± 0.06cd	0.5556	1.073

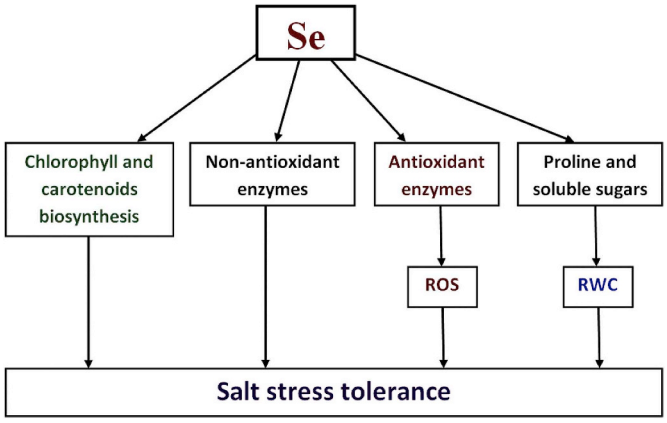

Fig. 6. Se model showing the regulation of salt stress tolerance.

at the chloroplast and mitochondrial electron transport chain into H_2O_2. Resulting H_2O_2 is neutralized either by CAT in the cytoplasm or by APX in the ascorbate-glutathione (AsA-GSH) pathway. Increased SOD activity in Se-supplied seedlings prevents the chances of hydroxyl (OH^-) radical formation (Alyemeni et al., 2018), resulting in greater protection of chloroplast functioning. Increased activities of antioxidant enzymes due to Se application have been reported by Kong et al. (2005), Nawaz et al. (2016) and Alyemeni et al. (2018) in sorrel, *Zea mays* and tomato, respectively. APX brings down the levels of H_2O_2, and GR mediates the maintenance of GSH and AsA content leading to balanced cellular redox (Foyer et al., 1994; Ahanger et al., 2017). In the present study, application of Se to wheat seedlings up-regulated AsA-GSH pathway by elevating the activities of APX and GR. In addition, increasing the AsA and GSH contents significantly resulted in the protection of photosynthetic electron transport chain by maintaining greater NADP levels and restricting the formation of toxic radicals. In particular, application of Se at low level potentially modulated osmolytes levels and induced the photosynthetic apparatus and enzymatic and non-enzymatic antioxidants, leading to reduced ROS levels and enhanced growth (Fig. 6). Research studies discussing the beneficial role of optimal Se levels for improved salt tolerance are rare, and further analysis for unraveling the actual mechanisms are still required.

5. Conclusion

The present study revealed that application of Se at low concentration imparts beneficial role in mitigating the deleterious effects of salt stress on the growth and biochemical attributes of wheat. NaCl stress potentially reduced the photosynthetic efficiency and negatively affected photosynthetic pigments contents. On the other hand, application of Se at low concentration mitigated salt stress effects, enhanced plant growth and up-regulated the antioxidants and osmolytes metabolism. Therefore, application of Se at lower levels could be suggested as an important strategy for improving wheat growth ad yield under NaCl stress.

Contributions

M.H.S, A.A.E, H.A and M.E designed the research and conducted the experiments. All authors collected and analyzed the data, wrote the final text and approved the final version of manuscript.

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