Plant Physiology and Biochemistry 145 (2019) 142–152

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Selenium mitigates the chromium toxicity in *Brassicca napus* L. by ameliorating nutrients uptake, amino acids metabolism and antioxidant defense system

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ARTICLE INFO

Keywords:
Amino acids
Antioxidants
Brassicca napus L
Chromium
Selenium
Nutrients

ABSTRACT

The phytotoxicity of chromium (Cr) makes it obligatory for the researchers to develop strategies that seek to hinder its accumulation in food chains. While, protective role of selenium (Se) has not been discussed in detail under adverse conditions in oilseed rape. Here, our aim was to investigate the potential use of Se (0, 5 and 10 μM) in alleviating the Cr toxicity (0, 100 and 200 μM) in *Brassica napus* L. Results delineated that Se-supplementation notably recovered the Cr-phytotoxicity by reducing the Cr accumulation in plant tissues and boosted the inhibition in plant growth and biomass. Under Cr stress, the exogenously applied Se significantly recovered the impairment in photosynthesis related parameters (chlorophyll *a*, chlorophyll *b*, carotenoids, net photosynthetic rate, stomatal conductance, and photochemical efficiency of photosystem II), and counteracted the reduction in nutrients uptake and improved the essential amino acids (EAAs) levels. In addition, Se activated the antioxidants enzymes included in AsA-GSH cycle (SOD, CAT, APX, GR, DHAR, MDHAR, GSH, and AsA) and glyoxalase (Gly) system (Gly I and Gly II) and minimized the excessive generation of reactive oxygen species (ROS) and methylglyoxal (MG) contents in response to Cr stress. In a nutshell, Se (more effective at 5 μM) alleviated the Cr and MG induced phytotoxicity and oxidative damages by minimizing their (Cr and MG) accumulation and enhanced the plant growth, nutrients element level, nutrition quality by improving EAAs, antioxidant and Gly system. By considering the above-mentioned biomarkers, the addition of exogenous Se in Cr polluted soils might be effective approach to decrease the Cr uptake and its linked phytotoxicity in *B. napus*.

1. Introduction

The by-products of rapid urbanization, industrial practices and technological innovations are the major sources of chromium (Cr), which is the non-essential toxic metal. The availability of Cr aggravated pollutant in soil and water bodies is posing serious risks to human health through its involvement in food chain (Shahid et al., 2017). Hexavalent (VI) and trivalent (III) are the two stable forms of Cr, in which Cr (VI) is considered more phytotoxic as compared to Cr (III) (Lopez-Luna et al., 2009). The excessive deposition of Cr in plant tissues is causing severe phytotoxicity as revealed by the growth and biomass reduction, chlorophyll degradation, retardation in nutrients uptake, overproduction of reactive oxidative species (ROS), disorganization in antioxidant defense machinery, destruction of cellular ultrastructure and ultimately no plant growth (Gill et al., 2014, 2015a; Bashri et al., 2016; Ahmad et al., 2017; Do et al., 2018). To handle the excessive production of reactive oxygen species, plants strengthen their immunity by activating various antioxidant scavengers including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase

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https://doi.org/10.1016/j.plaphy.2019.10.035
Received 6 August 2019; Received in revised form 27 September 2019; Accepted 24 October 2019
Available online 25 October 2019
0981-9428/

(DHAR), monodehydroascorbate reductase (MDHAR) besides other key defense agents including glutathione (GSH) and ascorbate (AsA) (Mostofa et al., 2017; Ulhassan et al., 2019). Amino acids are the reservoirs of proteins and precursors of phytohormones which helps in enhancing the plant tolerance against oxidative injuries (Choi et al., 2011). A cytotoxic aldehyde, methylglyoxal (MG) is well-known in plants under both stress/non-stress conditions which can be detoxified by glyoxalase (Gly) enzymes (Gly I and Gly II) (Hasanuzzaman et al., 2011).

Selenium (Se), chemically similar to sulphur (S), has gained reputation as essential element for human and animals. While, it is also considered beneficial for plants at lower doses as depicted in promoting growth, pigments, antioxidant defense system, metabolites accumulation, and reducing the over-generation of lipid peroxidation and reactive oxygen species (Alyemeni et al., 2018; Ulhassan et al., 2019). In natural soil environment, the concentrations of Se varies from 0.1 to 2 mg kg^{-1}. Globally, some areas are Se-rich (> 10 mg kg^{-1}) such as Hubei province, China (Zhu et al., 2009). Most leading and highly water-soluble Se-forms in soils are selenite (Se IV) and selenate (Se VI) (Mehdi et al., 2013). In aerobic soils, both Se forms can exist depending upon the soil pH and redox potential. Among the available Se forms, Se (IV) is easily taken by plants (remains adhere to soil) than Se (VI) (easily trickled from soil). It has been documented that Se (IV) has greater potential in mitigating heavy metals stress than Se (VI) (Liao et al., 2016; Tran et al., 2018). Numerous tactics have been carried out to alleviate the Cr-phytotoxicity such as the application of plant growth hormones (Gill et al., 2015b, 2016a; Singh et al., 2016; Ali et al., 2018) and metalloids (Huda et al., 2017; Handa et al., 2017, 2018a, 2019). Latest documentations have shown the potential of Se species (IV or VI) in alleviating the heavy metals induced phytotoxicities. Previously, role of Se species (0, 0.5, 1 and 2.5 mg kg^{-1}) in inhibiting the Hg (0, 1, 2 and 3 mg kg^{-1}) phytotoxicity has been studied in Pak choi (Tran et al., 2018). Recently, it has been investigated that Se (IV) (10 μM) alleviates the Cd (150 mg L^{-1}) induced oxidative injuries in tomato (Alyemeni et al., 2018). Further, it has been indicated that Se (IV) (0, 1, 5, 10, 15, 20 mg kg^{-1}) alleviates the Cd (0, 1 and 5 mg kg^{-1}) and Pb (0, 300 and 500 mg kg^{-1}) toxicity in *B. napus* by negating the oxidative damages and strengthening the antioxidant system (Wu et al., 2016). Recently, Handa et al. (2018a, 2018b, 2019) reported that Se (VI) (2, 4 and 6 μM) mitigates the Cr (300 μM) induced phytotoxicity in *B. juncea*. But, the role of selenium especially Se (IV) in mitigating Cr-phytotoxicity is least studied in *B. napus*.

Oilseed rape (*B. napus* L.) is well-grown crop, worldwide and potential candidate for phytoremediation due to their greater potential to alleviate heavy metals stress and higher biomass production (Grispen et al., 2006). Among the various member of *Brassicaceae* family, *B. napus* has been documented as the primary accumulator of Cr (Gill et al., 2015a) and Se (Ulhassan et al., 2018, 2019). Therefore, it is ideal plant to boost our knowledge concerning the optimized dose of Se in field study to mitigate the Cr-induce phytotoxicity. Based on the above discussion, current study was directed to explore the potential of Se (IV) in mitigating Cr toxicity in *B. napus* plants by evaluating the morpho-physiological, metabolic and phenotypical biomarkers. To testify this, alone or/combined impacts of Se (IV) and Cr were investigated on the plant growth and biomass production, photosynthesis attributes, intercellular Cr-accumulation, nutrient elements uptake, amino acid metabolism, ultrastructure alterations of chloroplasts, production of ROS, lipid peroxidation, as well as, antioxidant defense machinery and enzymes of glyoxalase system (Gly I and II) as scavenging agents for ROS and MG oxidants. For sustainable crop production, this study will provide basis for developing tactics (by using Se) to minimize the risks correlated with Cr-phytotoxicity.

2. Materials and methods

2.1. Plant materials and experimental conditions

The selected winter black-seeded cultivar (ZS 758) of *Brassica napus* (oilseed rape) had utmost tolerance ability against different metals/metalloids toxicity (Gill et al., 2016b, 2017; Ali et al., 2017, 2018a; Ulhassan et al., 2019). After taking seeds from the College of Agriculture and Biotechnology, Zhejiang University (China), the mature seeds were first sterilized and then allowed to germinate on wet filter paper in Petri dishes (in dark). Germinated seeds were allowed to grow up in plastic pots (170 mm × 220 mm) filled with peat soil under ambient conditions (20–24 °C, 350–400 μmol m^{-2} s^{-1} light intensity and 55–60% humidity) in greenhouse. After 28 days of sowing, the morphological identical-sized seedlings were covered with foam at shoot-root joint and shifted into 5-L plastic pots covering with holes in plates (five plants per pot) containing half strength Hoagland nutrient solution (Arnon and Hoagland, 1940). After allowing the seedlings to acclimatize in metal-free nutrient solution for one week, Se was supplied as sodium selenite (Na_2SeO_3) and Cr as potassium dichromate ($K_2Cr_2O_7$) by making the desired concentrations of Se (0, 5, 10 μM) and Cr (0, 100, 200 μM) in half strength Hoagland solution for four days at first and then converted to full strength Hoagland solution for next two weeks. The applied treatments were: (1) control (CK), (2) 5 μM Se (IV), (3) 10 μM Se (IV), (4) 100 μM Cr, (5) 200 μM Cr, (6) 5 μM Se (IV) + 100 μM Cr, (7) 5 μM Se (IV) + 200 μM Cr, (8) 10 μM Se (IV) + 100 μM Cr, (9) 10 μM Se (IV) + 200 μM Cr. The selection of treatment concentrations was based on the findings of preliminary experiments, with the application of different levels of Se (0, 2.5, 5, 10, 15 and 20 μM) and Cr (0, 50, 100, 200, 300 and 400 μM). Lower levels of Se and Cr showed no significant change in plant growth at 2.5 μM and 50 μM, respectively. It was observed that lower doses of Se (5 and 10 μM) performed better under applied Cr doses as compared with other Se doses. While, Se doses more than 10 μM depicted less significant changes when we compared it with lower Se doses (< 10 μM). In contrast, higher Cr levels > 200 μM were too toxic for the *Brassica* plant growth as revealed by the severe visible injuries in the form of leaf chlorosis and yellowing of leaves. During the experiment, a constant aeration system (air pump) was supplied into nutrient solution and renewed after every four days. On the daily basis, pH of the solution was maintained between 5.6 and 5.8 with one molar solution of NaOH and HCl. All the treatments were repeated thrice. After two-weeks of Se and Cr treatments, leaf and root samples were harvested for the physio-biochemical, metabolic and structural studies.

2.2. Measurement of morphological, endogenous elements, and photosynthesis traits

Instantly after harvesting, fresh biomass of plant tissues (leaf, stem and roots) (ten plants/treatment) were measured manually. Then, samples were oven-dried at 70 °C and weighted straightaway after taking out from the oven. After dry analysis, the extraction of endogenous contents of Se, Cr and nutrients elements (N, P, K, Zn, Fe, and Mn) in the leaves and roots were determined by following the previous protocols (Gill et al., 2015a; Ulhassan et al., 2019). The upper second fully stretched leaves were taken for the estimation of light harvesting pigments [chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*) and carotenoids)] (Ulhassan et al., 2019). Infrared gas analyzer (IRGA) portable photosynthesis system (Li-Cor 6400, Lincoln, NE, USA) was used to record the net photosynthetic rate and stomatal conductance as reported by Ali et al. (2018b). Fully expanded second leaves were chosen for the measurement of maximum quantum efficiency of photosystem II (*Fv/Fm*) via an imaging pulse-amplitude-modulated (PAM) fluorimeter (IMAG-MAXI; Heinz Walz, Effeltrich, Germany) (Farooq et al., 2016).

2.3. Quantification of oxidative stress indices, amino acids profiling and ultra-structures observation

The determination of MDA, H_2O_2 and $O_2^{\bullet-}$ contents in both leaves and roots was done by following the methods used in our previous study (Ulhassan et al., 2019). The quantification of amino acids was done by pico tag method on High Performance Liquid Chromatography (HPLC) (Bidlingmeyer et al., 1984). To examine the ultra-structures, root ends (around 2–3 mm) were mounted on copper grids and observed through transmission electron microscopic (JEOL TEM-1230EX, Japan) at accelerating voltage of 60.0 KV as performed by Gill et al. (2015b).

2.4. Assay of enzymes involved in ROS-detoxification and ascorbate-glutathione cycle

Samples (0.5 g) of leaf and root were first homogenized in 50 mM KH_2PO_4 buffer (pH 7.8) and centrifuged at 10,000 g for 20 min (Eppendorf AG, model 2231, Hamburg, Germany). The obtained liquid (lying on solid reside) was reserved for the assay of following enzyme activities. The estimation of reduced glutathione (GSH) and ascorbic acid (AsA) contents was carried out by following the modified protocols used by Ali et al. (2014). The activities of total superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6), glutathione reductase (GR, EC 1.6.4.2), and ascorbate peroxidase (APX, EC 1.11.1.11) were determined as reported earlier (Ulhassan et al., 2019). Activities of monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) and dehydroascorbate reductase (DHAR, EC 1.8.5.1) were assayed as described in earlier studies (Nakano and Asada, 1981; Hossain et al., 2010). Methylglyoxal (MG) content was estimated by Wild et al. (2012). The activities of glyoxalase I (Gly I, EC 4.4.1.5) and II (Gly II, EC 3.1.2.6)) were assayed by following the protocols of Hossain et al. (2009) and Mostofa and Fujita (2013), respectively.

2.5. Statistical analysis

Data with mean ± SD of at least four independent replicates was analyzed by statistical software package, Data Processing System (DPS). The analysis of variance (Two-way ANOVA) was done subsequently Duncan's Multiple Range Test (DMRT). The significance at P, 0.05 and 0.001 are considered as significant and highly significant, respectively. Origin Pro version 8.0 (Origin Lab corporation, Wellesley Hills, Wellesley, MA, USA) was used to produce graphs.

3. Results

3.1. Effects of selenium on intracellular chromium accumulation under chromium stress

The endogenous Se content in the plant tissues was slightly enhanced with the increasing Se alone concentrations (5 and 10 μM) in nutrient solution. The maximum increase in Se-accumulation was determined in roots (18.12 mg kg^{-1} DW) than leaves (15.9 mg kg^{-1} DW). The addition of Cr in nutrients solution reduced the endogenous Se levels with greater inhibition in roots (42%) than leaves (25%) at 200 μM Cr + 5 μM Se treatment as compared to other Cr + Se alone contents (Table 1). Thus, Cr declined the Se-accumulation mainly in roots than leaves. Under non-stress conditions of Se and Cr, no significant difference in endogenous Cr contents was noted in plant tissues. The endogenous Cr contents in plant tissues were notably elevated as we increased the Cr doses (100 and 200 μM) with higher accumulation in roots (1174.82 mg kg^{-1} DW) than leaves (59.51 mg kg^{-1} DW), when compared with control treatments. The application of Se in nutrient solution inhibited the Cr accumulation as revealed by the lower Se + Cr levels than Cr alone contents. However, maximum reduction in Cr (mainly 100 μM) uptake was observed at 5 μM Se (44% in leaves) and

Table 1
Interactive effects of selenium and chromium on morphological parameters and endogenous Se and Cr contents. Effects of selenium (Se) (0, 5 and 10 μM) and chromium (Cr) (0, 100 and 200 μM) treatments on the leaf fresh and dry weight (mg/plant), root fresh and dry weight (mg/plant), stem length (cm), root length (cm), and endogenous Cr and Se contents (mg/kg DW) in the leaves and roots of *Brassica napus* cv. ZS 758. Values were triplicate (minimum) of each treatment followed by the same letters with no significant difference at $P \leq 0.05$ according to Duncan's Multiple Range Test. "nd" indicates "not displayed".

Se conc. (μM)	Cr conc. (μM)	Leaf fresh weight	Leaf dry weight	Root fresh Weight	Root dry Weight	Stem length	Root length	Endogenous Cr contents (mg/kg DW)		Endogenous Se contents (mg/kg DW)	
								Leaves	Roots	Leaves	Roots
0	0	116.84 ± 9.34^{a}	8.24 ± 0.74^{ab}	19.97 ± 1.84^{ab}	4.56 ± 0.42^{a}	9.32 ± 1.02^{ab}	12.44 ± 1.1^{ab}	0.23 ± 0.05^{e}	1.25 ± 0.18^{e}	nd	nd
	100	84.85 ± 7.61^{bc}	6.41 ± 0.61^{cd}	15.43 ± 1.52^{bc}	3.91 ± 0.38^{ab}	6.82 ± 0.76^{cd}	8.12 ± 0.94^{cd}	22.90 ± 2.70^{c}	588.63 ± 61.12^{c}	nd	nd
	200	59.93 ± 6.20^{d}	3.97 ± 0.46^{e}	9.80 ± 1.14^{d}	2.89 ± 0.32^{b}	5.38 ± 0.58^{d}	5.76 ± 0.68^{d}	59.51 ± 6.52^{a}	1174.82 ± 106.2^{a}	nd	nd
5	0	119.31 ± 9.22^{a}	8.76 ± 0.73^{ab}	20.71 ± 1.84^{a}	4.70 ± 0.46^{a}	10.33 ± 1.16^{ab}	13.47 ± 1.26^{ab}	0.24 ± 0.04^{e}	1.46 ± 0.13^{e}	8.63 ± 0.98^{c}	10.28 ± 1.05^{cd}
	100	92.17 ± 7.71^{b}	7.61 ± 0.79^{abc}	19.30 ± 1.63^{ab}	4.63 ± 0.39^{a}	9.28 ± 1.04^{ab}	11.31 ± 1.15^{b}	12.65 ± 1.39^{d}	422.93 ± 48.25^{a}	7.59 ± 0.80^{c}	8.50 ± 0.94^{de}
	200	64.50 ± 6.15^{cd}	4.64 ± 0.46^{de}	11.61 ± 1.08^{cd}	3.40 ± 0.31^{b}	7.04 ± 0.77^{cd}	7.53 ± 0.68^{d}	35.91 ± 3.67^{b}	1035.4 ± 95.02^{ab}	6.44 ± 0.69^{bc}	5.94 ± 0.68^{e}
10	0	120.13 ± 9.43^{a}	8.91 ± 0.75^{a}	21.05 ± 1.86^{a}	4.78 ± 0.45^{a}	11.22 ± 1.09^{a}	14.25 ± 1.22^{a}	0.30 ± 0.03^{e}	1.60 ± 0.23^{e}	15.90 ± 1.31^{a}	18.12 ± 2.17^{a}
	100	90.07 ± 8.18^{b}	7.30 ± 0.64^{bc}	17.76 ± 1.58^{ab}	4.56 ± 0.40^{a}	8.65 ± 0.80^{bc}	10.66 ± 0.99^{bc}	17.52 ± 1.84^{cd}	229.16 ± 49.24^{d}	13.65 ± 1.28^{ab}	15.34 ± 1.23^{ab}
	200	62.39 ± 6.31^{cd}	4.57 ± 0.43^{e}	11.15 ± 1.21^{cd}	3.33 ± 0.41^{b}	6.64 ± 0.68^{cd}	7.15 ± 0.81^{d}	38.79 ± 4.34^{b}	947.44 ± 86.99^{b}	12.04 ± 1.16^{b}	13.38 ± 1.38^{bc}

Table 2
Interactive effects of selenium and chromium on photosynthesis traits. Effects of selenium supplementation (Se) (0, 5 and 10 μM) and chromium (Cr) (0, 100 and 200 μM) treatments on chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), carotenoids [(mg g^{-1} (FW)], net photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), stomatal conductance (mol H_2O m^{-2} s^{-1}), and photochemical efficiency of PS II (*Fv/Fm*) in the leaves of *Brassica napus* cv. ZS 758.

Se conc. (μM)	Cr conc. (μM)	*Chl a*	*Chl b*	Carotenoids	Net photosynthetic rate	Stomatal conductance	Photochemical efficiency (*Fv/Fm*)
0	0	19.27 ± 1.88^{ab}	8.13 ± 0.72^{ab}	9.17 ± 0.83^{abc}	14.63 ± 1.24^{a}	0.45 ± 0.04^{abc}	0.84 ± 0.06^{a}
	100	16.76 ± 1.53^{bc}	6.42 ± 0.58^{bc}	7.18 ± 0.61^{c}	10.78 ± 1.15^{bc}	0.30 ± 0.03d^{e}	0.62 ± 0.07^{b}
	200	12.23 ± 1.17^{d}	4.44 ± 0.42^{d}	4.26 ± 0.45^{d}	6.65 ± 0.54^{d}	0.17 ± 0.02^{f}	0.38 ± 0.03^{c}
5	0	21.34 ± 1.27^{a}	9.65 ± 0.81^{a}	9.85 ± 0.94^{ab}	15.09 ± 1.36^{a}	0.48 ± 0.04^{ab}	0.84 ± 0.06^{a}
	100	20.82 ± 1.61^{ab}	7.65 ± 0.74^{b}	8.40 ± 0.77^{bc}	12.88 ± 1.26^{ab}	0.39 ± 0.05^{bc}	0.73 ± 0.07^{ab}
	200	14.59 ± 1.32^{cd}	4.85 ± 0.49^{cd}	4.82 ± 0.44^{d}	7.87 ± 0.73^{cd}	0.22 ± 0.03^{ef}	0.45 ± 0.05^{c}
10	0	22.04 ± 1.21^{a}	10.03 ± 0.91^{a}	10.94 ± 0.93^{a}	15.28 ± 1.32^{a}	0.50 ± 0.04^{a}	0.85 ± 0.06^{a}
	100	19.82 ± 1.78^{ab}	7.45 ± 0.75^{b}	7.76 ± 0.72^{c}	12.22 ± 1.13^{ab}	0.37 ± 0.04^{cd}	0.69 ± 0.07^{ab}
	200	13.48 ± 1.28^{cd}	4.69 ± 0.42^{cd}	4.48 ± 0.55^{d}	7.47 ± 0.72^{d}	0.20 ± 0.02^{f}	0.42 ± 0.04^{c}

Values were triplicate (minimum) of each treatment followed by the same letters with no significant difference at P ≤ 0.05 according to Duncan's Multiple Range Test.

10 μM Se (46% in roots) (Table 1). These findings revealed the higher deposit of Cr in roots when compared with leaves and Cr-accumulation in plant tissues was greatly restricted by the Se-supplementation.

3.2. Selenium reduces the chromium toxicity by enhancing plant growth, biomass accumulation and photosynthetic traits

As expected, no significant dissimilarity in results was observed in Se and Cr contents under untreated control conditions. Among Cr alone treatments, 200 μM Cr depicted a significant decline in leaf fresh/dry (49/52%) biomass, root fresh/dry (51/37%) biomass, stem length (42%), root length (54%), *Chl a* (36%), *Chl b* (45%), carotenoids (53%), net photosynthetic rate (55%), stomatal conductance (62%), and *Fv/Fm* (54%) in parallel to their controls. While, Se-supplementation reversed the Cr-induced inhibition (particularly at 5 μM Se + 100 μM Cr) as revealed by the increase in leaf fresh/dry (9/19%) weight, root fresh/dry (25/18%) weight, stem length (36%), root length (38%), *Chl a* (24%), *Chl b* (19%), carotenoids (17%), net photosynthetic rate (19%), stomatal conductance (32%), and *Fv/Fm* (18%) in comparison with other Se + Cr treatments (Tables 1 and 2). Overall, Se at 5 μM was found more effective in mitigating the Cr stress on growth and photosynthetic parameters.

3.3. Selenium improves the nutrient homeostasis under chromium stress

Under Cr alone treatments, a significant inhibition in the transportation of macro (N, P, K), micro (Zn, Mn, Fe) nutrients was noticed in the leaves and roots. In comparison with Cr alone treatments, the decline in minerals uptake was maximum at 200 μM Cr dose in leaves/roots. The applied Se inverted the Cr-induced inhibition as noticed by the increase in nutrients uptake by leaves/roots, especially at 5 μM + 100 μM Cr (Table 3). Notably, both Se (5 and 10 μM) doses strengthen the nutrients homeostasis by restricting the inhibition in minerals transport indulged by Cr stress.

3.4. Selenium reduces the ROS-induced oxidative damages and maintains membrane integrity by reducing chromium stress

No substantial alteration in the values of H_2O_2, $O_2^{\bullet -}$ and MDA contents was noticed under non-stress conditions of Cr and Se alone treatments. Increasing Cr alone concentrations in growth media showed significant accumulation of H_2O_2, $O_2^{\bullet -}$ and MDA contents in leaves/roots, mainly at 200 μM Cr. Conversely, Se-supplementation restricted the Cr-induced accumulation in oxidative markers by reducing the H_2O_2 (23/17%), $O_2^{\bullet -}$ (15/19%) and MDA (16/17%) levels with maximum efficacy at 5 μM Se (Fig. 1A–C). These findings confirmed that lower Se-supply noticeably declined the Cr-induced oxidative damages and uphold membrane integrity.

3.5. Selenium inhibits the MG-induced oxidative injury and enhances glyoxalase defense system under chromium stress

Under Se and Cr alone treatments, no noticeable changes in MG levels were noticed. A significant upsurge in MG content was noted with the increasing Cr doses in nutrient solution. The extreme acceleration in MG content was observed at 200 μM Cr by 48% in leaves as compared with 100 μM Cr and relative controls. Contrarily, the application of Se doses considerably lowered the MG levels by 10% and 13% under 5 μM Se + 100 μM Cr and 10 μM Se + 100 μM Cr, respectively in comparison with other Se + Cr treatments (Fig. 1D). Whereas, Gly (I and II) enzyme activities showed increasing trend in values under Cr alone treatment. The maximum proliferation in glyoxalase enzyme activities was noted at 200 μM Cr by 49% (Gly I) and 38% (Gly II). Se-supplementation markedly enhanced the glyoxalase defense system by

Table 3
Interactive effects of selenium and chromium on the uptake of nutrients element. Effects of different treatments of selenium (Se) (0, 5 and 10 μM) and chromium (Cr) (0, 100 and 200 μM) on the macro- and micronutrients in the leaves and roots of *Brassica napus* cv. ZS 758.

Se conc. (μM)	Cr conc. (μM)	Leaves (mg g^{-1} DW)						Roots (mg g^{-1} DW)					
		N	P	K	Zn	Mn	Fe	N	P	K	Zn	Mn	Fe
0	0	29.05^{a}	17.65^{a}	48.71^{a}	0.19^{ab}	0.085^{a}	0.34^{a}	16.47^{a}	11.36^{a}	59.81^{a}	0.68^{a}	0.062^{a}	0.87^{a}
	100	24.01^{ab}	14.82^{ab}	42.94^{abc}	0.14^{bc}	0.071^{abc}	0.23^{bcd}	11.98^{bc}	8.59^{b}	51.21^{ab}	0.51^{bcd}	0.049^{abc}	0.70^{abc}
	200	17.24^{c}	10.68^{c}	33.94^{c}	0.08^{c}	0.063^{c}	0.17^{d}	6.84^{d}	4.38^{c}	42.37^{b}	0.36^{d}	0.037^{d}	0.58^{d}
5	0	27.40^{a}	14.9^{ab}	47.70^{ab}	0.20^{a}	0.080^{abc}	0.27^{abc}	17.03^{a}	10.57^{ab}	56.37^{a}	0.62^{ab}	0.055^{abc}	0.79^{abc}
	100	28.19^{a}	17.23^{a}	48.23^{a}	0.19^{ab}	0.083^{ab}	0.30^{ab}	15.5^{ab}	10.96^{ab}	58.57^{a}	0.60^{ab}	0.060^{ab}	0.83^{ab}
	200	19.66^{bc}	12.16^{bc}	38.06^{abc}	0.11^{c}	0.070^{abc}	0.22^{cd}	8.66^{cd}	5.54^{c}	47.96^{ab}	0.43^{cd}	0.046^{bcd}	0.67^{bcd}
10	0	27.94^{a}	16.86^{a}	47.61^{ab}	0.21^{a}	0.087^{a}	0.31^{a}	17.91^{a}	10.93^{ab}	57.54^{a}	0.69^{a}	0.059^{ab}	0.82^{abc}
	100	26.87^{a}	16.61^{a}	47.76^{ab}	0.17^{ab}	0.077^{abc}	0.27^{abc}	15.31^{ab}	10.14^{ab}	57.86^{a}	0.58^{abc}	0.054^{abc}	0.79^{abc}
	200	19.17^{bc}	11.67^{bc}	37.01^{bc}	0.10^{c}	0.065^{bc}	0.20^{cd}	8.17^{d}	5.11^{c}	46.87^{ab}	0.42^{d}	0.043^{cd}	0.63^{cd}

Values were triplicate of each treatment followed by the same letters with no significant difference at P ≤ 0.05 according to Duncan's Multiple Range Test.

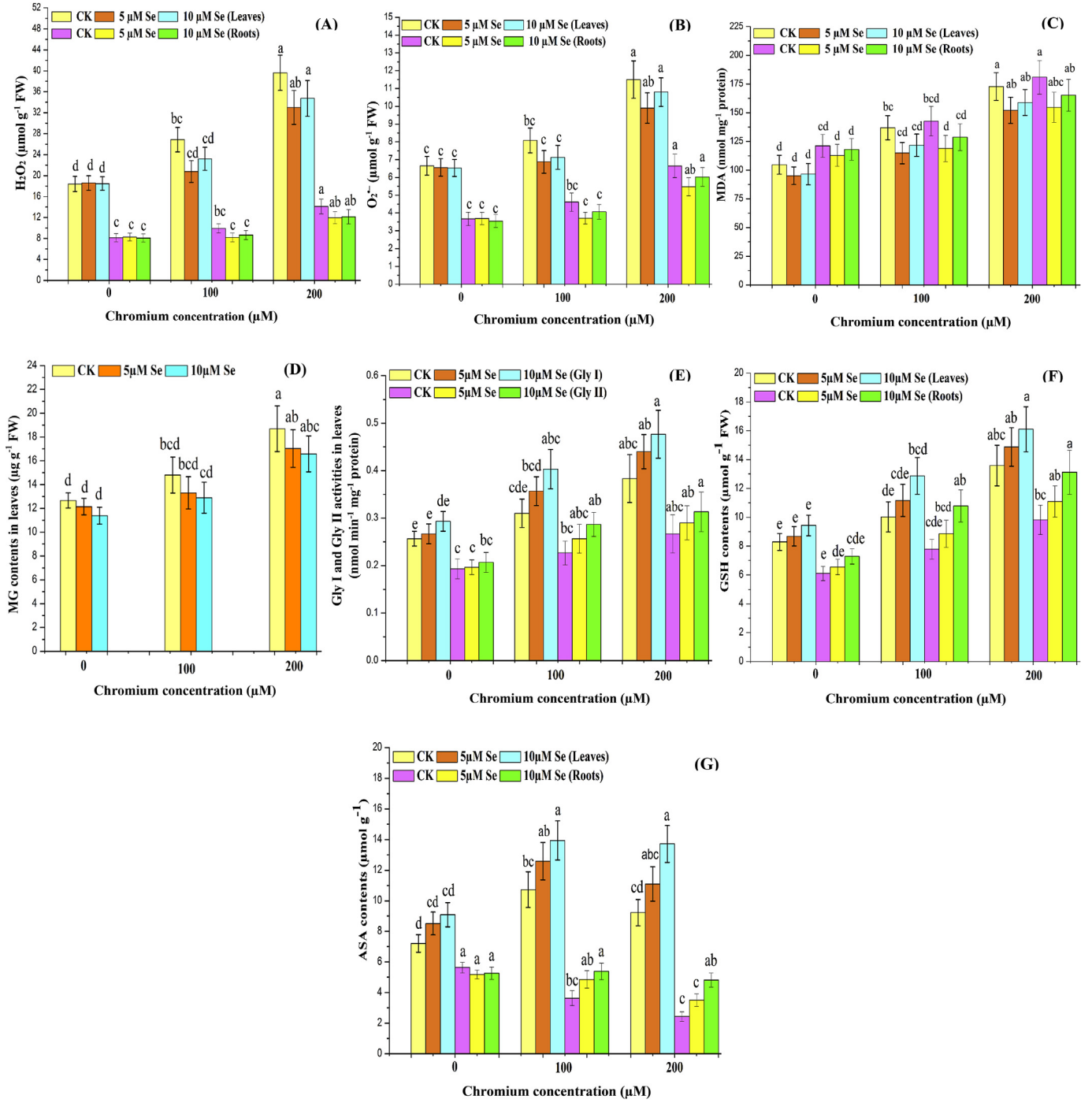

Fig. 1. Interactive effects of selenium and chromium on reactive oxidative species, glyoxalase system and AsA-GSH levels. Effects of different treatments of selenium (Se) (0, 5 and 10 µM) and chromium (Cr) (0, 100 and 200 µM) on the H_2O_2 (nmol mg^{-1} FW), $O_2^{\bullet -}$ (nmol mg^{-1} FW), MDA (nmol mg^{-1} protein), methylglyoxal (MG), glyoxalase enzymes (Gly I and Gly II), reduced glutathione (GSH) (µmol/g DW), and ascorbic acid (AsA) (µmol/g DW) in leaves and roots of *Brassica napus* cv. ZS 758.

escalating the Gly I and Gly II enzyme activities under different Cr treatments (100 and 200 µM), respectively (Fig. 1E). This proposed the protective role of Se against MG-induced oxidative injuries (mainly in roots).

3.6. Selenium regulates the GSH and AsA levels under chromium stress

The alone and non-stress treatments of Se and Cr showed no significant alterations in GSH and AsA levels. Higher levels of Cr alone (particularly at 200 µM) in nutrient solution significantly enhanced the GSH levels (64/61%) in leaves/roots. However, AsA levels were enhanced (49%) up to 100 µM Cr and then start declining (28%) until 200 µM Cr in leaves. But, decreasing trends in AsA levels were noted in roots mainly at 200 µM Cr (57%). The addition of Se in nutrient solution had greatly boosted the GSH and AsA levels as compared to relative controls. The maximum increase in GSH (48/97%) and AsA (28/39%) (leaves/roots) levels was observed under 10 µM Se + 100 µM Cr and 10 µM Se + 200 µM Cr treatments, respectively (Fig. 1F–G).

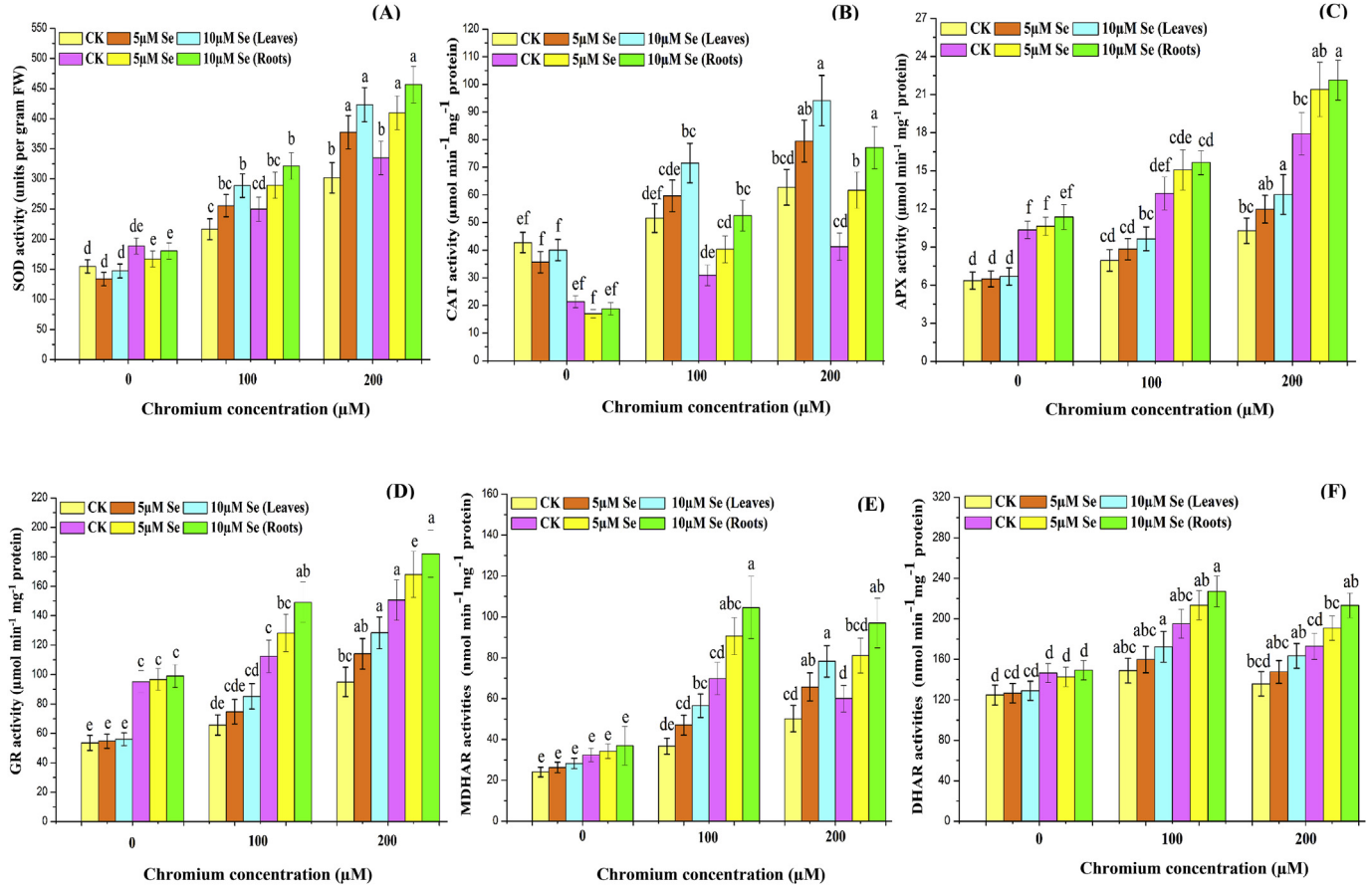

Fig. 2. Interactive effects of selenium and chromium on antioxidants and enzymes of AsA-GSH cycle. Effects of different treatments of selenium (Se) (0, 5 and 10 μM) and chromium (Cr) (0, 100 and 200 μM) on the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) in leaves and roots of *Brassica napus* cv. ZS 758.

3.7. Selenium strengthens the ROS-detoxifying enzymes of AsA-GSH cycle under chromium stress

Alone but non-stress treatments of Se and Cr showed no notable changes in the enzymes used for ROS-detoxification and AsA-GSH cycle. The Cr alone treatments significant induced the activities of AsA-GSH cycle (SOD, CAT, GR, APX, MDHAR and DHAR) enzymes in parallel to control conditions. The maximum induction in these enzymes was found at 200 μM Cr. Moreover, activities of DHAR (leaves/roots) and MDHAR (roots) enzyme were enhanced (19/33%) till 100 μM Cr and then showed decreasing trends (9/17%) until 200 μM Cr. Se-supplementation in nutrient solution further boosted the antioxidant protecting system against ROS as revealed by the increase in SOD (40/36%), CAT (49/86%), GR (35/21%), APX (28/24%), DHAR (20/23%), MDHAR (56/61%) in leaves/roots mainly at 10 μM Se + 200 μM Cr in comparison with other Se + Cr treatments (Fig. 2A–F). The enhancement in antioxidant enzymes under Se + Cr treatments than Cr alone stress suggested the greater potential of Se in scavenging the Cr-induced over production of ROS.

3.8. Selenium ameliorates essential amino acids level under chromium stress

The Cr-alone treatments in nutrient solution significantly declined the essential amino acids level (EAAs) including Isoleucine (Ile), Leucine (Leu), Valine (Val), Lysine (Lys), Threonine (Thr), Phenylalanine (Phe), Methionine (Met) in plant tissues (leaves and roots) (Fig. 3A–G). Most substantial decline in EAAs was observed at 200 μM Cr by 39/50% for Ile, 33/46% for Leu, 38/53 for Val, 35/47% for Lys, 46/60% for Thr, 25/37% for Phe, and 28/42% for Met in leaves/roots, respectively as compared to relative controls. The addition of Se doses in nutrient solution restored the EAAs by enhancing the Ile (21/28%), Leu (24/31%), Val (27/37%), Lys (29/36%), Thr (32/40%), Phe (27/41%), and Met (29/40%) levels in leaves/roots, mainly at 10 μM Se + 200 μM Cr. These results confirmed that Se improves the amino acid metabolism/biosynthesis by lowering the Cr-induced down-regulation in EAAs.

3.9. Selenium alleviates the chromium-induced ultrastructural damages

The ultra-structural alterations in the roots of *B. napus* were examined due to the greater Cr-accumulation in roots than leaves. Under non-stress conditions for Cr alone treatments, root tip cells showed distinct nucleus with well-shaped nucleolus and nuclear membrane. In addition, a visible and smooth cell wall and mature mitochondria (oval in shape) was observed. The organelles of cytoplasmic reticulum such as vacuoles were located abundantly near nuclei in cell wall (Fig. 4A). The Cr at 200 μM induced severe damages in root tip cells as depicted in the broken cell wall, ruptured nucleus with nucleolus and un-cleared nuclear membrane. A de-shaped and swollen mitochondrion with disrupted cristae was noticed. While, cytoplasmic organelles were depleted (Fig. 4B). The supply of Se in Cr-containing solution (5 μM Se + 200 μM Cr) significantly recovered the Cr-induced cellular damages which led to distinct and thick cell wall, well-shaped nucleus with smooth nucleolus, number of cytoplasmic organelle and mature mitochondria (some smaller in size) were observed in root tip cells (Fig. 4C).

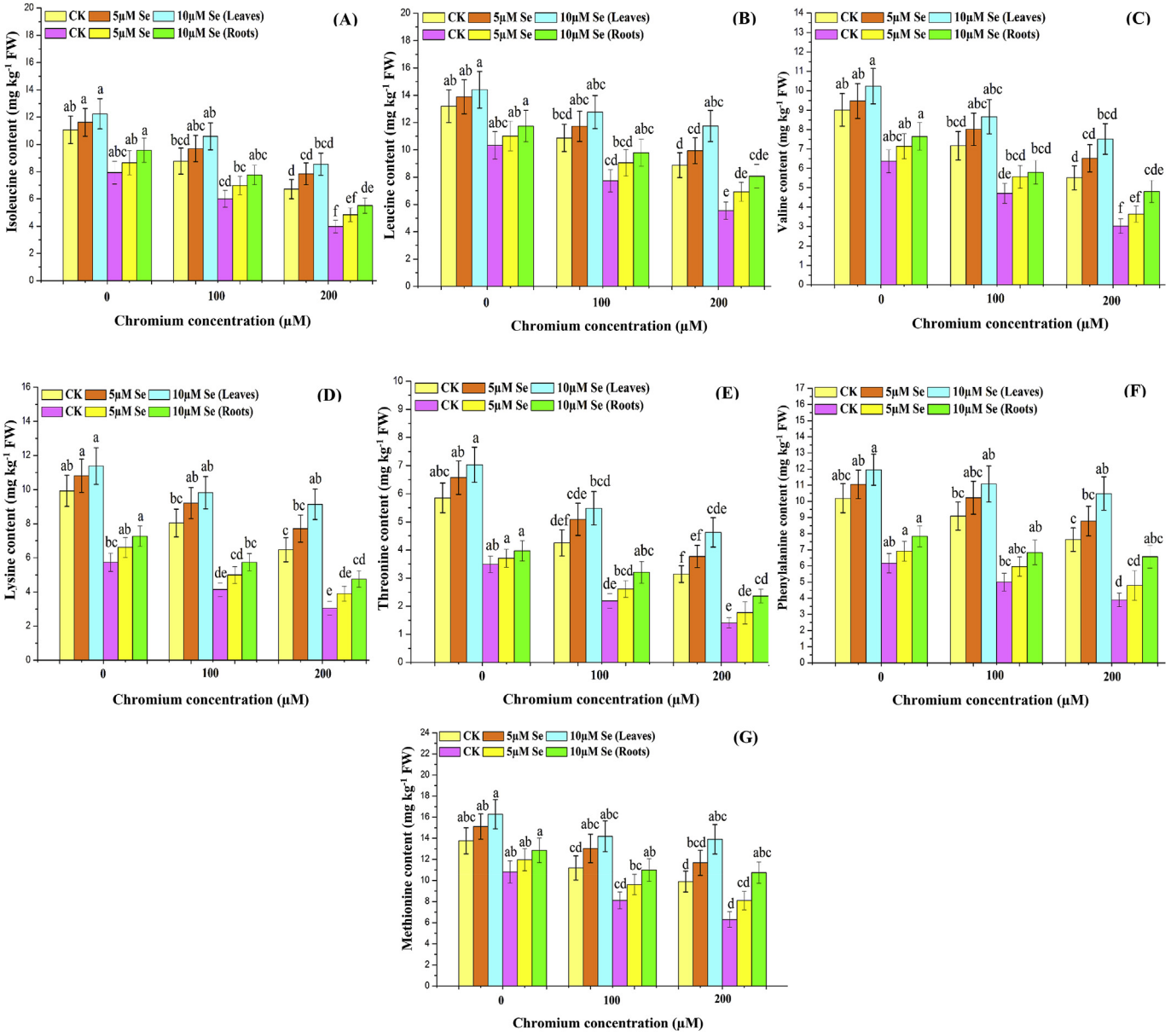

Fig. 3. Interactive effects of selenium and chromium on amino acids metabolism. Effects of different treatments of selenium (Se) (0, 5 and 10 μM) and chromium (Cr) (0, 100 and 200 μM) on the biosynthesis of essential amino acids (EAAs) including Isoleucine (Ile), Leucine (Leu), Valine (Val), Lysine (Lys), Threonine (Thr), Phenylalanine (Phe), and Methionine (Met) in leaves and roots of *Brassica napus* cv. ZS 758.

4. Discussion

The objective of the present study was to illuminate the potential mechanisms adopted by Se to alleviate the Cr-phytotoxicity by focusing on the nutrients uptake, antioxidants system and amino acids metabolism. The inhibitions in root growth (directly linked with Cr-accumulation) are the primary target sites of Cr exposure and its toxic symptoms (Table 1) as clearly matched with Handa et al. (2019) in the roots of *B. juncea*. Possibly, Cr may cause the delay in cell cycle duration, cell division and cell elongation rate that slowdown the root development (Singh and Prasad, 2019). Nevertheless, Se displayed potential in developing the root anatomy under heavy metals stress (Qin et al., 2018; Zhao et al., 2019). Similar results were obtained in our study that Se reversed the Cr-induced adverse effects on root growth (Table 1). Perhaps low Se levels improved the root elongation by inducing the auxin level in plant roots (Jia et al., 2018). In addition, our findings depicted that Cr alone concentrations considerably inhibited the overall plant growth (especially root length) (Table 1). The Cr-induced inhibition in root growth may hinders the nutrients availability to aerial plant parts (Table 3) and ultimately reduces the shoot growth (Table 1). Matched findings were observed by Handa et al. (2018a) and Zhao et al. (2019) in *B. juncea* and *B. campestris,* respectively that Cr inhibited the nutrients uptake which ultimately limited the shoot and root growth. Besides, the growth promoting effects of Se on *Brassica* plants under Cr stress can be linked with the positive role of Se on nutrients uptake (Table 3) and carbohydrate metabolism that ameliorates the plant growth (Malik et al., 2011).

The reduction in biomass accumulation (mainly fresh weight) by Cr can be explained on the basis of decline in light harvesting components (*Chl a, Chl b*, carotenoids) and PSII photochemistry (Table 1). The inhibition in photosynthetic electron transport chain may cause the dysfunction of photosystem II and thus reduced the plant biomass. The addition of Se in Cr treated plants enhanced the biomass accumulation which reflected the greater synthesis of chlorophyll pigments to gain biomass as reported by Chauhan et al. (2017) in *Oryza sativa* under As stress. In present study, the inhibition in light harvesting pigments (*Chl*

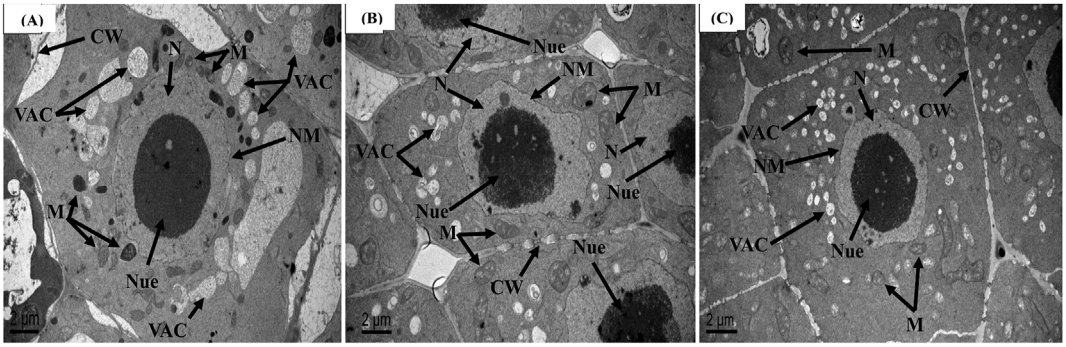

Fig. 4. Interactive effects of selenium and chromium on ultra-structure alterations. Transmission electron microscopic analysis of root tip cells of *Brassica napus* (cv. ZS 758) with different treatments of chromium (Cr) and selenium (Se); (A) control, (B) 200 μM Cr, and (C) 5 μM Se + 200 μM Cr. (A) Under non-stress control conditions, the micrographs of root tip cells displayed well-developed nucleus (N) with nucleolus (Nue) and nuclear membrane (NM). A mature mitochondria (M) and rich organelles of cytoplasmic reticulum such as vacuole (VAC) were observed. (B) Under 200 μM Cr alone stress, severe damages in the form of broken cell wall (CW), ruptured nucleus (N) with nucleolus (Nue) and de-shaped nuclear membrane (NM) were noted. A swollen mitochondria and depleted cytoplasmic organelles were observed. (C) Se-supply alleviated the ultrastructural damages induced by Cr which resulted in clear and thick cell wall (CW), well-developed nucleus (N) with smooth nucleolus (Nue), a number of small vacuole (VAC) and mature mitochondria (although some are smaller in size).

a, *Chl b* and carotenoids) induced by Cr (Table 2) maybe due to the reduced activities of chlorophyll biosynthetic enzymes (δ-aminolevulinic acid dehydratase and protochlorophyllide reductase) that impaired the biosynthesis pathways for chlorophyll pigments (Ganesh et al., 2008). Another justification for lower chlorophyll contents under Cr stress maybe the reduction in Fe accumulation required for chlorophyll biosynthesis and reduced activities of heme-based enzymes (Dixit et al., 2002). The reduced level of photosynthetic pigments due to the decline in the activities of δ-aminolevulinic acid dehydratase was also observed in Cr exposed *Nymphaea alba* (Rodriguez et al., 2012). The application of Se enhanced the total chlorophyll contents and carotenoids in Cr treated plants (Table 2) which suggested the protective role of Se on chlorophylls by improving the Fe accumulation, respiration rate and facilitated the biosynthesis of light harvesting pigments. Handa et al. (2018b, 2019) also reported that Se reversed the Cr-induced chlorophyll degradation in *B. juncea*. The increase in carotenoids (scavenger of singlet oxygen species) by Se suggested the importance of carotenoids in balancing the ROS production as reported by Saidi et al. (2014) in *Helianthus annuus* under Cd stress.

In current study, Cr adversely affected the gas exchange parameters (Table 2). The reduction in pigments biosynthesis by Cr may cause the inhibition in net photosynthetic rate and stomatal conductance (Singh et al., 2013; Gill et al., 2015a, Handa et al., 2019). Also, Cr might have obstructed the electron transport and carbon fixation that decline the light harvesting (Nichols et al., 2000). While, Se was noted to be beneficial by improving the gas exchange parameters in *B. napus* plants subjected to Cr stress (Table 2). The reason for enhanced photosynthetic efficiency by Se may be the improvement in respiration intensity and photosynthetic rate that reinforce the biosynthesis of chlorophyll pigments under stress conditions. Matched findings were observed by Qin et al. (2018) and Handa et al. (2019) in *T. aestivum* and *B. juncea* under Cd and Cr stress, respectively. The reduction in photochemical efficiency of PSII (*Fv/Fm*) by Cr declined the net photosynthesis (Table 2) which suggested the partial inhibition in electron transport (PSII to PSI) that destructed the antenna pigments (Mallick and Mohn, 2003). The efficiency of PSII was improved by Se application under Cr stress suggested that increase in electron transport chain may help plants to restrict the production of singlet oxygen species at PSII and thus boosted the PSII activity. A relatively higher Cr-accumulation in roots than leaves (Table 1) indicated the greater retention efficiency of Cr uptake by roots which limited the Cr transport into aerial plant parts (Do et al., 2018). Thus, higher Cr levels in roots and lower translocation from root to shoot which was clearly coordinated with previous outcomes (Gill et al., 2015a; Zhao et al., 2019). The addition of Se reduced the Cr-accumulation by restricting its uptake from roots to above plant parts. Probably, Se enhanced the pectin and hemicelluloses contents (used to bind metal ions) in root cell walls which was in accordance with the increase in Cr concentration in root cell wall (Zhao et al., 2019). Collectively, the presence of Se, pectin and hemicelluloses might be crucial in binding Cr ions to the root cell walls and thus affecting the intracellular distribution of Cr.

There are less reports regarding the potential effects of Se on the nutrient's uptake in Cr stressed plants. As expected, the Cr alone treatments significantly declined the macro and micro-nutrients uptake (Table 3). Due to the ionic resemblance with key nutrient elements (Ca, Mg, Fe, Zn and K), the excessive Cr levels may displace/interfere with nutrients uptake and their translocation (Handa et al., 2018a, 2019). While, Se showed the ability to boost the contents of mineral nutrients under Cr stress as shown by the enhanced mineral contents (N, P, K, Zn, Mn and Fe) mainly in roots than leaves (Table 3). Most probable reason is that Se-supply alleviated the Cr-induced membrane damages by reducing the production of lipid peroxidation, and ROS (H_2O_2 and $O_2^{\bullet-}$) that ameliorated the nutrients accumulation. Similar outcomes were reported by Handa et al. (2018a, 2019) in *B. juncea* and Zhao et al. (2019) in *B. campestris*. In the present study, Cr alone treatments induced the accumulation of ROS (H_2O_2 and $O_2^{\bullet-}$) and MDA levels (Fig. 1A–C) which may be the result of cellular damages in plant tissues induced by Cr (Gill et al., 2015a). The interaction of heavy metals with the carriers of electron transport chain might also have induced the ROS-accumulation (Singh et al., 2013) which was directly-linked with the lipid peroxidation (Fig. 1C) and chlorophyll fluorescence data (Table 2) that impaired the membrane stability (Singh et al., 2016). Se-supply mitigates the Cr-induced oxidative injuries by minimizing the excessive production of ROS and maintains cellular integrity by reducing the excessive production of lipid peroxidation (Fig. 1A–C). Similar research on Cr-stressed *B. juncea* revealed the protective role of Se against Cr-triggered membrane damages by minimizing the lipid peroxidation as MDA, H_2O_2, and $O_2^{\bullet-}$ contents (Handa et al., 2019), which provide the basis for the higher accumulation of nutrients element (Table 3). In current study, the Cr-induced ultrastructural damages in root tip cells (Fig. 4A–C) were predominantly due to the overproduction of ROS (Fig. 1A–B) as also reported previously (Gill et al., 2015a; Do et al., 2018). While, Se-supplementation reduced the Cr-induced ROS production and ultra-structure distortions (Fig. 4A–C) by minimizing the oxidative damages which ultimately maintain cellular structure, cellular integrity and increased membrane stability (Alyemeni et al., 2018; Zhao et al., 2019).

To counteract the provoked ROS, plant cells strengthen their antioxidant defense system. The present study revealed that Cr alone treatments enhanced the SOD and CAT activities in plant tissues

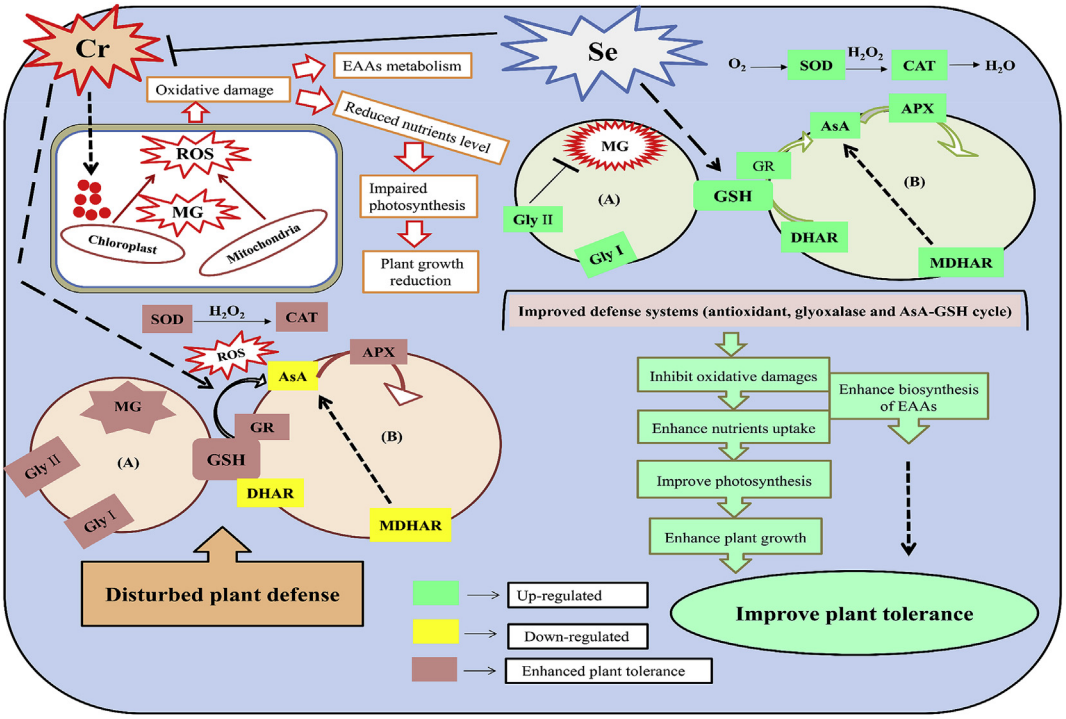

Fig. 5. Protective mechanisms of selenium against Cr phytotoxicity: A schematic diagram was illustrated to highlight the protective role of selenium in mitigating Cr toxicity on *Brassica napus* L. seedlings. Excessive Cr levels (mainly 200 μM Cr) induced the oxidative damages by overproduction of MG and ROS components that ultimately disturbed the EAAs metabolism, nutrients accumulation, photosynthetic efficiency and plant growth. In addition, Cr impairs the plant defense system by desynchronizing the AsA-GSH cycle and ROS-detoxifying enzymes by the down-regulation in AsA level, CAT, DHAR and MDHAR activates. The supplementation of Se enhanced the plant tolerance ability against Cr stress by up-regulating the antioxidants (SOD and CAT), glyoxalase enzymes (Gly I and II), AsA-GSH levels and enzymes of AsA-GSH cycle (APX, GR, MDHAR, and DHAR) and lowering the ROS and MG levels. The up-regulation in defense system caused the depletion in oxidative damages, increased nutrients uptake, enhanced EAAs levels, and improved photosynthesis which is directly linked with the plant growth and biomass production. (**A** and **B**) Indicates the glyoxalase system (Gly) and AsA-GSH cycle, respectively. $O_2^{\bullet-}$ (superoxide radical), H_2O_2 (hydrogen peroxide), SOD (superoxide dismutase), CAT (catalase), APX (ascorbate peroxidase), GR (glutathione reductase), MDHAR (monodehydroascorbate reductase), DHAR (dehydroascorbate reductase), GSH (reduced glutathione), AsA (ascorbic acid), MG (methylglyoxal), and essential amino acids (EAAs).

(Fig. 2A–B) which may be the result of activation in antioxidant defense system against Cr stress. The upregulation in SOD and CAT activities under Cr treatments was also found in *B. oleracea* (Ahmad et al., 2017), *B. juncea* (Handa et al., 2019), and *B. napus* (Gill et al., 2015a). In addition, comparatively lower Cr level (100 μM) enhanced the AsA-GSH levels but excessive Cr (200 μM) disturbed the AsA-GSH balance (increase in GSH but decrease in AsA) (Fig. 1F–G) At 200 μM Cr, the efficiency of enzymes participating in AsA-GSH cycle was disturbed as noticed by the increase in APX and GR activities, but decrease in MDHAR and DHAR activities (except MDHAR in leaves) (Fig. 2C–F). The increase in antioxidant and activities of AsA-GSH cycle enzymes under lower Cr levels suggested the greater plant tolerance against Cr stress. While the abnormalities in redox balance contributed at excessive Cr levels led to the overproduction of ROS and aggravated the oxidative stress. Nearly matched up-regulation/distortion in AsA-GSH levels and enzymes of AsA-GSH cycle were observed in *B. juncea* (Handa et al., 2019), *B. napus* (Zhang et al., 2018) under Cr stress. Addition of Se significantly boosted the activities of key antioxidant enzymes (SOD and CAT) and enzymes of AsA-GSH cycle (DHAR, APX, GR, and MDHAR) with Cr in same growth media (Fig. 2A–F). The upsurge in above enzymes suggested the greater potential of Se in enhancing plant tolerance against Cr stress and maintains redox balance by detoxifying the Cr induced overproduction of ROS. Previous studies also supported our results that Se-supplementation activated the antioxidants enzymes and those of AsA-GSH cycle under Cr (Handa et al., 2019), Cd (Hasanuzzaman et al., 2012), Cd and Pb (Wu et al., 2016) stress, respectively.

Further, we found a constant escalation in MG levels with the increasing Cr alone levels which indicated an acceleration in oxidative stress (Fig. 1D). While, the induction in Gly I and II activities under Cr alone treatments showed the potential of *B. napus* to handle the excessive production of MG (Fig. 1E). The exposure of Se improved the ability of *B. napus* to handle the oxidative damages by reducing the MG levels and further boosted the Gly enzyme activities (Gly I and II) (Fig. 1D–E). The mechanism of MG-detoxification may involve the adduct formation or cross-connections with Gly enzymes (Li, 2016). Similarly, the Se-induced up-regulation in glyoxalase system was reported in *B. napus* (Hasanuzzaman et al., 2012) under Cd stress. In current study, we observed a significant reduction in the levels of EAAs in response to Cr alone treatments (Fig. 3A–G). The Cr-mediated reduction in EAAs suggested the decrease in protein and nitrogen contents which are precursors of amino acids. The down-regulation of EAAs indicated the direct involvement of Cr in nitrogen metabolism, biosynthesis and degradation pathways for EAAs as reported by Finnegan and Chen (2012). The supply of Se along with Cr notably enhanced the EAAs in the tissues of *B. napus* seedlings. Because sulfur metabolism directly affects the nitrogen metabolism. Therefore, it is implicated that Se promotes the biosynthesis of amino acids (Kumar et al., 2016). Our study showed that an increase in the accumulation of nitrogen contents (Table 3) may also enhanced the quantification of EAAs. In current study, the ameliorative effects of Se on amino acids levels in the tissues of *B. napus* under Cr stress were clearly matched with previous reports by Kumar et al. (2016) in *Oryza sativa* and Handa et al. (2018a) in *B. juncea* under As and Cr stress, respectively.

5. Conclusions

A schematic diagram was illustrated to highlight the potential of Se

in alleviating Cr toxicity in *B. napus* plants (Fig. 5). In the present study, we found that increasing Cr alone (especially 200 μM) levels in nutrient solution significantly inhibited the plant growth and biomass accumulation, photosynthesis traits and nutrients uptake. Also, Cr impaired the cellular membrane integrity by inducing the lipid peroxidation, and overproduction of reactive species (H_2O_2 and $O_2^{\bullet-}$). Excessive Cr disturbed the antioxidant system by enhancing SOD but down-regulated the CAT activities, and imbalanced the AsA-GSH levels and their enzymes as observed by the increase in GSH, APX, GR but decline in AsA, DHAR and MDHAR. Moreover, EAAs level were significantly declined with the increase in Cr dose which indicated the Cr-induce inhibition in amino acids biosynthesis. Se-supplementation in nutrient solution markedly recovered the Cr-induced damages by maintaining the plant growth and biomass, photosynthesis, minerals accumulation and enhances the EAAs level to combat oxidative stress. In addition, Se reduced the Cr and MG induced over generation of reactive oxidative species by lowering their accumulation in plant tissues and enhances the plant ability to handle oxidative damages by up-regulating the enzymatic, non-enzymatic and glyoxalase enzymatic systems. Overall, Se-supplementation (mainly at 5 μM dose) was proved as potential candidate to minimize the Cr accumulation, toxicity and enhance nutritional value by improving EAAs levels in *B. napus*. Results of the present study can provide insights into the protective role of Se in handling the abiotic stress and ameliorate the plants ability to prevent the environmental stress. Further research is needed regarding the Se-pertaining mechanisms in mitigating the heavy metals toxicity to better understand the stress-tolerance strategies adopted by plants.

Author's contributions

ZU is the first and main author. ZU, RAG and WZ designed the experiment. QH, SA, RAG, and TMM helps in data interpretation and analysis. HH, YH, ARK and WZ helps in drafting the research article. BA, RAG, JW and WJ critically revised the manuscript.

Declaration of competing interest

“All authors declared that they have no conflicting concern regarding the submission of this article and its probable publication”.

Acknowledgements

The present work was supported by the National Key Research and Development Program of China (2018YFD0100601), the National Natural Science Foundation of China (31650110476), the Jiangsu Collaborative Innovation Center for Modern Crop Production, the Sino-German Research Project (GZ 1362), the Science and Technology Department of Zhejiang Province (2016C02050-8, LGN18C130007), and the Agricultural Technology Extension Funds of Zhejiang University. We thank Nianhang Rong and Junying Li from the Center of Analysis & Measurement, Zhejiang University for their assistance during Transmission Electron Microscopic (TEM) analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.10.035.

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