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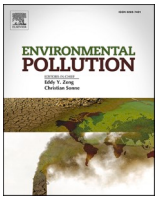

Brassica juncea and the Se-hyperaccumulator *Stanleya pinnata* exhibit a different pattern of chromium and selenium accumulation and distribution while activating distinct oxidative stress-response signatures☆

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ABSTRACT

Soils high in chromium and selenium exist in some countries, like China, India and the US. In the forms of chromate and selenate, these elements can compete during uptake by plants and lead to secondary effects on the absorption of the essential nutrient sulfur. In this study, we evaluated the potential of *Brassica juncea* and the Se-hyperaccumulator *Stanleya pinnata* to take-up and store chromium and selenium when applied individually or jointly, the effect on sulfur content, and the plant antioxidant responses. The aim is to advise the best use of these species in phytotechnologies. Plants were grown hydroponically with 50 μM chromate, 50 μM selenate and equimolar concentrations of both elements (50 μM chromate + 50 μM selenate). Our results suggest that *B. juncea* and *S. pinnata* possess transport systems with different affinity for chromate and selenate. The joint application of chromate and selenate restricted the accumulation of both elements, but the reduction of selenate uptake by chromate was more evident in *B. juncea*. On the other hand, selenate decreased chromium accumulation in *B. juncea*, whereas in *S. pinnata* such effect was evident only in roots. *B. juncea* plants stored more chromium and selenium than *S. pinnata* due to the higher biomass produced, but less selenium when treated with both elements. Chromate and selenate decreased sulfur accumulation in both species, but *B. juncea* was more sensitive to their toxicity when applied individually, as revealed by increased lipid peroxidation, hydrogen peroxide content in roots and antioxidant enzyme activity. This species can still be efficient for chromium and selenium phytoextraction as these elements in soil are less available than in hydroponics. In soils high in both elements, or low in selenium, *S. pinnata* is preferred for selenium phytoextraction and the biomass could be used for crop biofortification due its negligible chromium content.

1. Introduction

The contamination of agricultural soils with heavy metals and/or metalloids is global problem that poses a threat to the environment, humans and animals (He et al., 2015; Khan et al., 2021). Indeed, bioavailable forms of toxic inorganic contaminants can be taken up by plants and accumulated in edible parts, thereby eventually entering the food web. In particular, the increasing accumulation of chromium (Cr) in crops has become a major topic in the context of risk assessment and remediation studies (Costa and Klein, 2006).

Chromium is a notorious harmful contaminant that exists under various oxidation states ranging from −2 to +6. In the environment, Cr is mainly found in the trivalent (Cr^{3+}) and hexavalent (Cr^{6+}) forms (Ao et al., 2022), the former at least 100 times more toxic and carcinogenic than the latter (Pavesi and Moreira, 2020). These two forms are relatively stable and spontaneously interconvert. One of the major reasons these Cr species differ in toxicity lies in the degree of solubility and bioavailability of the element as a function of its valence. Cr^{3+} species are insoluble oxides or hydroxides that dominate in most soils, except in those with a neutral to alkaline pH and low organic matter in oxic

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environment (Zayed and Terry, 2003). In contrast, Cr^{6+} compounds such as chromate and dichromate salts are more soluble and mobile in both acidic and alkaline soils. In particular, sodium chromate (873 g/L at 30 °C) and potassium chromate (629 g/L at 20 °C) are considered highly soluble (OSHA, 2006). Also, the chromate and dichromate ions behave as powerful oxidizing agents under acidic conditions (ε_0 = 0.3–1.33 V) (Jardine et al., 2013).

Chromium naturally occurs in the environment, but its concentration can considerably increase as a result of either atmospheric emissions or discharge of Cr-containing industrial wastes (Coetzee et al., 2020). Almost 1.29×10^5 tons of Cr are released into the environment yearly, and approximately 896 tons of Cr are disposed into the soil, thus raising the risk of soil ecological safety (Shahid et al., 2017; Coetzee et al., 2020; Kapoor et al., 2022). Substantial quantities of Cr are also mined every year, particularly in Kazakhstan, South Africa, China and India (Das et al., 2020). The use of organic fertilizers containing Cr can additionally contribute to Cr pollution of agricultural soils, thus posing a potential health threat to humans via food contamination (Costa and Klein, 2006; Ertani et al., 2017). In fact, although trace amounts of Cr are required for human metabolism, Cr excess can induce carcinogenic effects (Alvarez et al., 2021; IARC, 2012; EFSA, 2014; den Braver-Sewradj et al., 2021).

So far, the acceptable level of Cr in farming soil and parkland for environmental and human health safety has been established at 64 mg kg^{-1} (CEPA, 2007; Srivastava et al., 2021). The transfer of Cr from soil to plants is controlled by several factors, primarily the plant species and the soil properties (Banks et al., 2006; Choppala et al., 2018; Cai et al., 2019). Although Cr hyperaccumulators exist, like *Leersia hexandra* (Zhang et al., 2023), most plants do not require Cr and most of them exhibit toxic symptoms when exposed to Cr concentrations ranging from 0.5 to 5 mg L^{-1} in nutrient solution and 5–100 mg g^{-1} in soil (Singh et al., 2013; Shahid et al., 2017; Sharma et al., 2020; Ao et al., 2022; Kapoor et al., 2022). Cr^{6+} can trigger oxidative stress in plants, alter the uptake of nutrients, impair the photosynthetic apparatus and cause tissue necrosis (Cervantes et al., 2001; Srivastava et al., 2021; Kapoor et al., 2022). Nevertheless, plants can take up both Cr^{3+} and Cr^{6+}. The transport of Cr^{3+} inside the plant's apoplast occurs via cation exchange sites present in the cell walls (Poschenrieder et al., 2019; Kapoor et al., 2022), whereas Cr^{6+} in the form of chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) anions enters the roots of most plants using active mechanisms that implicate nonspecific anionic transporters, such as those involved in the uptake of the analog oxyanions sulfate and phosphate (Schiavon et al., 2007, 2008; 2012; González et al., 2014; López-Bucio et al., 2014; 2022). Recently, Xu et al. (2021) demonstrated that the high-affinity sulfate transporter SULTR1; 2 has a major role in chromate uptake in *Arabidopsis thaliana*.

Chromate exposure can reduce the uptake of sulfur (S) by plants through competition for the sulfate transporters' binding sites, therefore inducing a S starvation-like condition (Schiavon et al., 2007, 2008). Similarly to chromate, selenate can use the sulfate transporters to enter the root cells, especially SULTR1; 2. Selenate is the most oxidized form of the element selenium (Se) (El Kassis et al., 2007). Low concentrations of this oxyanion allow higher sulfur uptake by plants via up-regulation of sulfate transporters' gene expression, while the opposite effect is observed at high concentrations, due to the greater competition of selenate over sulfate for the uptake (White et al., 2004; El Mehdawi et al., 2018; de Souza Cardoso et al., 2022). Selenium naturally occurs in soils and is particularly abundant in the so-called seleniferous soils (Schiavon and Pilon-Smits, 2017). Irrigated agriculture of seleniferous soils or mining of seleniferous fossil fuels can enhance Se release into the environment, which may have catastrophic consequences (Ohlendorf et al., 2020).

Since both Cr and Se contamination occurs in many soils and both oxyanions have similar chemical properties, it is plausible that selenate and chromate co-exist in polluted areas (Roca-Perez et al., 2010; Wolf et al., 2010; Shaheen et al., 2017). The effects of these two oxyanions in terms of absorption and stress physiology in plants may be relevant in those soils (Qing et al., 2015; Handa et al., 2018, 2019; Cai et al., 2019; Zhao et al., 2019). When selenate and chromate concentrations are high in the soil, these oxyanions could influence the uptake of each other by plants, and alter the accumulation of the essential element S. Selenium can also affect the rhizosphere bacterial communities altering Cr and Se uptake by plants (Cai et al., 2019). Therefore, Cr–Se interactions must be considered when plants are used for Cr and/or Se remediation or crop biofortification with Se.

Brassicaceae species are often employed in phytoremediation. Among them, *Brassica juncea* is a high biomass species frequently used in phytoextraction due to its great ability to sequester many inorganic contaminants (Schiavon et al., 2008; Singh and Fulekar, 2012). Another potentially interesting species from the Brassicaceae family is *Stanleya pinnata*: it is native to seleniferous soils in the Western United States and a Se-hyperaccumulator, i.e., a plant species with an exceptional ability to accumulate organic Se in the shoot (above 1% DW) and attain high Se: S ratios (White et al., 2007; Cappa et al., 2014). Although *S. pinnata* is slow-growing and tends to produce less biomass than *B. juncea*, it becomes sufficiently big in the long term to become a potential candidate for use in Se phytoremediation and can be further recycled as green manure for application in low Se soils to enrich crops with organic Se (Bañuelos et al., 2015; 2016; Schiavon et al., 2020). Such a combination of Se phytoremediation and biofortification is appealing because organic Se compounds are rapidly accumulated in crops and exert health promoting effects on consumers. *Stanleya pinnata* plants have been shown to constitutively overexpress root sulfate transporters, which explains why they hyperaccumulate Se and possibly can lead to a high accumulation of other analogous oxyanions, such as chromate (El Mehdawi et al., 2018; Wang et al., 2018).

So far, the interactions of Se and Cr during uptake processes in *B. juncea* and *S. pinnata* have not yet been comparatively investigated. Thus, the current study aims to compare *B. juncea* and *S. pinnata* with respect to their capacity to accumulate and translocate Se and Cr when the two elements are applied, jointly or separately, in order to develop recommendations about their use in different phytotechnologies.

2. Materials and methods

2.1. Plant growth and chlorophyll quantification

B. juncea L. Czern (Cv. PI 426314) and *S. pinnata* (Pursh) Britton seeds were surface-sterilized as described in Schiavon et al. (2015), and kept to germinate for 8 days in half-strength Murashige e Skoog (1962) medium inside a chamber with 14 h light/10 h dark cycle, 26/21 °C air temperature, relative humidity of 70/85%, and photon flux density (PFD) of 280 mol $m^{-2}s^{-1}$. After 9 days, germinated seedlings of similar height (6–8 cm) were transferred to 3 L pots containing a half-strength Hoagland nutrient solution with a density of 6 plants per pot. The nutrient solution was thoroughly aerated and changed every 2 days. After 10 days, plants were supplied for one week with the following Se and Cr concentrations: Se 50 μM, Cr 50 μM, Se 50 μM + Cr 50 μM. Plants that did not receive Se or Cr served as controls. Selenium was applied in the form of sodium selenate (Na_2SeO_4), whereas Cr as potassium chromate (K_2CrO_4). Se and Cr concentrations were chosen based on previous studies (Schiavon et al., 2008, 2015; El-Mehdawi et al., 2018) and preliminary tests. Furthermore, using equimolar concentrations of selenate and chromate served to better evaluate the competitive effects of these oxyanions during root uptake and differences in specificity of the sulfate transport system for Se and Cr between the two species.

For each experimental condition, three pots were prepared, with a density of 6 plants per pot. The experimental design for plant growth was randomized (the pots were re-arranged twice a week). Plants were harvested one week after Se and Cr treatments, washed with distilled water and dried with blotting paper. Nine plants per treatment (3 per pot) were divided into roots and shoots. The plant material was then placed inside a drying oven for 2 days at 70 °C to measure their dry

weight individually. The remaining plants were frozen in liquid nitrogen and kept at −80 °C for further analyses, with the exception of a subsample immediately used for microscopic analyses.

Chlorophyll *a* (λ = 665) and *b* (λ = 649) levels were determined spectrophotometrically by the method of Welburn and Lichtenthaler (1984).

2.2. Elemental content quantification

The plant material (100 mg) was dried for 48 h at 80 °C and further digested in nitric acid as described by Zarcinas et al. (1987). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used as described by Fassel (1978) to determine each digest's Cr, Se and S concentrations.

2.3. TF and enrichment factor

The translocation factor (TF) was calculated as the ratio of the concentration of the element (Se or Cr) in the aerial parts of the plant to the root concentration. The plant Se or Cr enrichment factors were calculated according to the following formulas:

Eq. 1: *Plant Se enrichment* = $(Se_{pt}/Cr_{pt} + S_{pt})/ (Se_{sol}/Cr_{sol} + S_{sol})$

Eq. 2: *Plant Cr enrichment* = $(Cr_{pt}/Se_{pt} + S_{pt})/ (Cr_{sol}/Se_{sol} + S_{sol})$

pt = plant tissues
sol = solution.

2.4. Soluble protein quantification and antioxidant enzyme activity

Frozen samples (200 mg) were ground in a mortar with liquid nitrogen and extracted with 100 mM phosphate buffer (pH 7.8) containing polyvinylpyrrolidone (PVP) (10 g/L) in the ratio of 1:10. Protein content was quantified using the Bradford's method (Bradford, 1976).

The analysis of enzyme activity was performed according to Ghirardelli et al. (2021) in frozen leaves (200 mg) extracted with 50 mM phosphate buffer (pH 7.8) containing PVP (10 g/L) and Triton X-100 (250 μL), in the ratio 1:10 (w/v). Guaiacol peroxidase (GPX) activity was determined by measuring the oxidation of guaiacol in the presence of H_2O_2 (ε_{470} = 26.6 mM cm^{-1}), ascorbate peroxidase (APX) activity was determined following the decrease of ascorbate in the presence of H_2O_2 (ε_{290} = 2.8 mM cm^{-1}), catalase (CAT) activity was determined by following the consumption of H_2O_2 (ε_{240} = 39.4 mM cm^{-1}).

2.5. Determination of total antioxidant activity and phenol content

The total antioxidant activity was evaluated by measuring the ferric-reducing antioxidant power (FRAP). The assay was based on the methodology of Benzie and Strain (1996). Calibration was performed against a standard curve (0–1200 mg mL^{-1} ferrous ion). FRAP values are presented as milligrams per kilogram of Fe^{2+} Eq (ferrous ion equivalents).

The concentration of total phenols was determined using the Folin-Ciocalteu (FC) assay according to Nicoletto et al. (2013) and calculated from a standard calibration curve obtained using gallic acid concentrations ranging from 0 to 600 mg mL^{-1}. Results were expressed as milligrams of gallic acid equivalent per kilogram of FW.

2.6. Lipid peroxidation

The malondialdehyde (MDA) assay was performed according to Ghirardelli et al. (2021). MDA is a product of lipid peroxidation by thiobarbituric acid reaction. The concentration of MDA was calculated from the absorbance at 532 nm (correction was done by subtracting the absorbance at 600 nm for unspecific turbidity) by using ε = 155 mM^{-1} cm^{-1}. Data were expressed as TBARS (thiobarbituric acid reactive substances).

2.7. Light and electron microscopy

Samples of leaves were fixed overnight at 4 °C in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 6.9) and post-fixed at 4 °C for 2 h in 1% osmium tetroxide in the same buffer. The specimens were dehydrated in a graded series of ethyl alcohol and propylene oxide and embedded in araldite. Sections were cut using an ultramicrotome (Ultracut S, Reichert-Jung, Wien, Austria). For light microscopy, thin sections (1 μm) stained with toluidine blue (1% basic toluidine and 1% Na tetraborate, 1:1, v/v) were observed by a DMR 5000 Leica (Sweden) microscope, equipped with a digital image acquisition system. For transmission electron microscopy, ultrathin sections (600 Å) stained with uranyl acetate and lead citrate were observed with a transmission electron microscope (TEM 300, Hitachi, Tokyo, Japan) operating at 75 kV.

2.8. Histochemical detection of H_2O_2 in roots

Hydrogen peroxide (H_2O_2) was detected via histochemical assay in roots of *B. juncea* and *S. pinnata* according to the protocol of Kumar et al. (2014) by using 3,3'-Diaminobenzidine (DAB) as the chromogenic substrate. H_2O_2 is visualized as a reddish-brown stain formed by the reaction of DAB with the endogenous H_2O_2.

2.9. Statistics

Analysis of variance (ANOVA) was performed using the SPSS software, and was followed by pair-wise post-hoc analyses (Student-Newman-Keuls test) to determine which means differed significantly at $p < 0.05$ (± SD). Statistically significant differences are reported in the text and shown in the figures. Biochemical analyses were conducted in triplicates (1 replicate = bunch of 3 plants per pot).

3. Results

3.1. Plant growth and chlorophyll content in response to Se and Cr application

Brassica juncea plants produced more biomass than *S. pinnata* plants during the study period (Fig. 1A and B); however, they were more sensitive to Se and Cr toxicity. In particular, the leaf dry weight of *B. juncea* was reduced by about 16% and 50% by Se and Cr, respectively, while in *S. pinnata* the leaf biomass was increased by Se by about 30% and was not affected by Cr exposure (Fig. 1A). The joint application of Se with Cr in part alleviated the detrimental effects of Cr alone in *B. juncea*, as the leaf biomass was less reduced (by about 28%). In *S. pinnata*, the concomitant supply of Se and Cr did not alter the leaf dry matter compared to the control plants and plants subjected to other Se and Cr treatments.

Selenium was particularly toxic to *B. juncea* roots, whose dry weight decreased by about 42% (Fig. 1B). Nevertheless, as in the case of leaves, the concurrent application of Se and Cr improved the root biomass compared to plants treated with individual Se or Cr. The growth response of *S. pinnata* roots was similar to that described for the leaves, except that the joint application of Se and Cr did not ameliorate the root growth compared to control plants and plants treated with Cr alone.

The content of chlorophyll was decreased in *B. juncea* under any Se and Cr treatment (about 35% by Se, and 50% by Cr and Se + Cr), while it was unchanged in *S. pinnata* (Fig. 1, Supplementary).

3.2. Effects of Se and Cr on leaf anatomy and ultrastructure

In leaves of Se-treated *B. juncea* plants, the mesophyll was not well organized in palisade and spongy parenchyma unlike control plants (Fig. 2A), but consisted of elongated cells, separated by large intercellular spaces. In plants exposed to Cr, a number of cellular layers was

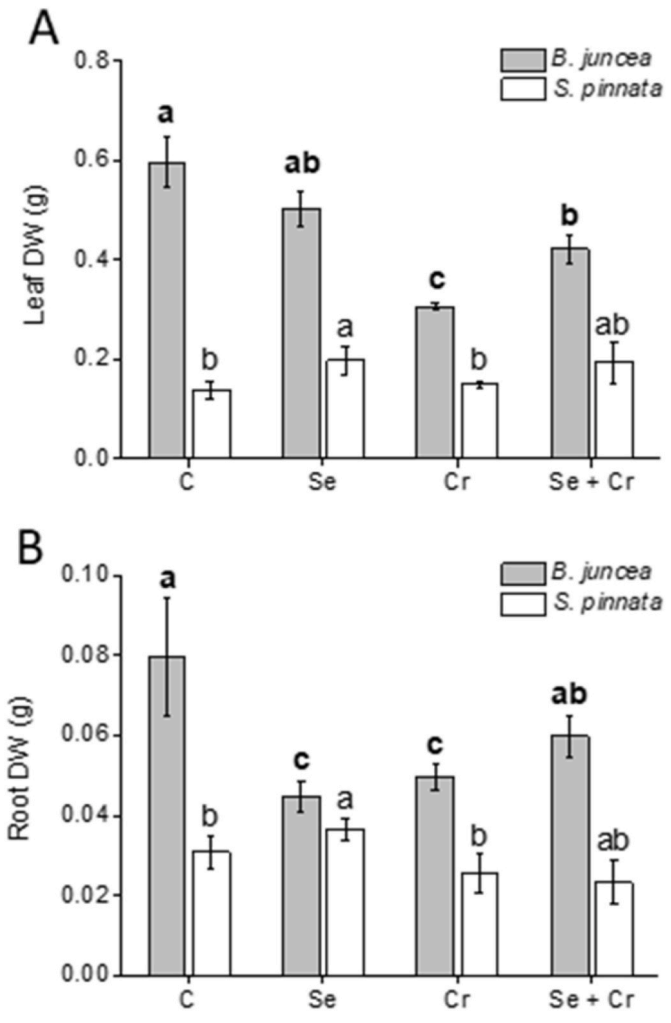

Fig. 1. Leaf (A) and root (B) dry weight (DW) of *B. juncea* and *S. pinnata* plants treated with Se, Cr or combined Cr and Se. Control plants were Cr- and Se-untreated. Different letters above bars indicate significant differences at $p < 0.05$ for *B. juncea* (in bold) and *S. pinnata* (not bolded). When differences are not detected, letters are not reported.

evident between the upper and bottom leaf epidermis. These layers were characterized by elongated cells, large intercellular spaces and a zone occupied by rounded cells and intercellular lacunae attributable to the spongy parenchyma. In plants supplied with both Se and Cr, the leaves showed well-organized palisade and spongy mesophyll layers. The leaf mesophyll of *S. pinnata* control plants was distinctly organized in palisade and spongy parenchyma, with several chloroplasts (Fig. 2B). No such clear anatomical organization was evident in leaves of plants treated with either Se or Cr. In this case, parenchymatic cells were roundish in shape and interspersed by intercellular spaces. In contrast, the leaf mesophyll of plants treated with a combination of Se and Cr was clearly differentiated in palisade and spongy parenchyma.

In *B. juncea*, significant differences were evident between chloroplasts of control plants and Se- and Cr-treated plants; representative images are shown in Fig. 2C and D. Chloroplasts in leaf mesophyll of untreated plants owned a thylakoid system well organized in stacked grana and stroma lamellae (Fig. 2C). Similarly, leaf chloroplasts of Se-treated plants displayed an organized thylakoid system, although grana in this case derived from the stacking of more and conspicuous membranes. In Cr-treated plants, chloroplasts still showed thylakoid membranes, but electrodense masses appeared in the stroma, some of them of fairly large size. In plants receiving both Se and Cr, the thylakoid system was not well arranged. In general, a number of bulky starch granules was observed in chloroplasts of plants treated with Se, Cr or Se plus Cr.

In *S. pinnata* control plants, leaf chloroplasts contained starch and finely organized thylakoid membranes (Fig. 2D). Similarly, the presence of starch granules and thylakoids forming prominent grana was evident in chloroplasts of plants treated with Se. On the contrary, leaf chloroplasts of plants exposed to Cr did not contain starch granules and their thylakoids were altered and dilated. However, when plants were subjected to a combined Cr and Se treatment, bulky starch granules occupied most of the chloroplast stroma. The thylakoid system in these chloroplasts was apparently intact.

3.3. Se and Cr accumulation

Brassica juncea and *S. pinnata* control plants contained negligible amounts of Se and Cr in their tissues (Fig. 3A–D). When exposed to Se and/or Cr, the two species held similar concentrations of Se in their leaves and roots, whereas Cr was preferentially stored in the roots.

S. pinnata plants treated with Se, either alone or jointly with Cr, contained more Se in leaves compared to *B. juncea* (Fig. 3A). However, Cr restricted Se accumulation in both species, and this reduction was more evident for *B. juncea* (84% vs. 50%). On the other hand, Cr accumulated more in *B. juncea* leaves, irrespective of Se addition (Fig. 3B), but Se decreased Cr concentration by 30%. The reduction of Cr accumulation by Se was hardly appreciable in *S. pinnata*.

Insignificant differences in root Se accumulation were determined between the two species treated with Se alone (Fig. 3C). However, the addition of Cr caused a remarkable reduction of Se concentration in *B. juncea* (by 58%). Such an effect was not observed in the roots of *S. pinnata*. Cr accumulated 5-fold more in roots of *B. juncea* than in *S. pinnata* (Fig. 3D). The combined Se and Cr application decreased Cr concentration in the roots of both species, which accounted for 20% in *B. juncea* and 51% in *S. pinnata*.

When the accumulated amount of Se and Cr in the total plant biomass was assayed, *B. juncea* was found to be the species that contained more of these elements per plant, especially in the leaves (Fig. 3E). The addition of Cr reduced Se content in both species, especially in *B. juncea*. Conversely, Se did not significantly affect the amount of Cr in either species (Fig. 3F).

The translocation factor (TF) was up to 40 fold higher for Se than for Cr (Fig. 3G and H). *S. pinnata* owned greater Se translocation capacity than *B. juncea*, but the concomitant application of Cr with Se decreased the Se TF in both species (Fig. 3G). *S. pinnata* was also apparently more efficient in Cr translocation compared to *B. juncea*, but TF was actually extremely low (Fig. 3H). Cr TF was not affected by Se in *S. pinnata*, but slightly reduced in *B. juncea*.

3.4. Effects of Se and Cr on S accumulation

Stanleya pinnata control plants contained 3-fold more S in leaves than *B. juncea* (Fig. 4A). Interestingly, S accumulation in *B. juncea* was enhanced by Se, while sharply reduced in *S. pinnata* (by 76%). Chromium application decreased leaf S concentration by approximately 50% in both species, but when combined with Se it caused a further reduction (80%) of S concentration in *S. pinnata*. The concentration of S in roots was comparable between control plants of the two species (Fig. 4B). The effect of Se and Cr on root S accumulation by *S. pinnata* was similar to that described for leaves, although differences in the degree of reduction were less evident. S accumulation in *B. juncea* was restricted by Se in roots (by 28%), and more prominently by Cr, both when supplied alone or with Se (by 44%).

The Se/S ratio was distinctly higher for *S. pinnata* compared to *B. juncea* (Fig. 4C,E). In leaves, the Se/S ratio was affected by the joint application of Se with Cr in both species, while in roots such an effect was evident only in *B. juncea*. The Cr/S ratio (Fig. 4D–F) was substantially greater in *B. juncea*, but decreased by Se. In *S. pinnata*, Se did not

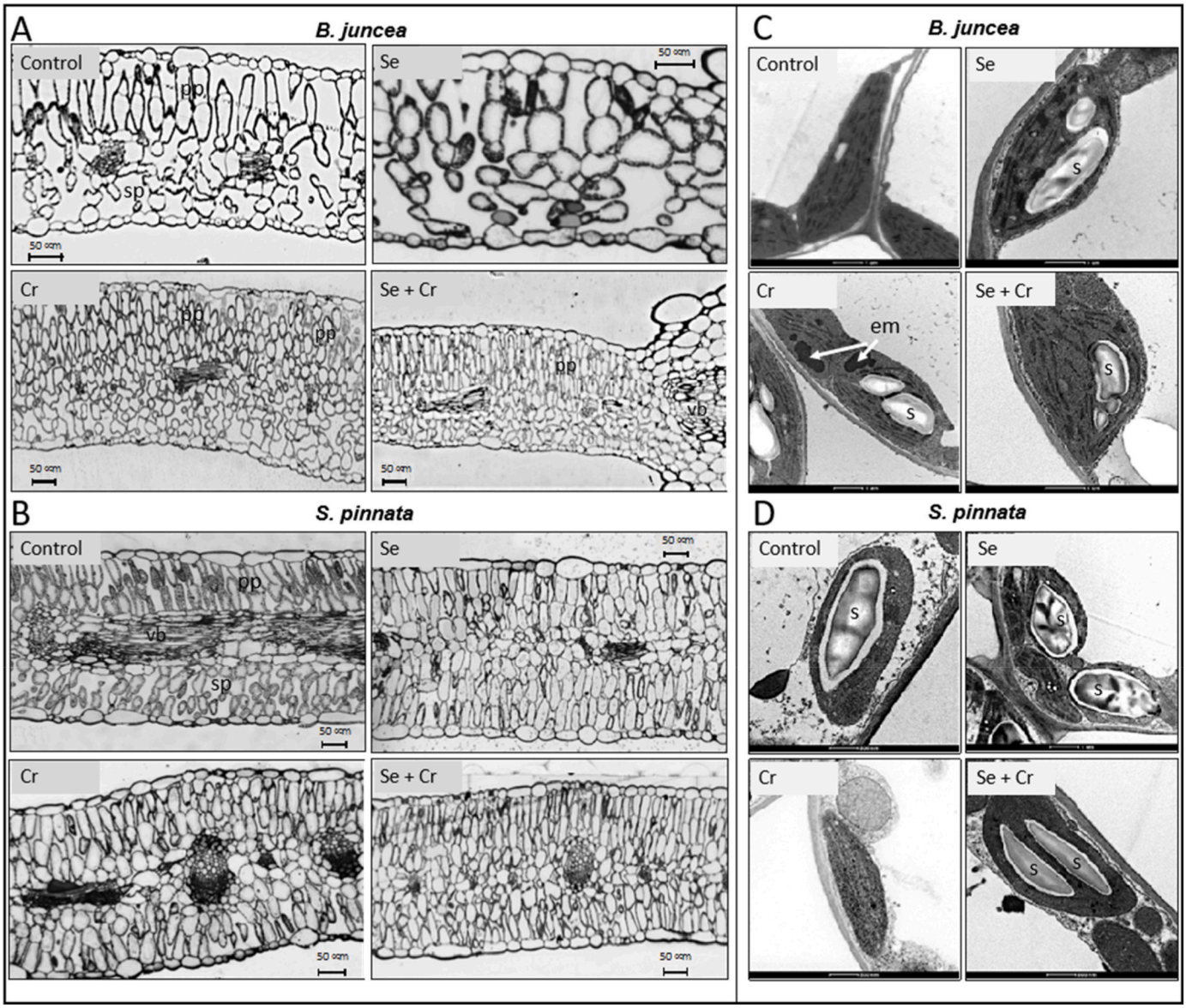

Fig. 2. Light microscopic images of representative leaves of *B. juncea* (A) and *S. pinnata* (B) plants treated with Se, Cr or combined Cr and Se. Control plants were Cr- and Se-untreated. pp: palisade parenchyma; sp: spongy parenchyma; vb: vascular bundle. Transmission electron microscope details of representative leaf cells of *B. juncea* (C) and *S. pinnata* (D) plants treated with Se, Cr or combined Cr and Se. Control plants were Cr- and Se-untreated. Electrodense masses are indicated by arrows. s: starch.

appreciably affect the Cr/S ratio.

The Se enrichment factor was more pronounced in *S. pinnata* (Fig. 4G), while the Cr enrichment factor was prominent in *B. juncea* (Fig. 4H). Treating the plants with combined Se and Cr reduced the Se enrichment factor in both species (Fig. 4G), and the Cr enrichment factor in *B. juncea* (Fig. 4H).

3.5. Effects of Se and Cr on plant antioxidant systems and lipid peroxidation

The antioxidant capacity of plants was markedly enhanced in leaves of *B. juncea* treated with Cr, while the application of Se, alone or jointly with Cr, resulted in weak increases (Fig. 5A). Conversely, the antioxidant capacity of *S. pinnata* leaves was enhanced under either Se or Se plus Cr treatments, while insignificant differences were observed between plants added with Cr and plants of the other conditions. The trend of total phenol compounds in leaves (Fig. 5B) was similar to that described for the leaf antioxidant capacity.

The leaf lipid peroxidation was high in leaves of *B. juncea* plants exposed to Se or Cr (Fig. 5C). However, the combined application of the two elements decreased the level of lipid peroxidation to values comparable to those of control plants. In *S. pinnata*, the application of Cr was the only treatment that resulted in leaf lipid peroxidation higher than the control. In the roots of both species, the amount of TBARS was increased by Cr, either alone or jointly with Se, whereas it was comparable between control plants and plants subjected to Se treatment (Fig. 5D). In most cases, the level of lipid peroxidation was higher in *B. juncea* than in *S. pinnata*, in leaves as in roots.

The activity of CAT, GPX and APX differed between *B. juncea* and *S. pinnata*, but the pattern of the three enzymes was the same for each species (Fig. 5E–G). In particular, the activity of all antioxidant enzymes in *B. juncea* was stimulated by Se (50–75%) and Cr (77–90%) when applied individually, while CAT and APX activities were comparable to those of the control when Cr and Se were concomitantly applied. GPX activity was increased by 66% in Se + Cr -treated plants. In the case of *S. pinnata*, no appreciable variation in antioxidant enzyme activity was

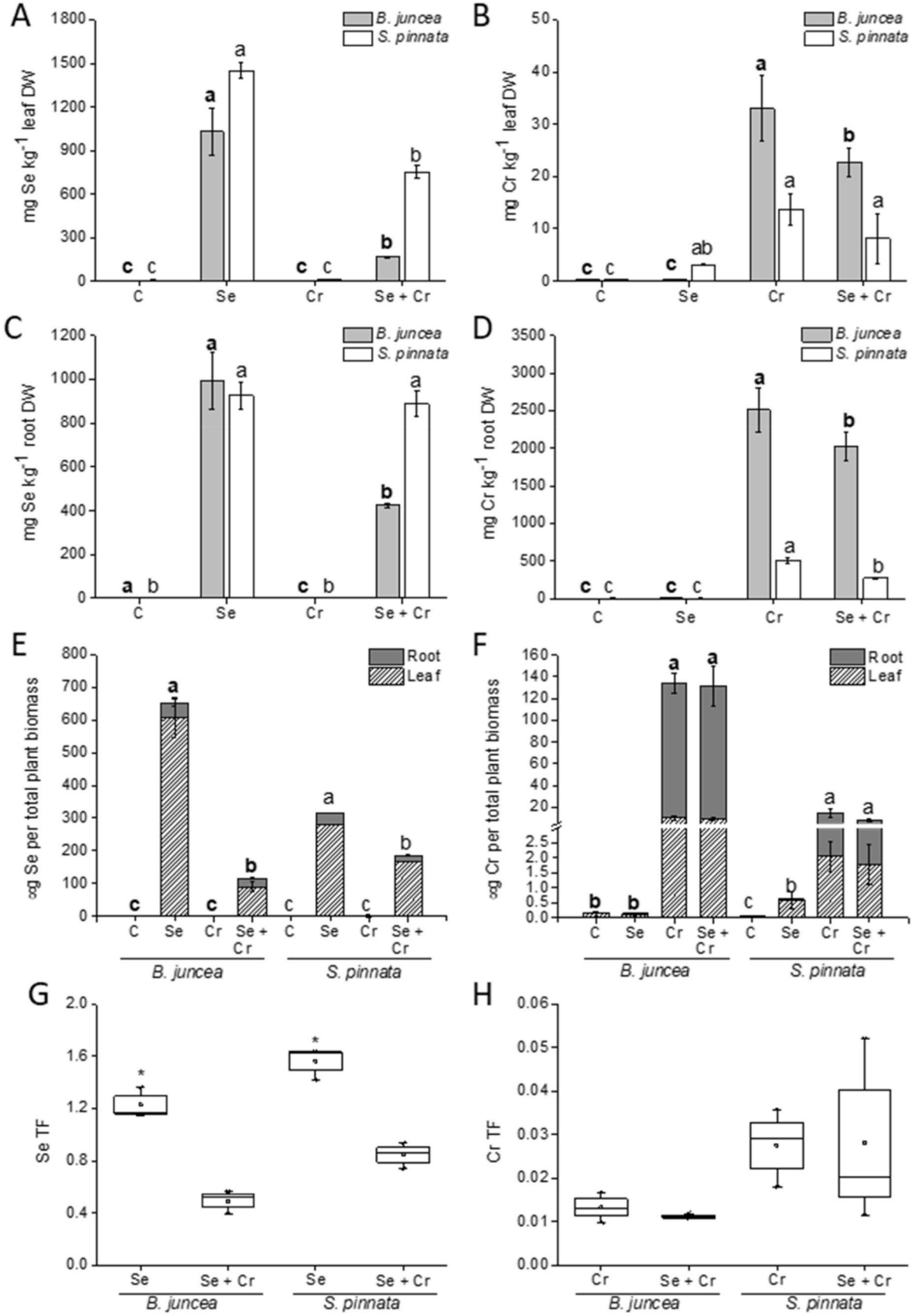

Fig. 3. Concentration of Se and Cr in leaves (A,B, respectively) and roots (C,D, respectively), total content of Se and Cr per plant biomass (E,F, respectively), Se and Cr translocation factors (G,H, respectively) in *B. juncea* and *S. pinnata* plants treated with Se, Cr or combined Cr and Se. Control plants were Cr- and Se-untreated. Different letters above bars indicate significant differences at $p < 0.05$ for *B. juncea* (in bold) and *S. pinnata* (not bolded). Asterisks indicate significant differences at $p < 0.05$ between *B. juncea* or *S. pinnata* plants treated with Se or Cr and Cr joint with Se.

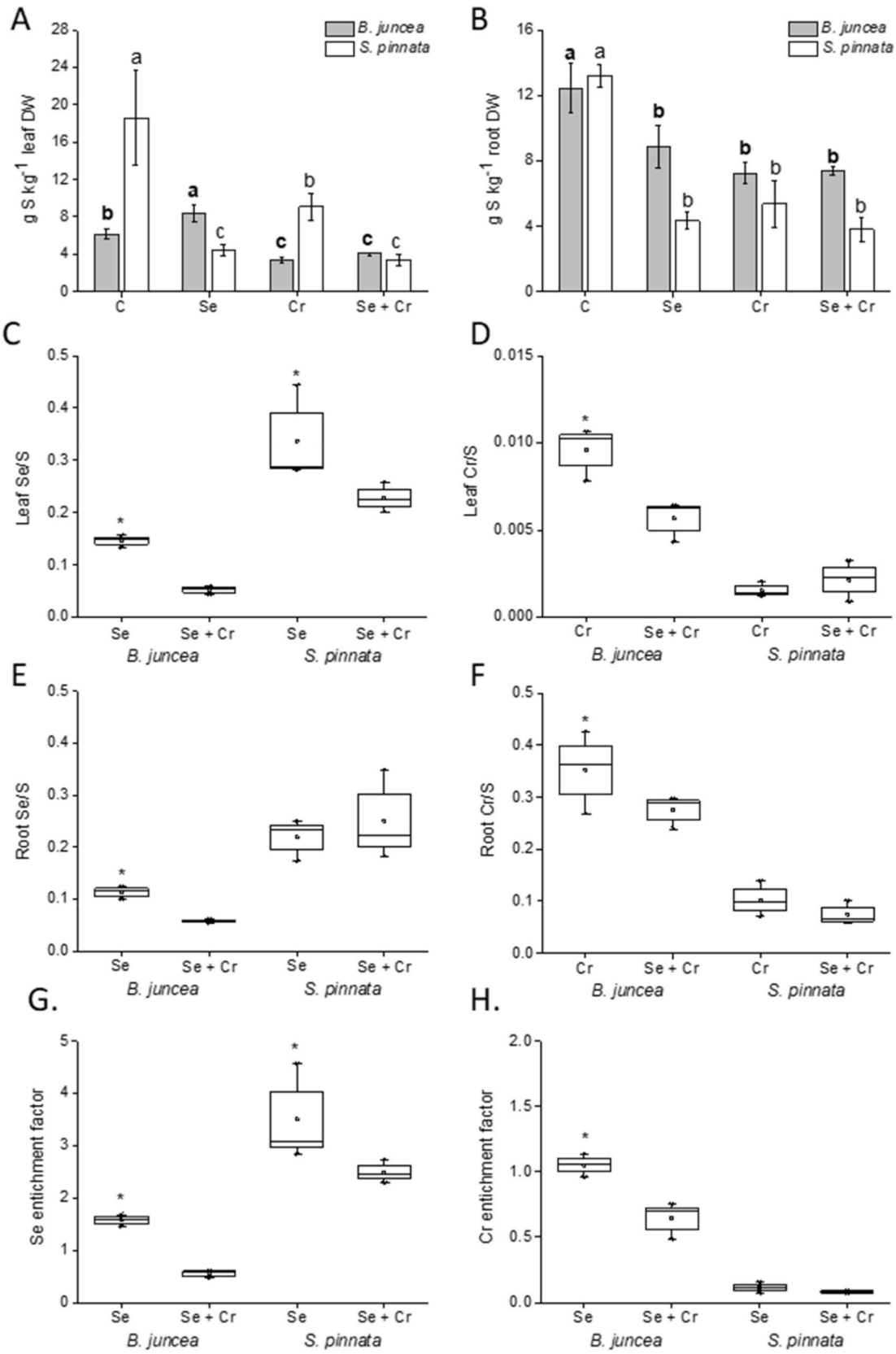

Fig. 4. Concentration of S in leaves (A) and roots (B), leaf Se/S and Cr/S ratios (C,D, respectively), root Se/S and Cr/S ratios (E,F, respectively), Se and Cr enrichment factors (G,H, respectively) in *B. juncea* and *S. pinnata* plants treated with Se, Cr or combined Cr and Se. Control plants were Cr- and Se-untreated. Different letters above bars indicate significant differences at $p < 0.05$ for *B. juncea* (in bold) and *S. pinnata* (not bolded). Asterisks indicate significant differences at $p < 0.05$ between *B. juncea* or *S. pinnata* plants treated with Se or Cr and Cr joint with Se.

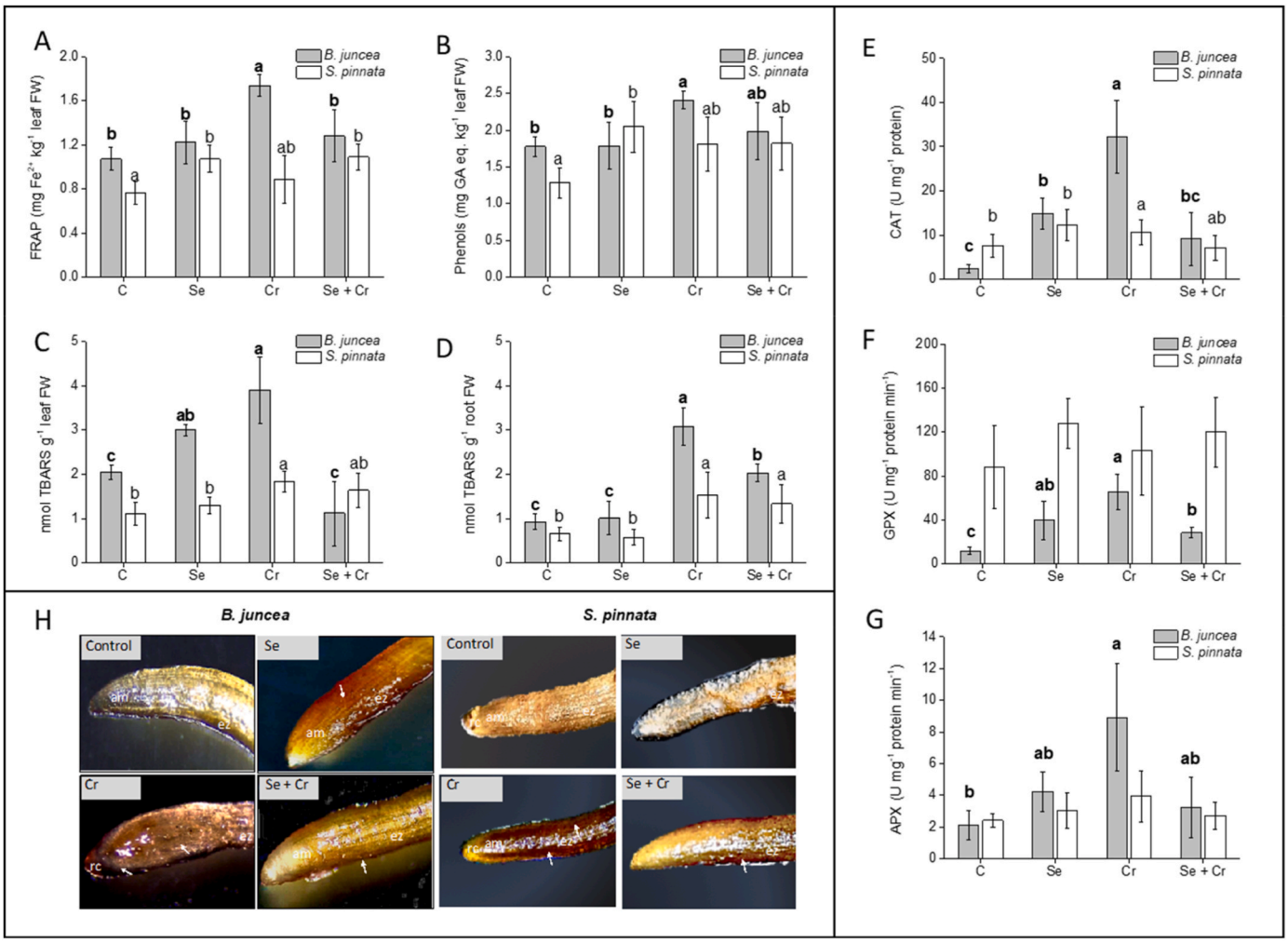

Fig. 5. Ferric-reducing antioxidant power (FRAP) expressed as milligrams per kilogram of ferrous ion equivalent (A), total phenols expressed as equivalent of gallic acid (B), lipid peroxidation expressed as thiobarbituric acid reactive substances (TBARS) (C,D in leaves and roots, respectively), catalase (CAT) (E), guaiacol peroxidase (GPX) (F) and ascorbate peroxidase (APX) (G) activity of *B. juncea* and *S. pinnata* plants treated with Se, Cr or combined Cr and Se. Control plants were Cr- and Se-untreated. Different letters above bars indicate significant differences at $p < 0.05$ for *B. juncea* (in bold) and *S. pinnata* (not bolded). When differences are not detected, letters are not reported. Histochemical detection of H_2O_2 using DAB in root tips of *B. juncea* and *S. pinnata* (H) plants treated with Se, Cr or combined Cr and Se. Control plants were Cr- and Se-untreated. Brown areas indicated by white arrows reveal the presence of H_2O_2. rc: root cap; am: apical meristem; ez: elongation zone.

detected, except GPX activity was higher than in *B. juncea* (Fig. 5F).

The histochemical detection of H_2O_2 showed greater abundance of this compound in roots of Se- and Cr-treated *B. juncea*, and in roots of Cr-treated *S. pinnata* (Fig. 5H). More specifically, in *B. juncea* Cr triggered H_2O_2 accumulation in the root tip mainly at the meristematic zone, while Se did it in the elongation zone. In *S. pinnata*, H_2O_2 primarily accumulated at the elongation zone, while insignificant staining was observed when Se was applied. In both species, the concurrent application of Se and Cr decreased H_2O_2 along the root tip, but the presence of H_2O_2 was still evident in the epidermis.

4. Discussion

In this study, *B. juncea* and the Se-hyperaccumulator *S. pinnata* were supplied with chromate and selenate to evaluate these species' capacity to take up Se and Cr and convey them to the aboveground parts with the aim to advise their use in suitable phytotechnologies according to the target. So far, selenate and sulfate interactions have been investigated in both species, as well as chromate and sulfate interactions in *B. juncea*, but the effects of selenate and chromate on the uptake of each other and those of their joint application on the accumulation of the essential element S are largely unknown. Nevertheless, since some soils contain appreciable amounts of Cr and Se, and S is involved in plant defense against multiple stresses, the interactions between these three elements in the form of competing oxyanions are worth studying.

Our results indicate that *B. juncea* and *S. pinnata* plants differ in their capacity to accumulate Cr, Se and S. In particular, *B. juncea* stored more Cr than *S. pinnata*, while S content was reduced by Cr in both species. According to previous studies (Schiavon et al., 2007, 2008; 2012), we hypothesize that chromate likely repressed the expression of root sulfate transporters, thus limiting the entry of sulfate ions into the plant. At the same time, chromate may have acted as a competitor of sulfate for root uptake, which would then explain why Cr was accumulated in high amounts by plants (Schiavon et al., 2007, 2008). Since S transporters can exhibit different affinity for other oxyanions beyond sulfate, sulfate transporters from *B. juncea* possibly had more affinity for chromate than those from *S. pinnata*, especially considering that the Se-hyperaccumulator was shown to have constitutive and elevated expression of sulfate transporters, which conceivably have high affinity for selenate (El-Mehdawi et al., 2018; Wang et al., 2018; Trippe III and Pilon-Smits, 2021). Also, *S. pinnata* might possess transporters specific only for selenate. In support of our hypothesis, the values of Se/S and

Cr/S ratios were the highest for *S. pinnata* and *B. juncea*, respectively. Furthermore, while Se accumulation was dramatically reduced by Cr in roots of *B. juncea*, it was not in *S. pinnata*. It should be noted, however, that the application of Cr significantly reduced Se translocation in both species, thus indicating that the effect of Cr is less species-specific for this process than for Se primary uptake.

With respect to the effect of Se on Cr accumulation by plants, we noticed a Se-mediated reduction in Cr concentration in *B. juncea*, which was less pronounced (about 20–30%) than the reverse reduction caused by Cr on Se concentration (about 60–85%). In *S. pinnata*, Se application significantly reduced the Cr concentration in roots, but not in leaves. This result might suggest that Cr is more effective in reducing Se translocation than Se is in restricting Cr translocation. A possible explanation is that the transport system involved in conveying chromate/selenate to the shoot may be more specific for chromate over selenate, which would be quite surprising for a Se-hyperaccumulator. Alternatively, Se may be largely assimilated into organic Se compounds within the root cells of the Se hyperaccumulator, and thus substantial amounts of organic Se rather than selenate are expected to be loaded into the xylem to be translocated to the shoot. As a result, the limited selenate pool of *S. pinnata* may not be sufficient to trigger a remarkable decrease in leaf Cr accumulation. According to this hypothesis, Se was previously reported to be sequestered in root cell vacuoles in *S. pinnata*, possibly to avoid selenite translocation to the shoot and favor Se metabolism towards the synthesis of nonprotein Se amino acids (Freeman et al., 2006, 2010; Amos et al., 2012). Furthermore, the gene encoding the enzyme ATP-sulphurylase isoform 2 involved in Se metabolism is overexpressed in the roots of this hyperaccumulator, supporting elevated Se assimilation in this organ (Jiang et al., 2018).

When the capacity of *B. juncea* and *S. pinnata* plants to remove Cr and Se was evaluated based on the total plant accumulation (the product of concentration and biomass), we noticed that *B. juncea* was more effective than *S. pinnata* in accumulating each of the elements. The reason was that this species produced from 2 to 4 times more biomass than *S. pinnata*. However, the distribution of Se was the same in both species, i.e., Se was more accumulated in the aerial parts, whereas Cr was equally accumulated in the leaves and roots of *S. pinnata* and preferentially in the roots of *B. juncea*. The effects of the joint application of Se and Cr on the total accumulation of these two elements in plants were partly different compared to those described for Se and Cr concentrations. Indeed, the total content of Se was reduced in *B. juncea* and *S. pinnata* by Cr, especially in the leaves, but the total content of Cr was not affected by Se. This was because the reduction of Cr content in both species was less prominent than that reported for Se.

Sulfur accumulation in plants was decreased by Se and/or Cr application. As previously mentioned, this effect can be ascribed to competitive events at the uptake sites, as well as to the downregulation of sulfate transporters (Schiavon et al., 2007, 2008; 2012). The reduction, however, was more pronounced in *S. pinnata* plants treated with Se, regardless of whether Cr was present or not. This result confirms previous studies where we observed a strong reduction of sulfate uptake by selenate in this species due to the strength of selenate as a competitor for binding to sulfate transporters (El Mehdawi et al., 2018). The joint application of Se and Cr did not substantially alter the Cr/S ratios in *S. pinnata* plants, although a reduction in leaf S content was observed. Conversely, this ratio was decreased in *B. juncea* leaves mainly because of the reduction in Cr accumulation with no effect on S content. The Se/S ratio declined in leaves of both species due to the negative effect of Cr on Se translocation, and in roots of *B. juncea* because Cr impacted on both Se uptake and translocation.

The enrichment factor is a proxy to evaluate Se and Cr accumulation in *B. juncea* and *S. pinnata* plants relative to all other analogous oxyanions available in the growth medium. The highest Cr and Se enrichment factors determined in *B. juncea* and *S. pinnata*, respectively, provide confirmation that the two species have preferential accumulation for Cr and Se, and that S does not significantly affect this preference.

B. juncea and *S. pinnata* were also evaluated for their capacity to tolerate Cr, Se and their combined application because plants that are used in phytotechnologies are expected to be relatively tolerant to metal/loids. Our results indicate that Cr induced relevant oxidative stress in *B. juncea*, which was most likely due to the large accumulation of this element in plant tissues. Chromate is a powerful oxidizing agent and its detrimental effects were evident on leaf anatomy (Wakeel and Ming, 2020). In addition, Cr treatment induced greater accumulation of H_2O_2 at the root tip, thereby impairing root development and overall plant growth. Similar Cr-induced effects were documented in this species by other authors (Pandey et al., 2005; Diwan et al., 2012; Ahmad et al., 2020; Sharma et al., 2022). The concomitant application of Cr with Se alleviated the damages induced by Cr alone. The protective role of low Se concentration against Cr, involving the activation of antioxidative systems and restriction of Cr uptake in plants was reported previously (Qing et al., 2015; Handa et al., 2018, 2019; Cai et al., 2019; Zhao et al., 2019), but in this study the relief from Cr injuries was probably due to the reduced accumulation of Cr by plants (Chauhan et al., 2019; Schiavon et al., 2020). In fact, plants supplied with Se and Cr grew better than Cr-treated plants and were less subjected to oxidative stress. Selenium in *B. juncea* plants induced less intense oxidative stress compared to Cr. Nevertheless, the supply of Cr was ineffective in decreasing Se-toxicity, except for TBARS generation in leaves. This is perhaps because selenate is a less potent oxidizing agent than chromate, so the positive effect of reduced Se concentration in *B. juncea* by Cr was compensated by the negative effect derived from Cr accumulation.

In *S. pinnata*, the application of Se increased the antioxidant activity and the content of phenols, but these effects were not related to either increased TBARS accumulation or increased antioxidant enzyme activity. Earlier studies reported the overexpression of several genes involved in the defense against stress when *S. pinnata* was supplied with Se (Freeman et al., 2010; Wang et al., 2018). Enzymes encoded by these genes and antioxidant metabolites produced in Se-elicited pathways may be responsible for low oxidative stress in the Se-hyperaccumulator. In our study, for instance, the activity of the GPX enzyme was very pronounced in *S. pinnata* and this was not dependent on any Cr or Se treatment. Despite this, Cr caused some detrimental effects in *S. pinnata*, like TBARS accumulation, which was not relieved by Se except for H_2O_2 production at the root tip.

5. Conclusions

Concluding, this study indicates that *B. juncea* and *S. pinnata* possess transport systems with distinct specificity for Cr and Se, and differ in Cr and Se translocation capacity. The negative effect on S accumulation was more pronounced in *S. pinnata* when supplied with Se, suggesting elevated affinity of sulfate transporters for selenate. *B. juncea* was more sensitive to Cr and Se, but it may still be considered a good candidate for use in Cr and Se phytoextraction. *Brassica juncea* had a higher total plant accumulation capacity because of its faster growth and higher biomass. However, when Se and Cr co-exist in soil, or soil Se is poorly available, *S. pinnata* may be preferred for Se phytoextraction. Cr scarcely accumulates in *S. pinnata* biomass, thus the plant material can be used as feed or fertilizer for crop biofortification. *B. juncea* plants instead, could be used as green manure only when available Cr is very low, because it accumulates high levels of this metal in the aboveground tissue, which might pose a hazard to the food web.

Author statement

M.S. Investigation, Methodology, Conceptualization, Writing – original draft, review & editing, Funding acquisition. F.D.V. Investigation, Methodology, Conceptualization, review & editing. V.S. Methodology, Data curation, review & editing. S.N. Resources, Writing – review & editing. E.P.S. Resources, Methodology, Writing – review & editing.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121048.

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