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# Selenium and its nanoparticles modulate the metabolism of reactive oxygen species and morpho-physiology of wheat (*Triticum aestivum* L.) to combat oxidative stress under water deficit conditions

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## Abstract

**Background** Wheat (*Triticum aestivum* L.) is one of the most important cereal crop species worldwide, but its growth and development are adversely influenced by drought stress. However, the application of trace elements is known to improve plant physiology under water-limited conditions. In this study, the effects of drought stress on wheat plants were investigated, with a focus on potential mitigation by foliar application of selenium nanoparticles (Se(np)) and sodium selenate (Na<sub>2</sub>SeO<sub>4</sub>). The experiment was conducted in a net house using a completely randomized design with four replications. The treatments involved three levels of drought stress (mild, moderate, and severe) started at 30 days after sowing (DAS), with foliar sprays of Se(np) and Se (both 25 μM) initiated at 27 DAS and repeated 4 times at 7-day intervals until 55 DAS.

**Results** Drought stress significantly reduced plant growth, whereas Se(np) and Se sprays enhanced it. Drought stress induced chlorophyll degradation, increased malondialdehyde and hydrogen peroxide levels, impaired membrane stability, and caused electrolyte leakage. Severe drought stress reduced the levels of antioxidants (e.g., proline, ascorbate, and glutathione by 4.18-fold, 80%, and 45%) and the activities of antioxidant enzymes (ascorbate peroxidase, dehydroascorbate reductase, and others). Conversely, treatment with Se(np) and Se restored these parameters, for example, 1.23-fold higher total chlorophyll content with Se(np) treatment, 26% higher APX activity with Se treatment, 15% lower electrolyte leakage with Se treatment in wheat plants under severe drought stress. This Se-associated enhancement facilitated rapid scavenging of reactive oxygen species and reduced methylglyoxal toxicity, thereby diminishing oxidative stress and positively affecting the morphophysiological and biochemical responses of the plants under drought.

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**Conclusions** Drought-stressed wheat plants exhibited reductions in physiological processes, including water uptake and photosynthetic activity. However, Se(np) and Se applied at 25  $\mu$ M mitigated the detrimental effects of drought. The application of Se(np) was notably more effective than the application of Se in mitigating drought stress, indicating the potential of the application of Se(np) as a sustainable agricultural practice under water-limited conditions.

**Keywords** Abiotic stress, Climate changes, Plant water relations, Oxidative stress, Nanoparticles, Trace elements

## Background

Global crop production is mostly threatened by water scarcity, and climatic variabilities signal that seasonal drought in crop fields will continue to be a major constraint limiting the future food and nutritional demands of the growing world population [1, 2]. Wheat (*Triticum aestivum* L.), which is grown globally on 220.76 million ha [3], is considered a staple crop worldwide, but wheat yields are severely limited by abiotic stresses, such as seasonal drought [4]. The Intergovernmental Panel on Climate Change (IPCC) emphasizes that more frequent and severe drought occurrences are anticipated as a result of climate change. This raises the threat to wheat production worldwide and more specifically in drought-prone areas. Wheat yield may be severely reduced by drought stress, particularly during critical growth stages for example flowering and grain filling stage. Yield losses of up to 50% or more are possible in cases of severe drought. The IPCC [5] projects that wheat yields would further fall due to the predicted 1–4 °C rise in global temperature by 2100. The limitations imposed by drought arise from biochemical changes that disrupt cellular homeostasis and trigger the overproduction of reactive oxygen species (ROS) in chloroplasts in response to stomatal closure and imbalances in photochemistry. ROS accumulation in plant cells causes lipid peroxidation and nucleic acid damage, inhibits enzyme activities, promotes electrolyte leakage, and dysregulates carbon metabolism [6].

Under nonstress conditions, ROS are effectively managed by intrinsic antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD); consequently, improvements in the activities of these enzymes during drought improve crop drought tolerance. Glyoxalase enzymes work alongside to negate the negative effects of overproduced methylglyoxal (MG) under drought stress. Studies have suggested that genotypes that show early and high activities of antioxidant defense compounds also show less hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) accumulation and lipid peroxidation, along with greater drought tolerance [7]. These findings suggest that the selection of plants with these traits would enable the breeding of drought-tolerant varieties; however, selective breeding of drought-tolerant wheat varieties is limited by the multifactorial nature of drought and variations in the intensity and duration of drought in the field, as well as the narrow genetic base and genome size of wheat

[8]. For these reasons, crop management approaches, such as the foliar application of selenium (Se), are gaining importance as on-time protocols for the management of drought and other abiotic stresses in different crops [9, 10]. In addition to providing timely drought management, these approaches also overcome the limitations of attempting to breed genotypes tailored to specific soil conditions.

The application of Se at low concentrations improves ROS scavenging in plant cells by enhancing intrinsic enzymatic and nonenzymatic antioxidant systems, thereby improving crop growth characteristics [10, 11]. However, recent studies have emphasized the benefits of using nanoparticles (NPs) as carriers of important metals, as NPs are readily taken up by plants and are able to cross cellular membranes. Foliar applications of different metal NPs have been shown to improve chlorophyll (Chl) contents, osmolyte levels, and antioxidant activities in different crops [10, 12]. Moreover, in addition to protecting against accumulated ROS, NP treatments also activate plant antioxidant defenses and induce the synthesis of protective secondary metabolites [13]. Notably, nanoparticles containing selenium (Se(np)) have been demonstrated to increase antioxidant levels and alter ROS signaling in plants [10, 11]. However, information is scarce regarding the potential benefits of Se and Se(np) application on the amelioration of drought effects or their influence on various physiological and biochemical parameters. Therefore, the aim of the current study was to assess the effects of foliar applications of Se and Se(np) on growth and antioxidant levels (both enzymatic and nonenzymatic) in wheat plants under drought conditions.

## Materials and methods

### Experimental setup, treatment, and design

Seeds of wheat (*Triticum aestivum* L.) cv. BARI Gom 30 were obtained from the Bangladesh Agricultural Research Institute (BARI) and healthy and uniformly sized seeds were selected for sterilization before sowing in plastic pots (14 L) filling up with sandy loam soils free of stubbles. Soil was air dried before preparation to kill weeds and insects. The experiment was carried out in a greenhouse with average day and night temperatures of 24.5 °C and 15.4 °C, respectively, and the relative humidity was approximately 52.5%. Following the BARI

recommendations [14], fertilizer was added during soil preparation, and 13.5 kg of soil was used to fill each pot.

Selenium nanoparticles were prepared following the method of El Lateef Gharib et al. [15], who used ascorbic acid as a reducing and stabilizing agent to reduce sodium selenate ( $\text{Na}_2\text{SeO}_4$ ). The reaction was initiated by slowly adding ascorbic acid powder (1.5% w/v) to a 10 mM aqueous  $\text{Na}_2\text{SeO}_4$  solution [16] and stirring at room temperature for 15 min. The reaction mixture was then left to stand until the solution turned light orange. The absorbance was measured using a spectrophotometer at 300 nm at 1 h and again every 15 min to confirm that no further changes occurred in the absorbance, indicating that the reaction was complete. When the color change was completed, the residue dried in a hot air oven at 200 °C for 72 h and then calcined at 450 °C in a Muffle furnace and preserved at 45 °C. The particles were characterized using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) which is presented in the Supplementary file (Fig. S1). The solution was diluted to 25  $\mu\text{M}$  with distilled water and used as the foliar Se(np) spray (pH 3.4).

Three levels of drought (50%, 25%, and 12.5% field capacity, denoted as mild [ $D_1$ ], moderate [ $D_2$ ], and severe [ $D_3$ ] drought, respectively) were maintained from 30 days after sowing (DAS) until physiological maturity. Field capacity was maintained by controlling the soil moisture based on the preliminary experiment and a soil moisture meter (Model no. WH0291) was used to measure the soil moisture level. The control plants were irrigated as needed. Spraying of sodium selenate ( $\text{Na}_2\text{SeO}_4$ ; Fuji-Film Wako Pure Chemical Corporation, Osaka, Japan) and Se(np) (both 25  $\mu\text{M}$ ) was initiated at 27 DAS, and repeated 4 times until 55 DAS at 7-day intervals. The study was conducted as a completely randomized design with four replications. Upon completion of the treatment, different morphological, physiological, and biochemical data were collected at 60 DAS.

#### Determination of plant height and biomass

Five plants were randomly selected from each treatment, and plant height was measured using a scale from the bottom of the plant to the tip of the longest leaf. The findings were averaged and are presented in centimeters (cm). The fresh weight (FW) of randomly selected plants from each set of treatments was determined. The entire plant was harvested, dust particles were removed, and the plant was carefully weighed using a digital balance. Each plant was air-dried and then transferred to an 80 °C oven to dry for 72 h, followed by dry weight (DW) measurements. The average FW and DW values are presented as  $\text{g plant}^{-1}$ .

#### Determination of chlorophyll levels

Chlorophyll content was estimated as per the method described by Arnon [17] by placing 0.25 g of chopped leaf tissue into 10 mL of 100% ethanol and boiling it in a water bath until the tissues turned white. Then spectrophotometric measurements were taken at 663, 645, and 470 nm and Chl *a*, Chl *b*, and Chl (*a*+*b*) pigments contents were calculated.

#### Determination of lipid peroxidation rates

The leaf malondialdehyde (MDA) concentrations were quantified according to Heath and Packer [18], using a thiobarbituric acid (TBA) reagent. Freshly harvested leaf tissue (approximately 0.5 g) was macerated with 3 mL of 5% (w/v) trichloroacetic acid (TCA) and centrifuged at  $11,500 \times g$ . A 1 mL volume of the supernatant was combined with TBA reagent (0.5% 4 mL TBA; 20% TCA) and incubated for 30 min at 95 °C in a water bath. After cooling, the absorbance at 532 nm was read, followed by a second reading at 600 nm for nonspecific values. After subtracting the nonspecific values, the final MDA content was determined utilizing an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  and expressed as  $\text{nmol g}^{-1} \text{ FW}$ .

#### Determination of hydrogen peroxide levels

The  $\text{H}_2\text{O}_2$  content was estimated by adding 3 mL of 5% TCA to freshly harvested leaf samples (approximately 0.5 g) and centrifuging at  $11,500 \times g$  at 4 °C. A 1 mL sample of the supernatant was combined with potassium iodide (1 mL) and potassium phosphate buffer (1 mL) (pH 7.0) and placed in the dark at room temperature for 1 h. The absorbance at 390 nm was then read, and the  $\text{H}_2\text{O}_2$  content was expressed as  $\text{nmol g}^{-1} \text{ FW}$  [19].

#### Quantification of electrolyte leakage

Electrolyte leakage (EL) was quantified as described by Dionisio-Sese and Tobita [20]. A freshly harvested leaf sample (0.5 g) was cut into small pieces, transferred to a Falcon tube containing 15 mL of  $\text{dH}_2\text{O}$ , and incubated in a 40 °C in a water bath for 60 min. After cooling the solution to room temperature, the electrical conductivity ( $\text{EC}_1$ ) was monitored using an EC meter. The Falcon tubes were then heated again at 121 °C, and a second EC reading ( $\text{EC}_2$ ) was taken after the cooling procedure. The EL was determined using the following formula:

$$\text{Electrolyte leakage \%} = \frac{\text{EC}_1}{\text{EC}_2} \times 100$$

#### Estimation of leaf proline content

The protocol described by Bates et al. [21] was used for proline (Pro) quantification. A leaf sample (approximately 0.5 g) was homogenized with an ice-cooled mortar and

pestle in 5 mL of 3% aqueous sulfosalicylic acid and centrifuged at  $11,500 \times g$  for 15 min at 4 °C. A 1 mL sample of the supernatant was then combined with 1 mL of ninhydrin reagent (glacial acetic acid and acid ninhydrin dissolved in 6 M phosphoric acid) and heated for 1 h in a 100 °C water bath. After the solution had cooled to room temperature, the Pro was extracted from the aqueous solution by adding 4 mL of toluene. The absorbance of the toluene layer was then read at 520 nm, and the Pro content was calculated against a standard curve of known Pro concentrations.

#### Measurement of ascorbate and glutathione pools

The protocol of Kampfenkel et al. [22] was used to quantify the content of the nonenzymatic antioxidants ascorbic acid (AsA) and glutathione (GSH) in freshly harvested leaf samples (0.5 g). The leaf samples were homogenized in 3 mL of 1 mM ethylenediaminetetraacetic acid (EDTA) in 5% meta-phosphoric acid and centrifuged at  $11,500 \times g$  at 4 °C. The AsA-GSH pool activities were estimated by neutralizing an aliquot with 0.5 M potassium phosphate buffer (pH 7.0) containing 0.1 M dithiothreitol (DTT) and adding  $dH_2O$  to determine the total AsA and reduced AsA contents. The neutralized aliquot was mixed with 100 mM potassium phosphate buffer (pH 6.5) and 0.5 units of ascorbate oxidase (AO), and the absorbance was read at 265 nm in a spectrophotometer. The amounts of total AsA and reduced AsA were determined by comparison to a standard curve of known AsA concentrations, and dehydroascorbate (DHA) amounts were quantified by subtracting the amounts of reduced AsA from the total AsA [23].

Another aliquot of 10  $\mu$ M supernatant was then neutralized with 0.5 M potassium phosphate buffer (pH 7.0), oxidized with 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB), and then reduced with nicotinamide adenine dinucleotide phosphate (NADPH) in the presence of glutathione reductase (GR). The GSH levels were determined by reading the absorbance at 412 nm. The oxidized glutathione (GSSG) levels were determined by neutralizing the extract with 2-vinylpyridine in potassium phosphate buffer. The contents of GSG and GSSG were estimated using a standard curve containing 12, 16, 20, and 24  $\mu$ g  $mL^{-1}$  GSH in 5% metaphosphoric acid, and then subtracting the values of GSSG from the total GSH to yield the GSH content [24].

#### Assays of antioxidant enzyme activities

A 0.5 g sample of fresh leaf tissue was harvested, homogenized in extraction buffer (1 mL) containing 50 mM potassium phosphate buffer (pH 7.0), 100 mM KCl, 5 mM  $\beta$ -mercaptoethanol, 1 mM L-ascorbic acid, and 10% (w/v) glycerol in an ice-cooled mortar and pestle, and then centrifuged at  $11,500 \times g$  for 15 min at 4 °C.

The protein content was determined using the Bradford method [25] by combining 5  $\mu$ L of supernatant with 5 mL of Bradford reagent and measuring the absorbance at 595 nm, followed by comparison to a standard curve constructed containing known concentrations of bovine serum albumin. The supernatant was used as the protein extract for all enzyme assays and was maintained at 4 °C. The ascorbate peroxidase (APX; EC: 1.11.1.11) activity was measured using the protocol of Nakano and Asada [26] by adding protein extract to a reaction mixture containing 15 mM potassium phosphate buffer (pH 7.0), 0.1 mM  $H_2O_2$ , 0.5 mM L-ascorbic acid, and 0.1 mM EDTA. The absorbance was then read at 290 nm, and an extinction coefficient ( $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ ) was used to estimate the APX enzyme activity.

The method of Hossain et al. [27] was used to determine the activity of monodehydroascorbate reductase (MDHAR, EC: 1.6.5.4). An aliquot of protein extract was added to the reaction mixture containing 50 mM Tris-HCl buffer (pH 7.5), 1 unit of AO, 0.2 mM NADPH, and 2.5 mM L-ascorbic acid. The absorbance was read at 340 nm, and the MDHAR activity was estimated using an extinction coefficient of  $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$ .

The method of Nakano and Asada [26] was followed to determine dehydroascorbate reductase (DHAR, EC:1.8.5.1) activity by adding protein extract to a reaction mixture containing EDTA, potassium phosphate buffer (pH 7.0), DHA, and GSH. The absorbance was read at 265 nm, and the activity of the DHAR was determined using an extinction coefficient of  $14 \text{ mM}^{-1} \text{ cm}^{-1}$ .

The activity of glutathione reductase (GR, EC:1.6.4.2) was estimated by adding protein extract to a reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.8), 1 mM oxidized GSSG, 0.2 mM NADPH, and 1 mM EDTA. The absorbance was read at 340 nm, and the activity was determined using an extinction coefficient of  $6.2 \text{ mM}^{-1} \text{ cm}^{-1}$  [24].

Catalase (CAT, EC: 1.11.1.6) activity was assessed by adding protein extract to a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0) and 15 mM  $H_2O_2$  and determining the decrease in absorbance at 240 nm, utilizing an extinction coefficient of  $39.4 \text{ M}^{-1} \text{ cm}^{-1}$  [24].

Glutathione peroxide (GPX, EC:1.11.1.9) activity was determined using the protocol of Nahar et al. [28] by adding 10  $\mu$ L of protein extract to a reaction mixture containing 1 mM EDTA, 1 mM sodium azide, 0.12 mM NADPH, 2 mM GSH, 1 unit of GR, potassium phosphate buffer (pH 7.0), and  $H_2O_2$ . The activity was determined by measuring the absorbance at 340 nm and using an extinction coefficient of  $6.62 \text{ mM}^{-1} \text{ cm}^{-1}$ .

The lipoxygenase (LOX, EC: 1.13.11.12) activity was determined using the protocol of Doderer et al. [29] by adding protein extract to a reaction mixture containing



sodium phosphate buffer (pH 6.5) containing Tween-20 and linolenic acid substrate. The activity was determined by reading the absorbance at 234 nm and using an extinction coefficient of  $25 \text{ mM}^{-1} \text{ cm}^{-1}$ .

The activity of glutathione-*S*-transferase (GST, EC: 2.5.1.18) was determined using the protocol of Hasanuzzaman et al. [24] by adding protein extract to a reaction mixture containing 0.25 M potassium phosphate buffer (pH 6.5), 1.5 mM GSH, and 1 mM 1-chloro-2,4-dinitrobenzene. The activity was determined by measuring the absorbance at 340 nm and using an extinction coefficient of  $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ .

The protocol of El-Shabrawi et al. [30] was used to determine the activity of superoxide dismutase (SOD, EC:1.15.1.1) by adding 5  $\mu\text{L}$  of protein extract to a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 2.24 mM nitro blue tetrazolium chloride, 2.36 mM xanthine, and xanthine oxidase. The activity was determined by measuring the absorbance at 560 nm.

The activity of peroxidase (POD, EC: 1.11.1.7) was estimated using the protocol of Hemeda et al. [31] by adding protein extract to a reaction mixture containing 1.5 mM guaiacol as the organic substrate, 0.5 M potassium phosphate buffer (pH 7.0), and 30 mM  $\text{H}_2\text{O}_2$ . The activity was determined by measuring the absorbance at 470 nm with a spectrophotometer and utilizing an extinction coefficient of  $26.6 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### Estimation of methylglyoxal content and glyoxalase enzyme activities

A 250 mg leaf sample was extracted using 5% perchloric acid and centrifuged at  $11,500 \times g$  for 12 min at  $4^\circ\text{C}$  [32]. Activated charcoal was added, the sample was centrifuged again, and the supernatant was neutralized with saturated sodium carbonate solution. The neutralized sample was mixed with sodium phosphate buffer (pH 7.0) containing 0.5 M *N*-acetyl-L-cysteine and reacted for 15 min. The absorbance was measured at 288 nm the methylglyoxal (MG) content was determined by comparison to a standard curve of known MG concentrations. The activity of glyoxalase I (Gly I, EC: 4.4.1.5) was assayed following the protocol of Hasanuzzaman et al. [24] by adding the protein extract to a reaction mixture containing 100 mM potassium phosphate buffer (pH 7.0), 16 mM magnesium sulfate, 35 mM MG, and 100 mM GSH. The enzyme activity was determined by measuring the absorbance at 240 nm and using an extinction coefficient of  $3.37 \text{ mM}^{-1} \text{ cm}^{-1}$ . Similarly, glyoxalase II (Gly II, EC: 3.1.2.6) activity was assayed by adding the protein extract to a reaction mixture containing 100 mM Tris-HCl buffer (pH 7.2), 0.2 mM DTNB, and 1 mM *S*-D-lactoylglutathione. The activity was determined by measuring the absorbance at 412 nm and using an extinction coefficient of  $13.6 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### Statistical analysis

CoStat v.6.400 (2008) software was used for the analysis of variance (ANOVA) of the data obtained from the measured parameters. The mean separation was then compared using Tukey's honest significant difference (HSD) test at the 5% level of significance.

## Results

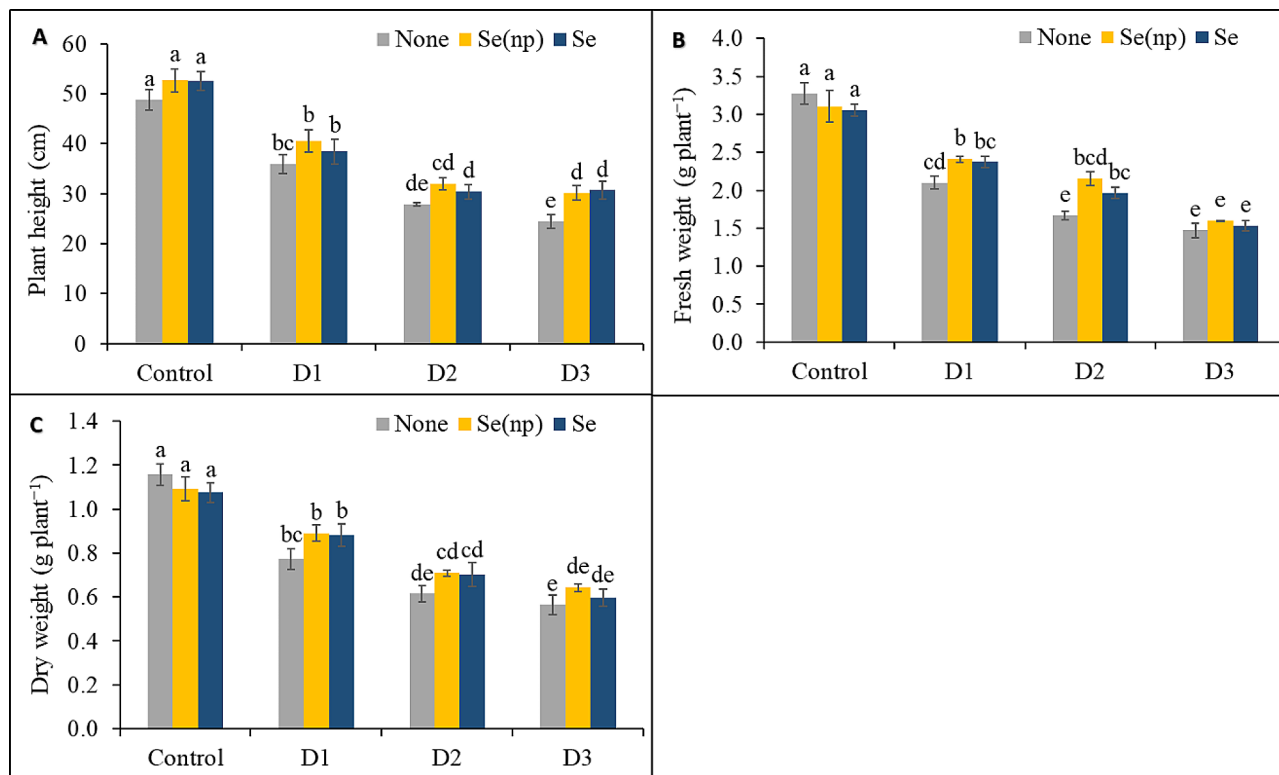
### Foliar Se(np) and Se application improved wheat growth under drought

Drought significantly impaired the growth of wheat, as indicated by decreases of 50%, 43%, and 26% in plant height in the  $D_3$ ,  $D_2$ , and  $D_1$  drought treatments, respectively, compared to those in the unsprayed, unstressed (henceforth untreated) controls (Fig. 1A). However, the Se(np) and Se treatments negated these drought effects, and plant height increased by 23% in response to the Se(np) treatment and by 26% in response to the Se treatment compared to the unsprayed, severe ( $D_3$ ) drought-stressed controls. Similarly, compared to the untreated controls (Fig. 1B), plants subjected to the  $D_3$ ,  $D_2$ , and  $D_1$  drought regimens also showed reduced fresh weight, with reductions of 55%, 49%, and 36%, respectively. Foliar spraying with Se(np) and Se significantly improved tissue water retention, and the fresh weights of plants in the  $D_1$  and  $D_2$  drought conditions were 15% and 29% greater after Se(np) treatment and 13% and 18% greater after Se treatment, respectively, compared to the respective untreated controls.

Drought stress also significantly decreased wheat dry weight, with reductions of 33%, 47%, and 51% in the  $D_1$ ,  $D_2$ , and  $D_3$  drought regimes, respectively (Fig. 1C). Dry weights were statistically similar to those of the unsprayed drought-stressed controls after foliar spraying of Se(np) and Se under all three drought conditions, although spraying tended to reduce the negative effects of drought. Under moderate drought conditions, the sprayed wheat plants attained a dry weight similar to that of the unsprayed controls under mild drought conditions. Similarly, under severe drought conditions, the dry weight of sprayed wheat plants was nearly equal to that of unsprayed plants under moderate drought conditions.

### Foliar Se(np) and Se application reduced drought-induced chlorophyll degradation

The Chl *a*, Chl *b*, and total Chl contents of the wheat plants decreased significantly in response to drought. The Chl *a* content was reduced by 1.38-fold, 1.67-fold, and 2.79-fold under the  $D_1$ ,  $D_2$ , and  $D_3$  drought regimes, respectively, compared to the untreated controls (Fig. 2A). However, the plants subjected to foliar spraying of Se(np) and Se application showed greater Chl *a* content under drought conditions, with Se(np)-sprayed plants showing up to 1.28-fold greater Chl levels in  $D_1$ ,



**Fig. 1** Wheat plant height (A), fresh weight (B) and dry weight (C) as influenced by foliar spraying of Se(np) and Se under different water regimes [Control=well-watered; D<sub>1</sub>=50% field capacity (mild drought); D<sub>2</sub>=25% field capacity (moderate drought); D<sub>3</sub>=12.5% field capacity (severe drought)]. The values are the means  $\pm$  SEMs. The means were compared by Tukey's honestly significant difference test at  $p=0.05$ . Bars with the same letter do not differ significantly from each other

1.48-fold greater Chl levels in D<sub>2</sub>, and 1.36-fold greater Chl levels in D<sub>3</sub> compared to the respective unsprayed drought-stressed control plants. Similarly, plants sprayed with Se had 1.19-fold and 1.25-fold greater Chl contents under D<sub>1</sub> and D<sub>2</sub>, respectively, compared to the respective unsprayed drought-stressed controls.

The drought-induced reductions in Chl *b* were 1.38-fold, 1.61-fold, and 1.67-fold greater under D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>, respectively, compared to the untreated controls (Fig. 2B). Plants sprayed with Se(np) showed 1.21-fold and 1.34-fold higher Chl *b* levels under the D<sub>1</sub> and D<sub>2</sub> conditions, respectively, compared to the unsprayed drought-treated controls. Plants treated with Se also showed Chl *b* levels that were 1.26-fold and 1.22-fold higher in the D<sub>1</sub> and D<sub>2</sub> treatment groups, respectively, than in the respective unsprayed drought-stressed plants.

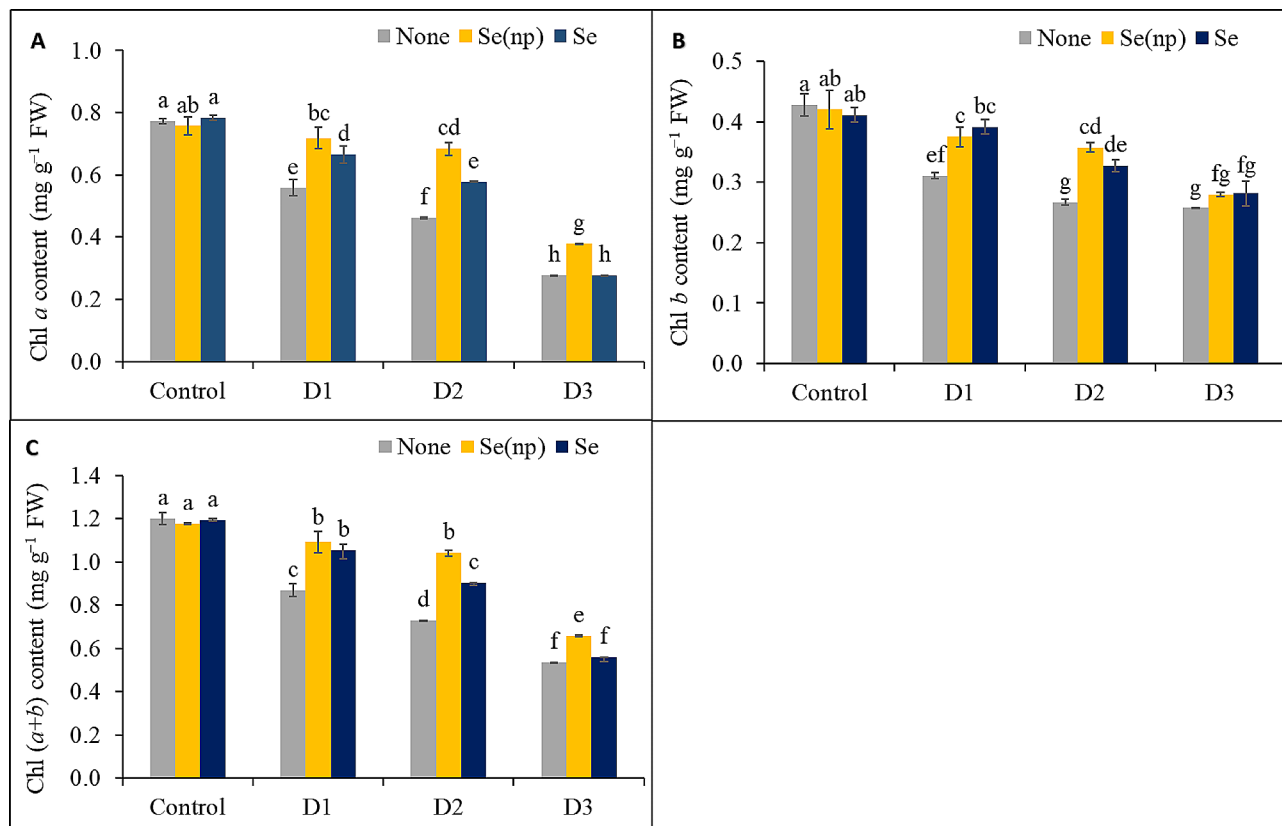
The decreases in Chl *a* and Chl *b* were also reflected in the total Chl content, which declined by 1.38-fold, 1.69-fold, and 2.25-fold, respectively, in unsprayed plants grown under the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> conditions (Fig. 2C). Plants sprayed with Se(np) showed 1.26-fold, 1.43-fold, and 1.23-fold higher total Chl levels in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> treatment groups than in the unsprayed drought-stressed control groups, respectively. Plants sprayed with Se showed 1.21- and 1.24-times higher total Chl levels

under D<sub>1</sub> and D<sub>2</sub>, respectively, compared to the respective untreated controls.

#### Foliar Se(np) and Se application reduced oxidative stress and protected membrane integrity

Drought stress significantly increased oxidative stress in wheat leaves, as indicated by increases in the MDA and H<sub>2</sub>O<sub>2</sub> contents. Compared with the untreated controls, the plants in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> treatment groups showed increases in MDA levels of 1.59-fold, 2.17-fold, and 2.77-fold, respectively (Fig. 3A). Similarly, the H<sub>2</sub>O<sub>2</sub> content was 1.5-fold, 2.02-fold, and 2.81-fold greater in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> treatment groups, respectively than in the untreated control groups (Fig. 3B). Plants sprayed with Se(np) showed reduced oxidative damage during drought stress, as indicated by lower MDA and H<sub>2</sub>O<sub>2</sub> contents under the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> water regimes compared to the untreated control plants. By contrast, plants sprayed with Se showed reduced MDA levels under the D<sub>1</sub> and D<sub>2</sub> conditions and lower H<sub>2</sub>O<sub>2</sub> contents under D<sub>2</sub> conditions when compared with the respective unsprayed drought-stressed plants.

Drought stress also impaired plant membrane stability, as indicated by 22% and 26% increases in EL under the D<sub>1</sub>, and D<sub>3</sub> water regimes, respectively, compared to



**Fig. 2** The chlorophyll *a* content (**A**), chlorophyll *b* content (**B**) and total chlorophyll content (**C**) of wheat influenced by foliar spraying of Se(np) and Se under different water regimes [Control=well-watered; D<sub>1</sub>=50% field capacity (mild drought); D<sub>2</sub>=25% field capacity (moderate drought); D<sub>3</sub>=12.5% field capacity (severe drought)]. The values are the means ± SEMs. The means were compared by Tukey's honestly significant difference test at  $p=0.05$ . Bars with the same letter do not differ significantly from each other

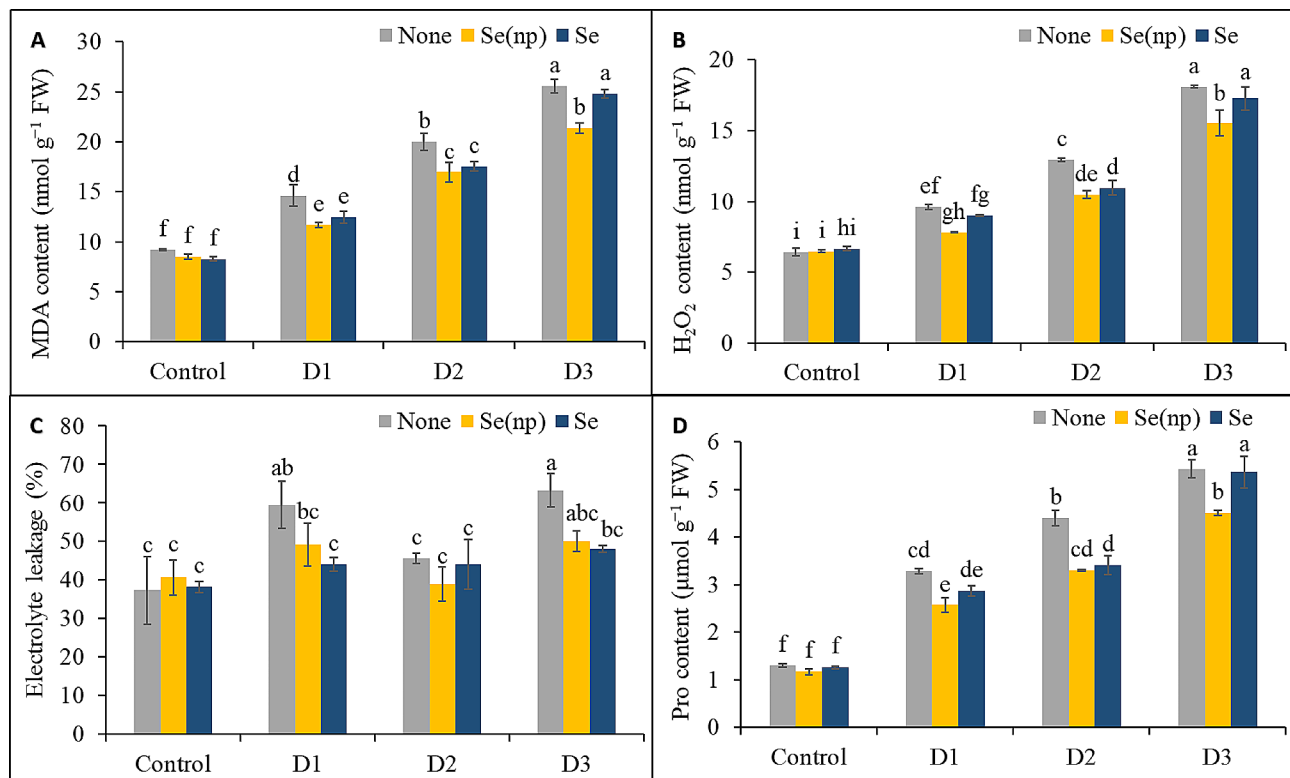
the untreated controls (Fig. 3C). However, plants treated with Se under drought conditions exhibited greater membrane stability, with lower electrolyte leakage in the D<sub>1</sub> (15%) and D<sub>3</sub> (15%) treatment groups only, respectively, than in the respective unsprayed drought-stressed control plants. On the contrary, the plants sprayed with Se(np) did not show significant changes in EL% when subjected to drought stress.

#### Foliar Se(np) and Se application altered the leaf proline content

The proline levels showed a notable increase under drought conditions, which was increased 2.52-fold, 3.39-fold, and 4.18-fold in the plants treated with D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> drought stress, respectively, compared to those in the untreated controls (Fig. 3D). Plants treated with Se(np) showed lower accumulation of proline under the D<sub>1</sub> (22%), D<sub>2</sub> (25%), and D<sub>3</sub> (17%) water regimes, respectively, than did their respective unsprayed drought-stressed controls. Plants treated with Se showed a 22% lower proline content in the D<sub>2</sub> treatment group than in the respective unsprayed drought-stressed control group.

#### Foliar Se(np) and Se application enhanced ascorbate–glutathione cycle activity

Drought stress enhanced the conversion of AsA to DHA, as plants showed 51%, 82%, and 92% lower AsA/DHA ratios under the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> conditions, respectively, than did the untreated controls (Fig. 4E), whereas the DHA contents were increased by 21%, 106%, and 163%, and the AsA contents were decreased by 41%, 64%, and 80%, respectively, under the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> water regimes, compared to the untreated controls (Fig. 4A). Plants sprayed with Se(np) showed 33%, 92%, and 49% higher AsA levels and 16%, 20%, and 23% lower DHA levels under D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>, respectively (Fig. 4A, C), as well as 58% and 140% higher AsA/DHA ratios under D<sub>1</sub> and D<sub>2</sub>, respectively, compared to their respective unsprayed drought-stressed controls (Fig. 4E). Plants sprayed with Se had 28% and 56% greater AsA contents, 17% and 16% lower DHA contents, and 54% and 85% greater AsA/DHA ratios under D<sub>1</sub> and D<sub>2</sub> conditions, respectively, than the respective unsprayed drought-stressed controls. Spraying of Se(np) or Se under the D<sub>3</sub> condition did not result in noticeable changes in the AsA/DHA ratio.



**Fig. 3** The MDA content (A), H<sub>2</sub>O<sub>2</sub> content (B), electrolyte leakage (C) and proline content (D) of wheat influenced by foliar spraying of Se(np) and Se under different water regimes [Control=well-watered; D<sub>1</sub>=50% field capacity (mild drought); D<sub>2</sub>=25% field capacity (moderate drought); D<sub>3</sub>=12.5% field capacity (severe drought)]. The values are the means  $\pm$  SEMs. The means were compared by Tukey's honestly significant difference test at  $p=0.05$ . Bars with the same letter do not differ significantly from each other

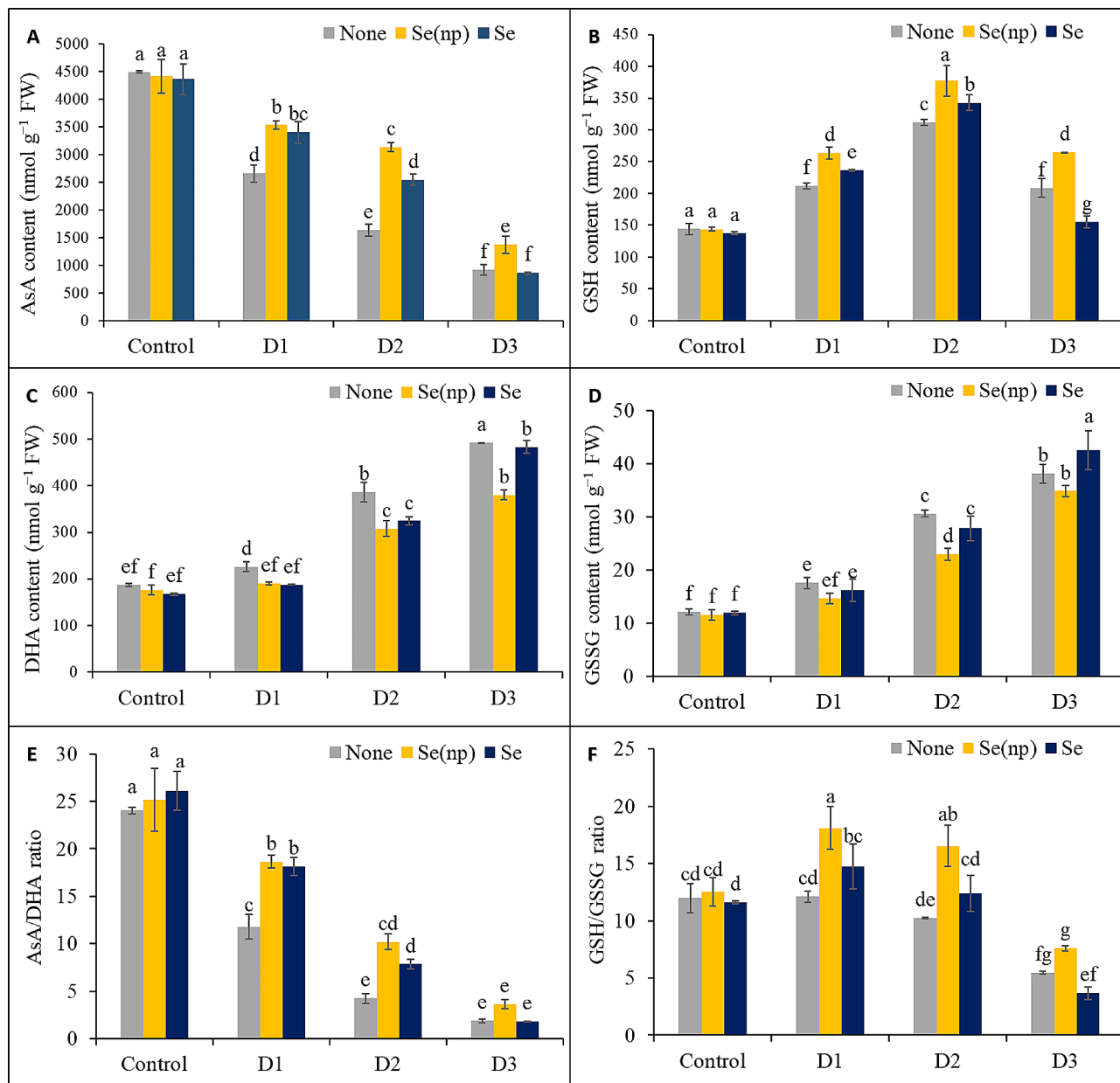
Plants exposed to the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> water regimes showed 47%, 116%, and 45% higher levels of GSH and 45%, 153%, and 215% higher levels of GSSG, respectively, compared to the untreated controls (Fig. 4B, D). The ratio of GSH/GSSG was notably reduced by 54% under the D<sub>3</sub> water regime compared to the untreated controls (Fig. 4F). Plants treated with Se(np) showed 25%, 21%, and 27% greater GSH contents under D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub>, respectively, as well as a 25% lower GSSG content under D<sub>2</sub> compared to the respective unsprayed drought-stressed controls. The plants sprayed with Se(np) also had 50% and 62% greater GSH/GSSG ratios under D<sub>1</sub> and D<sub>2</sub>, respectively, than the unsprayed drought-stressed controls. Moreover, plants sprayed with Se showed 26% lower GSH levels under D<sub>3</sub> conditions but 12% and 10% greater GSH levels under D<sub>1</sub> and D<sub>2</sub> conditions, respectively, than the drought-stressed controls. Plants sprayed with Se also had 12% greater GSSG content in the D<sub>3</sub> treatment group than in the respective unsprayed drought-stressed control group. However, the ratio of GSH/GSSG did not change notably in the Se-sprayed plants in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> drought-stressed groups.

#### Foliar Se(np) and Se applications upregulate antioxidant enzymes

Unsprayed plants showed 17%, 41%, and 51% lower APX activity (Fig. 5A), 55%, 64%, and 70% lower DHAR activity (Fig. 5B), and 41%, 54%, and 64% lower MDHAR activity (Fig. 5C) under the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> conditions, respectively, than their respective untreated controls. Compared to the untreated controls, the unsprayed drought-stressed plants also exhibited 45% lower GR activity under the D<sub>1</sub> condition but 57% and 112% greater GR activity under the D<sub>2</sub> and D<sub>3</sub> conditions, respectively (Fig. 5D).

Plants sprayed with Se(np) under the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> water regimes showed a notable increase in APX activity (by 36%, 32%, and 14%, respectively), DHAR activity (by 71%, 66%, and 36%, respectively), and MDHAR activity (by 36%, 51%, and 50%, respectively) compared to the respective unsprayed drought-stressed controls, whereas GR activity was increased by 68% and 26% in the D<sub>1</sub> and D<sub>2</sub> conditions compared to the respective unsprayed drought-stressed controls. Plants sprayed with Se showed 19% and 26% greater APX activity, 27% and 34% greater DHAR activity, 29% and 26% greater MDHAR activity, and 41% and 18% greater GR activity under D<sub>1</sub> and D<sub>2</sub>, respectively, compared to the respective unsprayed



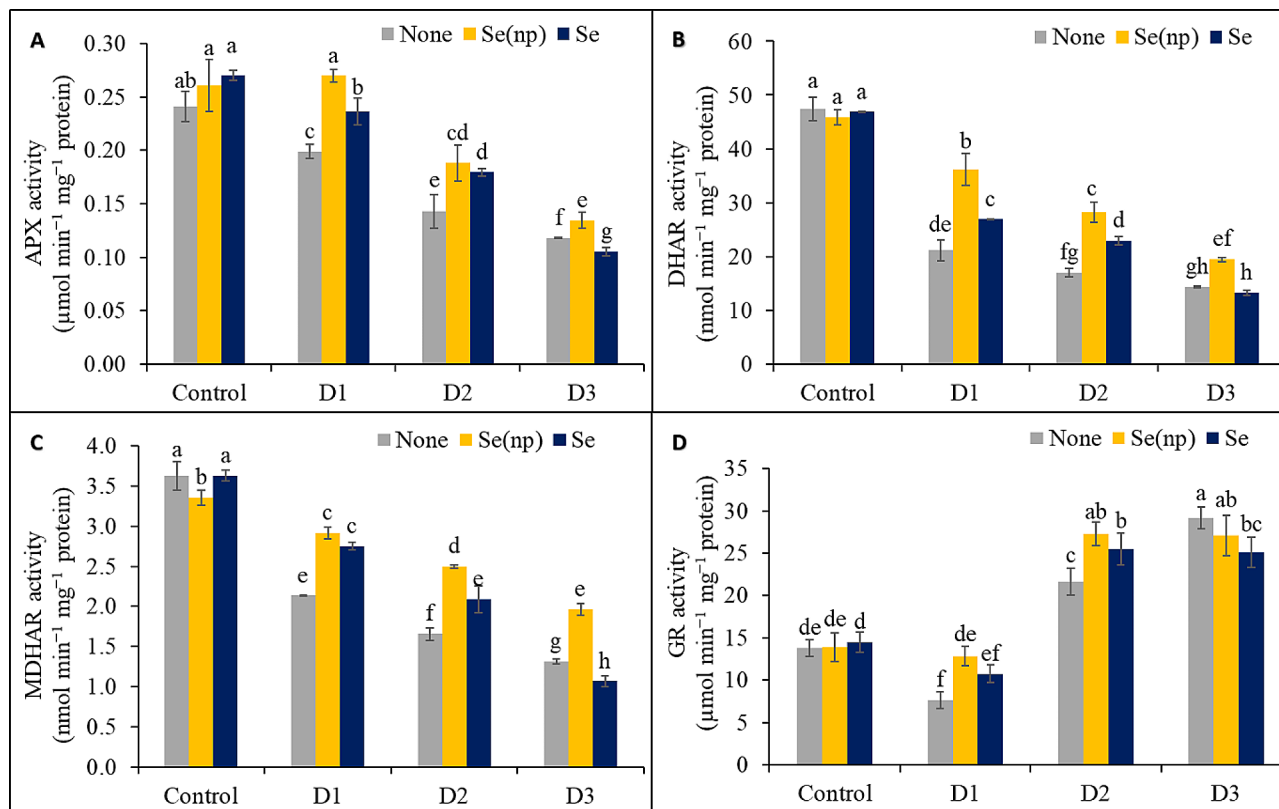


**Fig. 4** The AsA content (A), GSH content (B), DHA content (C), GSSG content (D), AsA/DHA ratio (E) and GSH/GSSG ratio (F) of wheat as influenced by foliar spraying of Se(np) and Se under different water regimes [Control=well-watered; D<sub>1</sub>=50% field capacity (mild drought); D<sub>2</sub>=25% field capacity (moderate drought); D<sub>3</sub>=12.5% field capacity (severe drought)]. The values are the means ± SEMs. The means were compared by Tukey's honestly significant difference test at *p* = 0.05. Bars with the same letter do not differ significantly from each other

drought-stressed controls. Notably, under D<sub>3</sub> conditions, plants sprayed with Se had 11% lower APX activity, 19% lower MDHAR activity, and 14% lower GR activity than the respective unsprayed drought-stressed controls.

The activity of CAT was not significantly influenced by the different drought regimes (Fig. 6A). Drought decreased GPX activity by 31%, 47%, and 57% (Fig. 6B), and SOD activity by 34%, 42%, and 60% (Fig. 6E), respectively, in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> water regimes compared to the unsprayed drought-stressed controls. On the

contrary, the LOX activity was increased by 1.79-fold, 2.37-fold, and 2.78-fold, the GST activity by 1.42-fold, 1.95-fold, and 1.75-fold, and POD activity by 1.48-fold, 1.7-fold, and 2.28-fold, respectively, in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> conditions than in the untreated controls (Fig. 6C and D, and 6F). Spraying of Se(np) increased the activities of GPX, SOD, and POD in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> treatment groups and the GST activity in the D<sub>1</sub> and D<sub>2</sub> conditions, whereas the activity of LOX was decreased in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> treatment groups compared to the drought stress



**Fig. 5** The APX activity (A), DHAR activity (B), MDHAR activity (C) and GR activity (D) of wheat as influenced by foliar spraying of Se(np) and Se under different water regimes [Control = well-watered; D<sub>1</sub> = 50% field capacity (mild drought); D<sub>2</sub> = 25% field capacity (moderate drought); D<sub>3</sub> = 12.5% field capacity (severe drought)]. The values are the means  $\pm$  SEMs. The means were compared by Tukey's honestly significant difference test at  $p=0.05$ . Bars with the same letter do not differ significantly from each other

alone. Moreover, Se treatment increased the GPX, GST, SOD, and POD activities under mild (D<sub>1</sub>) and moderate (D<sub>2</sub>) drought stress, and reduced the LOX activity in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> conditions compared to the unsprayed drought-stressed plants.

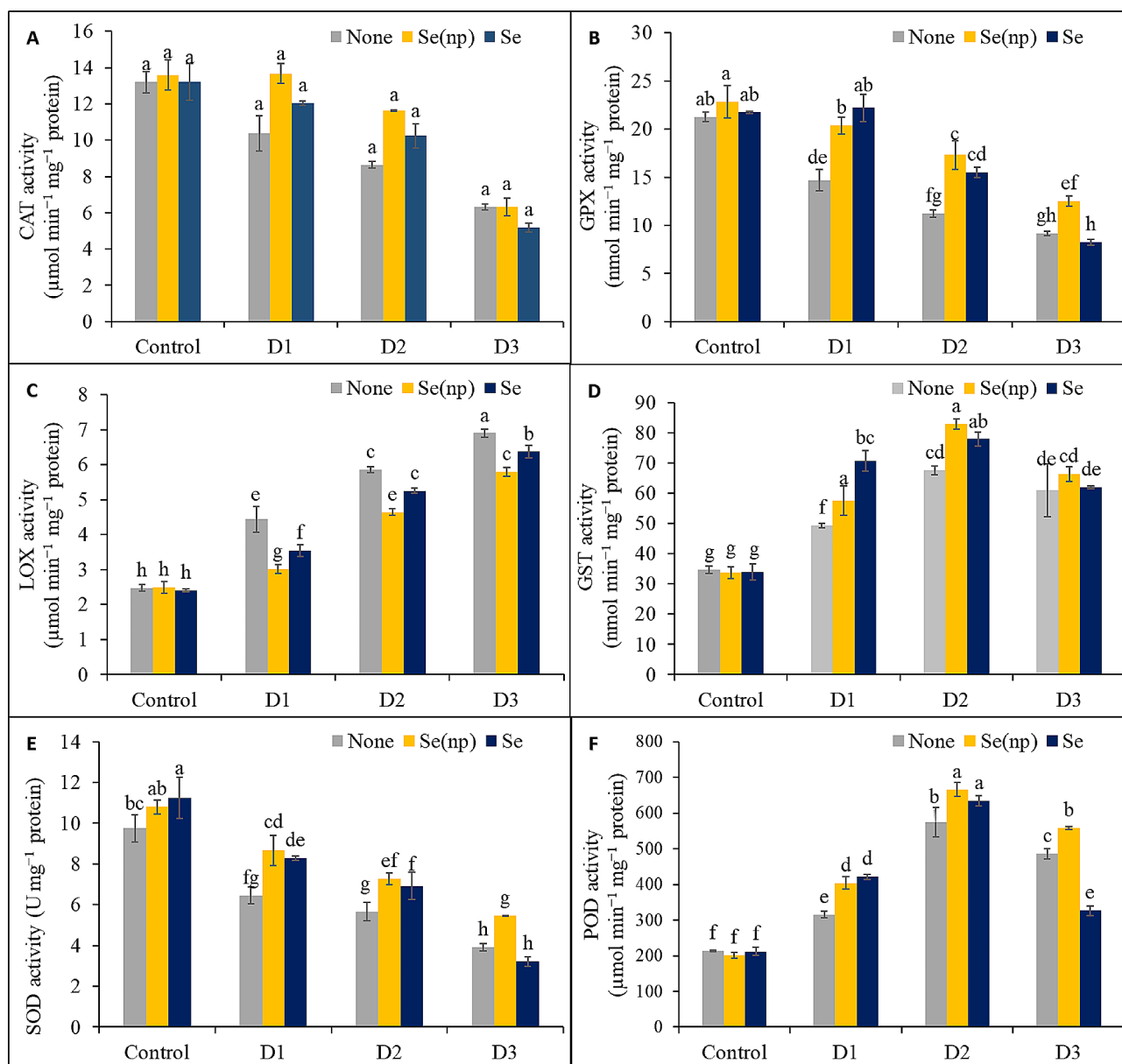
#### Foliar Se(np) and Se application altered glyoxalase system activity

Plants exposed to drought showed 1.77-fold, 2.16-fold, and 2.73-fold higher MG contents (Fig. 7A), but 1.76-fold, 2.17-fold, and 3.23-fold lower activities of Gly I (Fig. 7B) and 1.49-fold, 1.76-fold, and 2.14-fold lower activities of Gly II (Fig. 7C) in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> conditions, respectively, compared to the untreated controls. However, spraying with Se(np) under the D<sub>1</sub> and D<sub>2</sub> conditions increased the Gly I and Gly II activities, but reduced the MG levels in the D<sub>1</sub>, D<sub>2</sub>, and D<sub>3</sub> conditions compared to the unsprayed drought-stressed plants. Plants sprayed with Se also showed a similar trend of reduction of MG levels under the D<sub>1</sub> and D<sub>2</sub> treatment, increased Gly I activity in the D<sub>1</sub> condition, and increased Gly II activity in the D<sub>1</sub> and D<sub>2</sub> conditions compared to the respective unsprayed drought-stressed controls.

#### Discussion

Plant growth processes are negatively impacted by water deficit conditions or drought stress due to the resulting disruptions in cytosolic metabolism, reductions in assimilate production, suppression of vegetative growth and development, and decreases in productivity. Drought stress reduces the photosynthesis rate in several ways—by altering the levels of photosynthetic pigments in leaves, decreasing leaf area, reducing stomatal conductivity, enhancing lipid peroxidation in cell membranes, and suppressing protein and Chl synthesis—with deleterious effects on plant growth [33]. In the present study, the growth of wheat plants was clearly decreased under drought conditions. Similar decreases in plant biomass (dry weight and fresh weight) have been previously reported in many plants, including *Salvia officinalis* L. by Ostadi et al. [34] and *Lallemantia iberica* by Javanmard et al. [35], when grown under mild and moderate drought stress. These declines in plant growth attributes have increased interest in the use of chemical elicitors and nanotechnology to improve plant growth and development under abiotic stresses, such as water stress.

In the present study, foliar applications of Se and Se(np) clearly mitigated the negative effects of drought stress

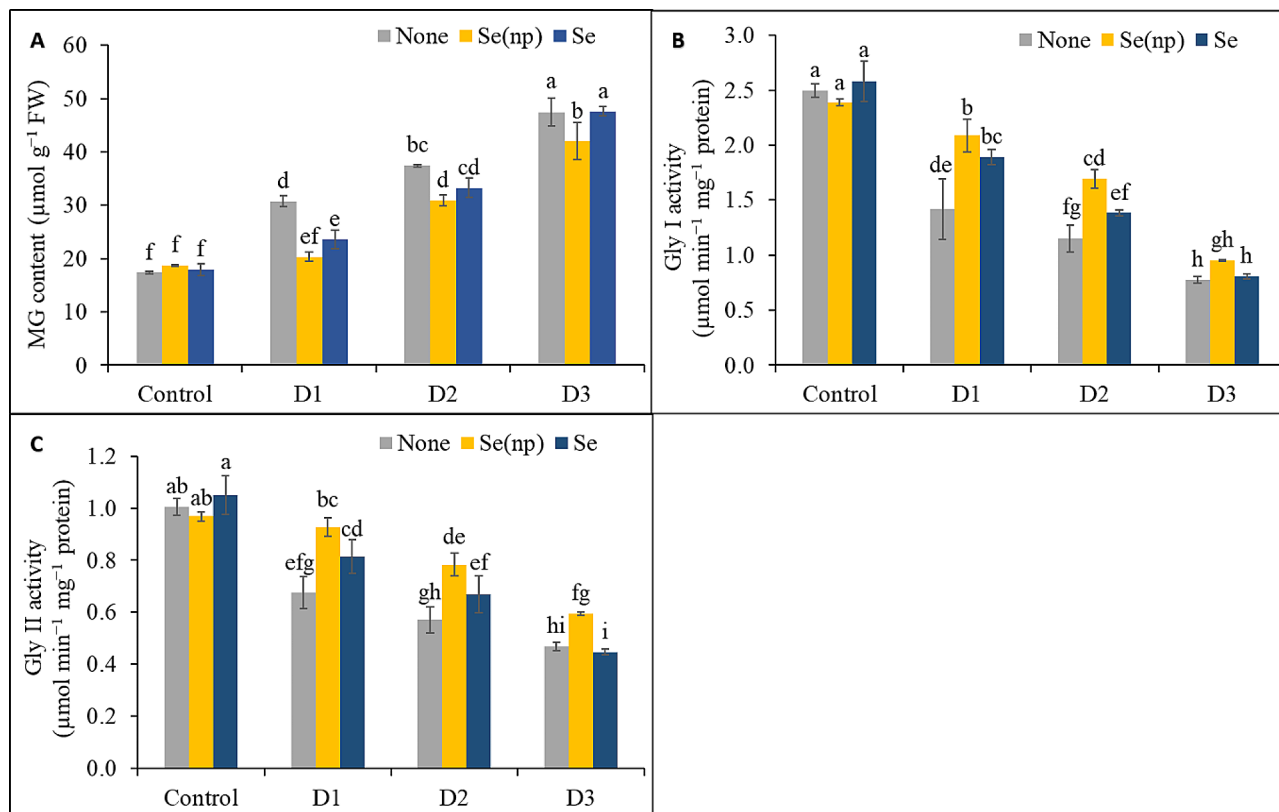


**Fig. 6** The CAT activity (A), GPX activity (B), LOX activity (C), GST activity (D), SOD activity (E) and POD activity (F) of wheat as influenced by foliar spraying of Se(np) and Se under different water regimes [Control=well-watered; D<sub>1</sub>=50% field capacity (mild drought); D<sub>2</sub>=25% field capacity (moderate drought); D<sub>3</sub>=12.5% field capacity (severe drought)]. The values are the means  $\pm$  SEMs. The means were compared by Tukey's honestly significant difference test at  $p=0.05$ . Bars with the same letter do not differ significantly from each other

on wheat growth and development. Several studies have demonstrated positive effects of Se on growth parameters and the enhancement of dry matter accumulation, plant height, and leaf area in drought-affected plants. A previous study also revealed improved plant growth under drought in wheat seedlings of two different genotypes after treatment with a foliar Se spray [36]. Overall, the use of Se and Se(np) appears to accelerate plant root growth, thereby enhancing nutrient absorption from the soil and improving the rates of photosynthesis, with a

consequent suppression of the adverse effects of stress on plant growth characteristics [37].

In the present study, different levels of drought stress significantly reduced the Chl pigment content of wheat leaves. This reduction enhanced ROS generation and subsequent membrane damage, eventually leading to the further destruction of leaf Chl pigments [38]. A reduction in photoassimilate production would also limit the energy needed for the absorption of nutrients (e.g., Mg) from the soil, which would also affect Chl biosynthesis [39]. However, foliar sprays of Se and Se(np) restored Chl pigment



**Fig. 7** The MG content (A), Gly I activity (B) and Gly II activity (C) of wheat as influenced by foliar spraying of Se(np) and Se under different water regimes [Control = well-watered; D<sub>1</sub> = 50% field capacity (mild drought); D<sub>2</sub> = 25% field capacity (moderate drought); D<sub>3</sub> = 12.5% field capacity (severe drought)]. The values are the means  $\pm$  SEMs. The means were compared by Tukey's honestly significant difference test at  $p = 0.05$ . Bars with the same letter do not differ significantly from each other

contents in drought-stressed wheat plants in this study. This effect might be a result of the Se-induced suppression of membrane lipid peroxidation in plants. Seliem et al. [40] reported a similar increase in leaf Chl *a* and Chl *b* contents in *Chrysanthemum morifolium* in response to Se(np) application. Other studies using Se(np), including experiments on *Solanum lycopersicum* by Haghighi et al. [41] and *Vigna unguiculata* L. by El Lateef Gharib et al. [15], have reported that Se(np) are more effective than Se alone in improving Chl pigment levels, in agreement with the findings of the present study. Higher rates of CO<sub>2</sub> assimilation, enhanced rates of photosynthesis, and improved soil nutrient uptake are the supposed causes of these Se(np) effects [15].

Another possible mechanism that could explain Se(np) effects may involve H<sub>2</sub>O<sub>2</sub> production. Although H<sub>2</sub>O<sub>2</sub> is an important signaling molecule, its levels become elevated during oxidative stress to defend against environmental stresses. The generation of ROS also triggers lipid peroxidation in the plasma membrane, with negative effects on cellular functioning that further increase oxidative stress. The excessive production of H<sub>2</sub>O<sub>2</sub> and MDA can therefore serve as indicators of oxidative stress generated by various abiotic stresses in plants. In the

present study, the H<sub>2</sub>O<sub>2</sub> and MDA levels were notably elevated in response to all three levels of drought stress, confirming that plants need external elicitors to activate the antioxidant defense systems needed to supplement their innate antioxidant enzymes in times of stress. Possibly, the Se and Se(np) foliar sprays decreased the generation of H<sub>2</sub>O<sub>2</sub> and MDA to levels below those observed in the unsprayed drought-stressed plants through the activation of antioxidant enzymes, in agreement with numerous previous results [10, 42–44]. Se itself cannot scavenge H<sub>2</sub>O<sub>2</sub>; instead, it facilitates the reduction of the cellular H<sub>2</sub>O<sub>2</sub> content by other H<sub>2</sub>O<sub>2</sub> quenchers [10]. Similarly, Pro [44, 45] and leaf EL% [40, 46] were reduced in plants growing in stressful environments after Se(np) and Se supplementation, in agreement with the findings of the present experiment. Foliar application of Se and Se(np) reduced wheat leaf EL% and Pro content under different levels of drought stress, indicating stress alleviation. This effect presumably arose because Se and Se(np) maintained the structural integrity of cells by enhancing ROS scavenging, ensuring proper fatty acid content, and triggering amino acid regulation of the TCA cycle to enhance lipid metabolism [46].

The deleterious effects of abiotic stresses are observed if ROS generation surpasses the capability of the antioxidant mechanisms that deal with ROS generation during normal metabolism. In this case, oxidative stress occurs in plant cells, and integrated efforts are made to activate enzymatic and nonenzymatic antioxidant activities, which are part of the antioxidant defense mechanisms required under these circumstances [47]. The APX enzyme creates MDHA as part of the nonenzymatic antioxidant pool of AsA-GSH, where AsA provides electrons. This reduction in MDHA generates DHA, which is subsequently oxidized to AsA with the help of GSH (another electron donor) under stressful conditions [48]. Through this AsA-GSH cycle, DHA and GSSG can also confer tolerance against stress in plants. In the present study, lower AsA and higher DHA levels indicated a reduction in the AsA/DHA ratio, whereas the GSH and GSSG levels showed an opposite shift, which eventually lowered their ratio. The upregulation of DHA and GSSG in wheat under different drought conditions again indicates enhanced oxidative metabolism, but the ratios are reversed by treatment with Se and Se(np) foliar sprays. Here, the activity of GR increased after Se and Se(np) foliar sprays, eventually increasing the GSH content and decreasing the GSSG content, indicating that Se and Se(np) have the potential to mitigate drought in wheat. These results corroborate similar findings from previous experiments with Se [24, 49] and Se(np) [50, 51] in different plants.

In the AsA-GSH pathway, APX reduces  $H_2O_2$  to  $H_2O$  [50]. In wheat leaves, the APX activity decreased significantly under different levels of drought stress, but the activity notably increased after treatment with Se and Se(np). This might reflect the detoxification of intracellular  $H_2O_2$  because APX has a strong affinity for  $H_2O_2$ . Other studies have reported similar findings of APX activation following Se and Se(np) application [52–54].

The regeneration of AsA is significantly affected by MDHAR and DHAR metabolism, and both metabolites are crucial parts of plant antioxidant defense systems. Dehydroascorbate reductase increases the level of AsA under stressful conditions to facilitate the scavenging of  $H_2O_2$ . Moreover, NADPH is needed for the reduction of GSSH to GSH, so its levels indirectly maintain the GSH/GSSG ratio. The wheat plants in the present study displayed a notable decrease in MDHAR and DHAR activities and concomitantly greater GR activities when exposed to the three different levels of drought stress. However, the Se- and Se(np)-treated plants showed increased levels of MDHAR, DHAR, and GR activity when exposed to the same drought stresses, clearly supporting the distinctive roles of antioxidant enzymes in mitigating drought-induced oxidative damage by

maintaining AsA recycling. These findings are also corroborated by previous reports [55, 56].

The wheat plants in the present study also exhibited notably increased GST, POD, and LOX activities and decreased activities of other enzymes (e.g., CAT, GPX, and SOD) under different drought stress levels. The POD enzyme converts  $H_2O_2$  to  $H_2O$ , while the SOD enzyme detoxifies  $O_2^{\bullet-}$  to  $H_2O_2$  [57]. Catalase subsequently turns  $H_2O_2$  into  $H_2O$  and  $O_2$ . The effects of Se and Se(np) sprays on GST, POD, and LOX activities were notable and paralleled the reductions in  $H_2O_2$  levels and lipid peroxidation, indicating that Se and Se(np) were effective at mitigating drought stress in wheat plants, in agreement with Se [53, 58–60] and Se(np) [37, 56, 61] studies on other plants under different kinds of stress. A decrease in CAT activity is important for the detoxification of peroxides, whereas SOD helps detoxify  $O_2^{\bullet-}$  to less toxic  $H_2O_2$ . During water stress, the accumulation of  $H_2O_2$  might inactivate CAT, an enzyme known to undergo photoinactivation [62]. POD assists in plant respiration and changes phenols into quinines, which may help with stress reduction under stressful conditions [63].

Glyoxalase enzymes work together to detoxify the harmful effects of MG that accumulates in stressful situations [64]. Therefore, the downregulated activities of Gly I and Gly II by drought stress in this study suppressed wheat growth and development, as previously reported [55, 60]. However, foliar application of Se and Se(np) enhanced the activities of Gly I and Gly II, indicating improved detoxification of MG. This was further supported by the elevated GSH levels, because GSH recycling relies on the glyoxalase system. Previous results with Se and Se(np) treatments in stressed plants also corroborate the findings of the present study [56, 60, 65, 66].

## Conclusion

This study highlights the protective roles of selenium and its nanoparticles in alleviating the adverse effects of drought stress on wheat plants, with a focus on enhancing various morphophysiological and biochemical parameters. The application of Se(np) and Se significantly enhanced the activity of several enzymes, such as the glyoxalases Gly I and Gly II, APX, MDHAR, DHAR, GR, SOD, CAT, and POD, which are key components of plant antioxidant defense systems. This enhancement facilitated rapid ROS scavenging while suppressing MG toxicity, thereby diminishing oxidative stress in wheat plants. Selenium nanoparticles and Se foliar sprays had positive effects on wheat growth and Chl synthesis, while reducing oxidative stress through the moderation of MDA and  $H_2O_2$  levels and decreasing EL% by preserving membrane integrity under drought conditions. These findings suggest that Se is an effective alleviator of drought stress that acts by modifying plant ROS-scavenging and



antioxidant defense mechanisms, as well as activating the glyoxalase system, thereby enhancing plant growth and development. Notably, in most aspects, Se(np) was more effective than the Se spray at mitigating drought stress. This observation indicates a need for further research to explain the molecular mechanisms and signaling pathways by which Se(np) enhances stress tolerance in plants.

#### Abbreviations

ANOVA	Analysis of variance
APX	Ascorbate peroxidase
CAT	Catalase
Chl	Chlorophyll
DHA	Dehydroascorbate
DTNB	5,5-dithio-bis-(2-nitrobenzoic acid)
DTT	Dithiothreitol
EC	Electrical conductivity
EDTA	Ethylenediaminetetraacetic acid
EL	Electrolyte leakage
FW	Fresh weight
Gly	Glyoxalase
GPX	Glutathione peroxidase
GR	Glutathione reductase
GSSG	Oxidized glutathione
GST	Glutathione S-transferase
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
LOX	Lipoxygenase
MDHAR	Monodehydroascorbate reductase
MG	Methylglyoxal
NADPH	Nicotinamide adenine dinucleotide phosphate
NPs	Nanoparticles
POD	Peroxidase
ROS	Reactive oxygen species
Se	Selenium
SEM	Scanning electron microscopy
SLG	S-D-lactoylglutathione
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloroacetic acid
TEM	Transmission electron microscopy

#### Supplementary Information

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Supplementary Material 1

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#### Author contributions

MH: Methodology, conceptualization, visualization, supervision, visualization, writing—review and editing. MRHR: Methodology, investigation; AS: Investigation, writing—original draft preparation; K.B.: Methodology; writing—original draft preparation; MSH: Investigation, writing—original draft preparation, data curation; PVVP: funding acquisition, writing—review and editing.

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#### Data availability

Data are provided within the manuscript.

#### Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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#### References

1. Prodhon FA, Zhang J, Pangali Sharma TP, Nanzad L, Zhang D, Seka AM, Ahmed N, Hasan SS, Hoque MZ, Mohana HP. Projection of future drought and its impact on simulated crop yield over South Asia using ensemble machine learning approach. *Sci Total Environ*. 2022;807:151029.
2. Vicente-Serrano SM, Peña-Angulo D, Beguería S, Domínguez-Castro F, Tomás-Burguera M, Noguera I, Gimeno-Sotelo L, Kenawy AE. Global drought trends and future projections. *Philos Transact R Soc Math Phys Eng Sci*. 2022;380:20210285.
3. FAOSTAT. Statistical database. Rome: Food and Agriculture Organization of the United Nations; 2021.
4. Wan C, Dang P, Gao L, Wang J, Tao J, Qin X, Feng B, Gao J. How does the environment affect wheat yield and protein content response to drought? A meta-analysis. *Front Plant Sci*. 2022;13:896985.
5. IPCC. Summary for Policymakers. In: Masson-Delmotte V, Zhai P, Portner H-O, Roberts D, Skea J, Shukla PR, Pirani A, Moufouma-Okia W, P'ean C, Pidcock R, Connors S, Matthews JBR, Chen Y, Zhou X, Gomis MI, Lonnoy E, Maycock T, Tignor M, Waterfield T, editors. *Global warming of 1.5°C; an IPCC special report on the impacts of global warming of 1.5°C above preindustrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty*. World Meteorological Organization: Geneva, Switzerland. 2018. p. 32.
6. Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Mahmud AJ, Fujita M, Fotopoulos V. Reactive oxygen species and antioxidant defense in plants under abiotic stress: revisiting the crucial role of a universal defense regulator. *Antioxidants*. 2020;9:681.
7. Dvojković K, Plavšin I, Novoselović D, Šimić G, Lalić A, Čupić T, Horvat D, Vuletić MV. Early antioxidative response to desiccant-stimulated drought stress in field-grown traditional wheat varieties. *Plants*. 2023;12(2):249.
8. Bapela T, Shimelis H, Tsilo TJ, Mathew I. Genetic improvement of wheat for drought tolerance: Progress, challenges and opportunities. *Plants*. 2022;11(10):1331.
9. Lanza MGDB, dos Reis AR. Roles of selenium in mineral plant nutrition: ROS scavenging responses against abiotic stresses. *Plant Physiol Biochem*. 2021;164:27–43.

10. Djanaguiraman M, Belliraj N, Bossmann SH, Prasad PV. High-temperature stress alleviation by selenium nanoparticle treatment in grain sorghum. *ACS Omega*. 2018;3(3):2479–91.
11. Khan E, Panthri M, Pandey C, Sahay S, Gupta M. Silicon modulates expression of pin genes and genotoxicity during arsenic stress in rice (*Oryza sativa*). *J Soil Sci Plant Nutr*. 2023;23:1660–77.
12. Al-Khayri JM, Rashmi R, Surya Ulhas R, Sudheer WN, Banadka A, Nagella P, Aldaej MI, Rezk AAS, Shehata WF, Almaghasla MI. The role of nanoparticles in response of plants to abiotic stress at physiological, biochemical, and molecular levels. *Plants*. 2023;12(2):292.
13. González-García Y, González-Moscoso M, Hernández-Hernández H, Méndez-López A, Juárez-Maldonado A. Induction of stress tolerance in crops by applying nanomaterials. In: Ingle AP, editor. *Nanotechnology in Plant Growth Promotion and Protection*. Hoboken, NJ, USA: John Wiley & Sons Ltd.; 2021. pp. 129–69.
14. BARI (Bangladesh Agricultural Research Institute). *Krishi Projukti Hatboi*; Bangladesh Agricultural Research Institute. Gazipur, Bangladesh; 2021.
15. El Lateef Gharib FA, Zeid IM, Ghazi SM, Ahmed EZ. The response of cowpea (*Vigna unguiculata* L.) plants to foliar application of sodium selenate and selenium nanoparticles (SeNPs). *J Nanomater Mol Nanotechnol*. 2019;8(4):1000274.
16. Deepa B, Ganesan V. Bioinspired synthesis of selenium nanoparticles using flowers of *Catharanth usroseus* (L). *Int J Chem Tech Res*. 2015;7:725–33.
17. Arnon DT. Copper enzymes in isolated chloroplasts. Polyphenoloxidase. *Q25 Beta vulgaris*. *J Plant Physiol*. 1949;24:1–15.
18. Heath RL, Packer L. Photo peroxidation in isolated chloroplast: I. kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys*. 1968;125(1):189–98.
19. Yu CW, Murphy TM, Lin CH. Hydrogen peroxide induced chilling tolerance in mung beans mediated through ABA-independent glutathione accumulation. *Funct Plant Biol*. 2003;30(9):955–63.
20. Dionisio-Sese ML, Tobita S. Antioxidant responses of rice seedlings to salinity stress. *Plant Sci*. 1998;135(1):1–9.
21. Bates LS, Waldren RP, Teari D. Rapid determination of free proline for water stress studies. *Plant Soil*. 1973;39:205–7.
22. Kampfenkel K, Van Montagu M, Inz'e D. Extraction and determination of ascorbate and dehydroascorbate from plant-tissue. *Anal Biochem*. 1995;225(1):165–7.
23. Huang C, He W, Guo J, Chang X, Su P, Zhang L. Increased sensitivity to salt stress in ascorbate-deficient Arabidopsis mutant. *J Exp Bot*. 2005;56(422):3041–9.
24. Hasanuzzaman M, Nahar K, Alam MM, Fujita M. Modulation of antioxidant machinery and the methylglyoxal detoxification system in selenium-supplemented *Brassica napus* seedlings confers tolerance to high-temperature stress. *Biol Trace Elem Res*. 2014;161:297–307.
25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976;72(1–2):248–54.
26. Nakano Y, Asada K. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*. 1981;22(5):867–80.
27. Hossain MA, Nakano Y, Asada K. Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol*. 1984;25(3):385–95.
28. Nahar K, Hasanuzzaman M, Alam MM, Rahman A, Suzuki T, Fujita M. Polyamine and nitric oxide crosstalk: antagonistic effects on cadmium toxicity in mung bean plants through upregulating the metal detoxification, antioxidant defense and methylglyoxal detoxification systems. *Ecotoxicol Environ Saf*. 2016;126:245–55.
29. Doderer A, Kokkelink I, Van der Veen S, Valk B, Schram A, Douma A. Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim Biophys Acta*. 1992;1120(1):97–104.
30. El-Shabrawi H, Kumar B, Kaul T, Reddy MK, Singla-Pareek SL, Sopory SK. Redox homeostasis, antioxidant defense, and methylglyoxal detoxification as markers for salt tolerance in Pokkali rice. *Protoplasma*. 2010;245:85–96.
31. Hemeda HM, Klein BP. Effects of naturally occurring antioxidants on peroxidase activity of vegetable extracts. *J Food Sci*. 1990;55(1):184–5.
32. Wild R, Ooi L, Srikanth V, Münch G. A quick, convenient and economical method for the reliable determination of methylglyoxal in millimolar concentrations: the N-acetyl-L-cysteine assay. *Anal Bioanal Chem*. 2012;403:2577–81.
33. Gao S, Wang Y, Yu S, Huang Y, Liu H, Chen W, He X. Effects of drought stress on growth, physiology and secondary metabolites of two adonis species in Northeast China. *Sci Hortic*. 2020;259:108795.
34. Ostadi A, Javanmard A, Amani Machiani M, Sadeghpour A, Maggi F, Nouraein M, Morshedloo MR, Hano C, Lorenzo JM. Co-application of TiO<sub>2</sub> nanoparticles and arbuscular mycorrhizal fungi improves essential oil quantity and quality of sage (*Salvia officinalis* L.) in drought stress conditions. *Plants*. 2022;11(13):1659.
35. Javanmard A, Ashrafi M, Morshedloo MR, Machiani MA, Rasouli F, Maggi F. Optimizing phytochemical and physiological characteristics of Balangu (*Lallemantia Iberica*) by foliar application of chitosan nanoparticles and myco-root inoculation under water supply restrictions. *Horticulturae*. 2022;8(8):695.
36. Nawaz F, Ahmad R, Ashraf MY, Waraich EA, Khan SZ. Effect of selenium foliar spray on physiological and biochemical processes and chemical constituents of wheat under drought stress. *Ecotoxicol Environ Saf*. 2015;113:191–200.
37. Morales-Espinoza MC, Cadenas-Pliego G, Pérez-Alvarez M, Hernández-Fuentes AD, De La Fuente MC, Benavides-Mendoza A, Valdés-Reyna J, Juárez-Maldonado A. Se nanoparticles induce changes in the growth, antioxidant responses, and fruit quality of tomato developed under NaCl stress. *Molecules*. 2019;24(17):3030.
38. Abd Elbar OH, Farag RE, Shehata SA. Effect of putrescine application on some growth, biochemical and anatomical characteristics of *Thymus vulgaris* L. under drought stress. *Ann Agric Sci*. 2019;64(2):129–37.
39. Amani Machiani M, Javanmard A, Morshedloo MR, Janmohammadi M, Maggi F. *Funneliformis mosseae* application improves the oil quantity and quality and eco-physiological characteristics of soybean (*Glycine max* L.) under water stress conditions. *J Soil Sci Plant Nutr*. 2021;21:3076–90.
40. Seliem MK, Hafez Y, El-Ramady H. Using of Nano - Selenium in reducing the negative effects of high temperature stress on *Chrysanthemum morifolium* Ramat. *J Sustain Agric Sci*. 2020;46:47–59.
41. Haghghi MR, Abolghasemi JA, Teixeira DS. Low and high-temperature stress affect the growth characteristics of tomato in hydroponic culture with Se and nano-Se amendment. *Sci Hortic*. 2014;178:231–40.
42. Balal RM, Shahid MA, Javaid MM, Iqbal Z, Anjum MA, Garcia-Sanchez F, Mattson NS. The role of selenium in amelioration of heat-induced oxidative damage in cucumber under high temperature stress. *Acta Physiol Plant*. 2016;38:158.
43. Huang C, Qin N, Sun L, Yu M, Hu W, Qi Z. Selenium improves physiological parameters and alleviates oxidative stress in strawberry seedlings under low temperature stress. *Int J Mol Sci*. 2018;19(7):1913.
44. Zahedi SM, Abdelrahman M, Hosseini MS, Hoveizeh NF, Tran LSP. Alleviation of the effect of salinity on growth and yield of strawberry by foliar spray of selenium nanoparticles. *Environ Pollut*. 2019;253:246–58.
45. Hemmati M, Delkosh B, Rad AHS, Mohammadi GN. Effect of the application of foliar selenium on canola cultivars as influenced by different irrigation regimes. *J Agric Sci*. 2019;25(3):309–18.
46. Shalaby TA, Abd-Elkarim E, El-Aidy F, Hamed ES, Sharaf-Eldin M, Taha N, El-Ramady H, Bayoumi Y, Dos Reis AR. Nano-selenium, silicon and H<sub>2</sub>O<sub>2</sub> boost growth and productivity of cucumber under combined salinity and heat stress. *Ecotoxicol Environ Saf*. 2021;212:111962.
47. Dumanović J, Nepovimova E, Natić M, Kuča K, Jačević V. The significance of reactive oxygen species and antioxidant defense system in plants: a concise overview. *Front Plant Sci*. 2021;11:552969.
48. Sofo A, Scopa A, Nuzzaci M, Vitti A. Ascorbate peroxidase and catalase activities and their genetic regulation in plants subjected to drought and salinity stresses. *Int J Mol Sci*. 2015;16(6):13561–78.
49. Yildiztugay E, Ozfidan-Konakci C, Cucukoduk M, Tekis SA. The impact of selenium application on enzymatic and non-enzymatic antioxidant systems in *Zea mays* roots treated with combined osmotic and heat stress. *Arch Agron Soil Sci*. 2017;63:261–75.
50. Farooq MA, Islam F, Ayyaz A, Chen W, Noor Y, Hu W, Hannan F, Zhou W. Mitigation effects of exogenous melatonin-selenium nanoparticles on arsenic-induced stress in *Brassica napus*. *Environ Pollut*. 2022;292:118473.
51. Soliman MH, Alnusairi GS, Khan AA, Alnusaire TS, Fakhr MA, Abdulmajeed AM, Aldesuquy HS, Yahya M, Najeeb U. Biochar and selenium nanoparticles induce water transporter genes for sustaining carbon assimilation and grain production in salt-stressed wheat. *J Plant Growth Regul*. 2023;42(3):1522–43.
52. Zahedi SM, Hosseini MS, Daneshvar Hakimi Meybodi N, Pejmanburg W. Mitigation of the effect of drought on growth and yield of pomegranates by foliar spraying of different sizes of selenium nanoparticles. *J Sci Food Agric*. 2021;101(12):5202–13.
53. Sardar R, Ahmed S, Shah AA, Yasin NA. Selenium nanoparticles reduced cadmium uptake, regulated nutritional homeostasis and antioxidative system in *Coriandrum sativum* grown in cadmium toxic conditions. *Chemosphere*. 2022;287:132332.

54. Ali J, Jan I, Ullah H, Fahad S, Saud S, Adnan M, Ali B, Liu K, Harrison MT, Hassan S, Kumar S. Biochemical response of okra (*Abelmoschus esculentus* L.) to selenium (Se) under drought stress. *Sustainability*. 2023;15(7):5694.
55. Rahman M, Rahman K, Sathi KS, Alam MM, Nahar K, Fujita M, Hasanuzzaman M. Supplemental selenium and boron mitigate salt-induced oxidative damages in *Glycine max* L. *Plants*. 2021;10(10):2224.
56. Ishtiaq M, Mazhar MW, Maqbool M, Hussain T, Hussain SA, Casini R, Abd-ElGawad AM, Elansary HO. Seed priming with the selenium nanoparticles maintains the redox status in the water stressed tomato plants by modulating the antioxidant defense enzymes. *Plants*. 2023;12(7):1556.
57. Deng J, Fu D, Hu W, Lu X, Wu Y, Bryan H. Physiological responses and accumulation ability of *Microcystis aeruginosa* to zinc and cadmium: implications for bioremediation of heavy metal pollution. *Bioresour Technol*. 2020;303:122963.
58. Yang H, Yang X, Ning Z, Kwon SY, Li ML, Tack FM, Kwon EE, Rinklebe J, Yin R. The beneficial and hazardous effects of selenium on the health of the soil-plant-human system: an overview. *J Hazard Mater*. 2022;422:126876.
59. Çatav ŞŞ, Köşkeröğlu S, Tuna AL. Selenium supplementation mitigates boron toxicity induced growth inhibition and oxidative damage in pepper plants. *S Afr J Bot*. 2022;146:375–82.
60. Sakouhi L, Mahjoubi Y, Labben A, Kharbech O, Chaoui A, Djebali W. Effects of cadmium–selenium interaction on glyoxalase and antioxidant systems of *Pisum sativum* germinating seeds. *J Plant Growth Regul*. 2023;42(5):3084–99.
61. Nasibi F, Aminian F, Mohammadinejad G, Hassanshahian M. Seed priming with selenium nanoparticle and plant growth promoting rhizobacteria improve seedling development of foxtail millet (*Setaria italica*) under salinity stress. *Res Square*. 2022. <https://doi.org/10.21203/rs.3.rs-1809244/v1>.
62. Zhang JX, Kirkham MB. Drought stress induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant Cell Physiol*. 1994;35(5):785–91.
63. Cheng J, Qiu H, Chang Z, Jiang Z, Yin W. The effect of cadmium on the growth and antioxidant response for freshwater algae *Chlorella vulgaris*. *SpringerPlus*. 2016;5:1290.
64. Shivani GSK, Gill RK, Virk HK, Bhardwaj RD. Methylglyoxal detoxification pathway explored first time for imazethapyr tolerance in lentil (*Lens culinaris* L.). *Plant Physiol Biochem*. 2022;177:10–22.
65. Hasan MM, Alharbi BM, Alhailoul HAS, Abdulmajeed AM, Alghanem SM, Al-Mushhin AA, Jahan MS, Corpas FJ, Fang XW, Soliman MH. Spermine-mediated tolerance to selenium toxicity in wheat (*Triticum aestivum* L.) depends on endogenous nitric oxide synthesis. *Antioxidants*. 2021;10(11):1835.
66. El-Badri AM, Batool M, Mohamed IA, Wang Z, Wang C, Tabl KM, Khatab A, Kuai J, Wang J, Wang B, Zhou G. Mitigation of the salinity stress in rapeseed (*Brassica napus* L.) productivity by exogenous applications of bio-selenium nanoparticles during the early seedling stage. *Environ Pollut*. 2022;310:119815.

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