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Efficient superoxide scavenging and metal immobilization in roots determines the level of tolerance to Vanadium stress in two contrasting *Brassica napus* genotypes

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ABSTRACT

Brassica napus also known as Rapeseed is a member of the Brassicaceae family which is mainly cultivated for its oil-rich seeds. Indeed, *B. napus* is ranked the third-largest source of vegetable oil in the world. *Brassica napus* growth, development and yield are negatively affected by heavy metals. Vanadium is a heavy metal and presence in high concentrations impact plant growth and development negatively. However, the impact of Vanadium on *B. napus* growth and development is unknown. Therefore, in this study we assessed the effects of Vanadium stress on leaf physiology and biochemistry response of two *B. napus* cultivars (namely Agamax and AV Garnet). A randomised pot-experiment under controlled conditions was used to grow *B. napus* cultivars under control (distilled water) and Vanadium (350 μM $NaVO_3$) treatments. Results showed that Vanadium caused yellowing of AV Garnet leaves but not Agamax leaves. Furthermore, Vanadium stress caused a more severe decrease in leaf dry and fresh weight of AV Garnet as compared to the decrease in leaf dry and fresh weight of Agamax. We also observed that Vanadium stress only decreased leaf chlorophyll content (a, b and total) of AV Garnet but had no effect on chlorophyll content of Agamax. In addition, Vanadium stress induced an increase in toxic superoxide (O_2^-) content in leaves of both AV Garnet and Agamax however; we observed more O_2^- content in AV Garnet leaves than Agamax leaves. Furthermore, we observed a more drastic increase in leaf lipid peroxidation and leaf cell death (assessed by Evans blue uptake) of AV Garnet when compared to Agamax. In order to investigate whether Vanadium regulates O_2^- metabolising enzymes we assessed superoxide dismutase (SOD) activity (total SOD enzymatic activity and SOD isoform activity). Vanadium inhibited the total leaf SOD activity of AV Garnet more than the total leaf SOD activity of Agamax. The SOD isoform analyses displayed that Vanadium treatment did not alter the leaf MnSOD as well as leaf Cu/ZnSOD isoforms of both Agamax and AV Garnet. However, we observed that FeSOD 1 and FeSOD 3 activity was upregulated in Agamax leaves in response to Vanadium treatment but decreased in AV Garnet following Vanadium treatment. Furthermore, we observed that the leaf FeSOD 2 activity was inhibited in both Agamax and AV Garnet. In addition, we also analysed the Vanadium contents in the two cultivars following Vanadium treatment and observed more Vanadium content in Agamax roots than in AV Garnet roots. Furthermore, translocation factor (TF) analysis showed that AV Garnet had a higher Vanadium TF from roots to leaves than Agamax following Vanadium treatment. In conclusion, Vanadium stress tolerance in *B. napus* is possibly controlled by SOD activity and Vanadium content root immobilisation.

1. Introduction

Heavy metal pollution is rapidly increasing, therefore polluted areas often overlap with agricultural soils to cause decreases in the yield and quality of crop plants (Singh et al. 2017). To combat heavy metal stress in the field, plants have developed various physiological and biochemical strategies which vary among different plant species and also between the different parts of plants (Sytar et al. 2016). Vanadium (V) is the fifth most abundant transition metal found in the earth's crust. Furthermore, Vanadium is also ranked 22 among all discovered elements in

Abbreviations: AAS, Atomic Absorption Spectrophotometer; ANOVA, analysis of variance; AVG, average value; ICP-OES, Inductively coupled plasma optical emission spectrometer; MDA, malondialdehyde; PAGE, polyacrylamide gel electrophoresis; ROS, reactive oxygen species; SE, standard error; SDS, Sodium dodecyl sulphate; SOD, superoxide dismutase; TBA, thiobarbituric acid; TF, translocation factor..

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the earth's crust (Amorim et al. 2007). Vanadium mining occurs extensively in China, South African, Russia and the United States of America (USA) (Imtiaz et al. 2015b). Due to mining, Vanadium can leach into the surrounding soils causing pollution (Imtiaz et al. 2015a). Indeed, Lazaridis et al. (2003) stated that Vanadium should be recognised as a potentially dangerous pollutant in the same class as mercury, lead and arsenic. Therefore, studies have determined Vanadium content in the earth's crust and have found that Vanadium concentrations varies from 10 to 200 $\mu g.g^{-1}$ (Teng et al. 2006; Wazalwar et al. 2011). At these testing sites, Vanadium most often exists as vanadium pentaoxide (V_2O_5); however, other forms such as ammonium metavanadate (NH_4VO_3), sodium metavanadate ($NaVO_3$) and sodium orthovanadate (Na_3VO_4) can also be present. Due to the existence of the various Vanadium forms in soils, Vanadium can get into contact with various life forms on earth.

Imtiaz et al. (2015a) stated that Vanadium at low concentrations can be beneficial for the growth of microorganisms and animals such as rats but that the role of Vanadium in plant and human nutrition is not clearly defined. Taylor and Van Staden (1994) stated that Vanadium acts as a growth promoting factor and may participate in nitrogen fixation in plants. Contrastingly, Imtiaz et al. (2016) showed that elevated concentrations of Vanadium reduced the root and shoot growth in Chickpea plants. Furthermore, Yang et al. (2017) showed that exogenous Vanadium applications reduced Soybean growth. Similarly, Nawaz et al. (2018) observed a decrease in Watermelon seedling growth after Vanadium treatment. These studies highlight the need for more research to establish the role of Vanadium in plants. Nevertheless, plants treated with excessive Vanadium display chlorosis due to chlorophyll degradation (Olness et al. 2005) as well as protein degradation (Tanyolac et al. 2007). Imtiaz et al. (2015b) attributed the toxic effects of Vanadium to over production of reactive oxygen species (ROS). It is known that heavy metal stress induces ROS production such as superoxide (O_2^-) content, hydrogen peroxide (H_2O_2) content and hydroxyl radical ($OH^\bullet$) (Shah et al. 2001). These ROS molecules will interact with lipid membranes ultimately leading to lipid peroxidation and eventually cellular death. To date, no study has revealed changes in O_2^- content following Vanadium treatments in plants; however, Imtiaz et al. (2016) showed that exogenous application of Vanadium increased the H_2O_2 content of Chickpea plants. Furthermore, studies by Imtiaz et al. (2015b) and (2016) have showed that Chickpea plants experiences an increase in lipid peroxidation following Vanadium treatment.

In order to regulate ROS levels under stressful conditions and to avoid oxidative damage, plants possess various antioxidant enzymes. Within plant cells superoxide dismutases (SODs; EC 1.15.1.1) constitute the first line of defence against ROS by converting toxic O_2^- into H_2O_2 and oxygen. SODs exist as various isoforms in plants depending on the metal cofactor in the active site. These isoforms include manganese SOD (MnSOD), iron SOD (FeSOD) and Cu/Zn SOD (Cu/ZnSOD). Rizhsky et al. (2003) showed that a Cu/ZnSOD *Arabidopsis* knockdown mutant displayed suppressed growth and development. Furthermore, Myouga et al. (2008) observed arrested chloroplast development in FeSOD *Arabidopsis* knockdown mutants. Under Aluminium (Al) stress conditions, Basu et al. (2001) observed Al tolerance in transgenic *Brassica napus* plants which overexpresses a MnSOD. These studies have highlighted the importance of SOD in plant growth and development under normal (unstressed) and stress conditions. To our knowledge, to date no study has investigated the effect of Vanadium stress on the various SOD isoforms in plants. However, the effect of Vanadium stress on total SOD activity has been investigated. Imtiaz et al. (2015b) observed an increase in total SOD activity in Chickpea in response to Vanadium treatment. Contrastingly, Nawaz et al. (2018) observed a decrease in total SOD activity in Watermelon seedlings in response to Vanadium treatments.

In addition to controlling ROS levels under Vanadium stress, plants have also developed mechanisms to deal with the toxic Vanadium metal directly. In general, plants will try to avoid accumulation or bioconcentration of Vanadium in tissues (Aihemaiti et al. 2017); however, under very high Vanadium concentrations; plants will mainly immobilise the Vanadium in the roots (Kaplan et al. 1990). This strategy is mainly used to protect the photosynthetic machinery from the toxic Vanadium. Indeed, Imtiaz et al. (2015b) suggested that Vanadium sensitivity in plants might be linked to the inefficient immobilisation of toxic Vanadium by the roots. Xiao et al. (2012) used a pot-experiment to study the effects of exogenously applied Vanadium on *Brassica chinensis* plants. In that study, the authors observed a decrease in leaf and root biomass with an increasing in Vanadium concentration as well as that the plants had more Vanadium in the roots than in the leaves. The authors then concluded that the plants became more sensitive due to the higher translocation of Vanadium into the leaves especially at higher Vanadium concentrations (Xiao et al. 2012).

Brassica napus is the third largest oil crop after soybean and oil palm. Furthermore, *B. napus* is used for the production of margarine, spreads, dairy blends, animal feed, emulsifiers, and a source of vitamin E as well as healthy cooking oils (Maheshwari and Kovalchuk 2016). Due to the nutritional benefit of *B. napus* for human consumption and animal feed, its demand has increased during the last two decades (Ahmadi et al. 2018). However, *B. napus* yield can be seriously limited by environmental stresses such as heavy metal stress. More specifically, *B. napus* growth and development is negatively affected by cadmium (Zheng et al. 2017), lead (Ferreyroa et al. 2017), arsenic (Farooq et al. 2018), chromium (Nafees et al., 2018), mercury (Shen et al. 2011) and nickel (Kazemi et al. 2010), to name a few. However, to our knowledge no study has investigated the effect of Vanadium stress on *B. napus* growth and development. Thus, in this study we hypothesise that the ROS-antioxidant system and Vanadium restrictive translocation from root to leaf are key processes in determining Vanadium tolerance and sensitivity in *B. napus*. To study this hypothesis, we investigated the physiological and biochemical effect of exogenous Vanadium on two *B. napus* cultivars namely Agamax and AV Garnet. These two cultivars are conventional cultivars which are commercially available and often used in Canola farming worldwide. Our objectives were as follows: (1) to investigate the effect of Vanadium on *B. napus* leaf biomass as well as leaf morphology; (2) to analyse the effect of Vanadium on leaf O_2^-, leaf lipid peroxidation, leaf chlorophyll content and leaf cell death in the two *B. napus* cultivars; (3) to study the responses of SOD (total enzymatic activity and isoforms) to Vanadium stress; and (4) to measure Vanadium content in roots and leaves of both cultivars following Vanadium and control treatments in order to identify possible Vanadium translocation strategies in *B. napus*.

2. Materials and methods

2.1. Plant growth

Plant growth was performed as described by Gokul et al. (2016). The pot experiments were performed in 15 cm diameter plastic pots containing a nutrient rich potting mix [Stodels Nurseries South Africa; 1 part Double grow weed-free compost and 1 part Double grow potting soil] under a 25/19 °C day/night temperature cycle with a 16/8 h light/dark cycle, at a photosynthetic photon flux density of 300 μmol photons.$m^{-2}.s^{-1}$ (during the day phase), in a complete randomised design. *Brassica napus* (Agamax and AV Garnet) seeds (purchased from Agricol South Africa) were surface-sterilised in 0.35% (v/v) sodium hypochlorite for 10 min and then rinsed five times with sterile distilled water. The seeds were subsequently imbibed in sterile distilled water for 15 min and sown in the pre-soaked (distilled water) pots (containing the nutrient rich mix). The sand was kept moist by watering with distilled water until the rosette stage (two unfold true leaves). Once the plants reached the rosette stage, the uniform sized plants were thinned to one plant per pot.

2.2. Treatment of plants

Five days after the plants have reached the rosette stage, control plants were supplied with distilled water. For Vanadium treatments, the distilled water was supplemented with 350 μM sodium metavanadate ($NaVO_3$; Sigma 99.9% trace metals basis). Control or Vanadium treatments (200 ml per pot) were applied to each plant directly to the sand at the base of the stem of the plant in the pot every three days. After 21 days of treatment, plants were carefully removed from the sand and the roots were separated from shoots to prevent erroneous data interpretation caused by possible root damage from plant removal from the soil. However, roots were stored for Vanadium content determination only. The second youngest leaves were separated from the stems and used for all experiments. Photographs and images were captured using a Canon 80D digital camera (lens; Canon EF-S 10–18 mm f/4.5–5.6 IS STM) and storing of plant material was done by snap-freezing the material in liquid nitrogen and stored at -80 °C for future analysis. Three leaves were weighed to obtain the fresh weight and dry weight analysis was performed by drying three leaves per treatment at 80 °C for 48 h followed by weight recording.

2.3. Chlorophyll content determination

Chlorophyll a, b and total were assessed using the method of Nxele et al. (2017).

2.4. Measurement of superoxide content

Using intact leaves (excised at the base and above the petiole), the O_2^- content was detected using the method described by Gokul et al. (2016).

2.5. Determination of protein concentration

Leaf proteins were extracted using the method described by Egbichi et al. (2014) and the protein concentrations were determined using the RC DC Protein Assay Kit 11 (Bio-Rad Laboratories).

2.6. Determination of superoxide dismutase activity

SOD activity assays were performed on leaf protein extracts using the method of Stewart and Bewley (1980).

2.7. Measurement of lipid peroxidation

Lipid peroxidation was determined in leaves by measuring malondialdehyde (MDA) formation, using the thiobarbituric acid (TBA) method as previously described by Zhang et al. (2007).

2.8. Evaluation of cell viability

Cell viability was assessed by using the method of Gokul et al. (2016). Briefly, the undamaged leaf was immersed in 0.25% (m/v) Evans Blue (sigma; Dye content ≥75%). Dye uptake was assessed by spectrophotometry at 600 nm and calculations were performed as described by Sanevas et al. (2007).

2.9. Analysis and classification of superoxide dismutase isoforms

Protein extracts from various treatments (containing 200 μg protein per sample) were separated on a 13% (v/v) resolving native polyacrylamide gel at 4 °C. SOD activity was detected by photochemical staining with riboflavin and nitrotetrazolium blue chloride (NBT) as described by Beauchamp and Fridovich (1971). SOD isoforms were identified by incubating gels in 5 mM H_2O_2 to inhibit Cu/ZnSOD and FeSOD, or in 5 mM KCN to inhibit only Cu/ZnSOD, with MnSOD activity assigned on the basis of its resistance to both H_2O_2 and KCN (Archibald and Fridovich 1982). In addition, gels were incubated in 2% (m/v) sodium dodecyl sulfate (SDS) to inhibit MnSOD and FeSOD as described by Brou et al. (2007). Densitometry analysis, indicative of the level of SOD activity, was performed using the Spot Denso tool (AlphaEase FC Imaging software, Alpha Innotech Corporation). SOD activity of each isoform was scored as described by Wu et al. (2007).

2.10. Vanadium content determination

Sample digestion of stored root and leaf material of treated *B. napus* plants was done according to Vachirapatama and Jirakiattikul (2008). The vanadium concentration was determined by using a Unicam Solaar M-series Atomic Absorption Spectrophotometer (AAS) (Unicam, United Kingdom) and a Varian Vista Pro CCD Simultaneous Inductively coupled plasma optical emission spectrometer (ICP-OES) (Varian, Australia) using certified standards (sigma; TraceCERT®).

2.11. Data interpretation and statistical analysis

The translocation factor (TF) was estimated as follows: TF = concentration of metal found in leaf/Concentration of metal found in root. One-way analysis of variance (ANOVA) was used to evaluate the statistical significance of the data obtained using the GraphPad Prism 5.03 software by applying the Tukey–Kramer test at 5% level of significance for three replicates. All experiments in this study were repeated three times (independently) for repeatability and reproducibility purposes.

3. Results

3.1. Effect of Vanadium on leaf size, appearance and biomass

Vanadium treatment ($NaVO_3$) induced a reduction in leaf size in both *B. napus* cultivars (Agamax and AV Garnet) when visually compared to respective controls. Furthermore, Vanadium treatment induced yellowing in AV Garnet leaves only (Fig. 1). Vanadium treatment induced a reduction in leaf fresh weight of both Agamax and AV Garnet, respectively. Vanadium treatment led to a 22% decrease in Agamax leaf fresh weight when compared to the control. Furthermore, Vanadium treatment led to a 51% decrease in AV Garnet leaf fresh weight (Fig. 2A). The leaf biomass (dry weight) of both Agamax and AV Garnet was decreased by Vanadium treatment. Vanadium treatment led to a 8% decrease in Agamax leaf dry weight when compared to the control. Furthermore, Vanadium treatment led to a 62% decrease in AV Garnet leaf dry weight (Fig. 2B).

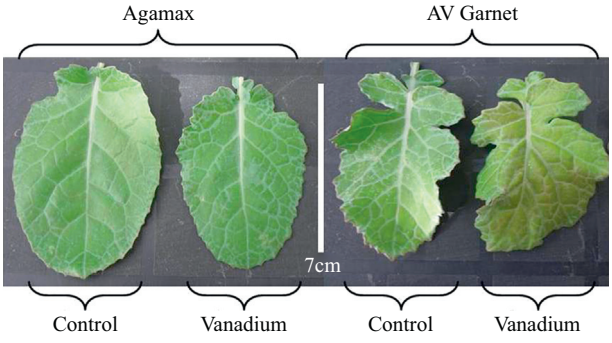

Fig. 1. Responses of two *B. napus* cultivar leaves to control or 350 μM Vanadium treatments. Representative photographs of 21-day-old control and V-exposed *B. napus* leaves (2nd youngest). Scale bar = 7 cm.

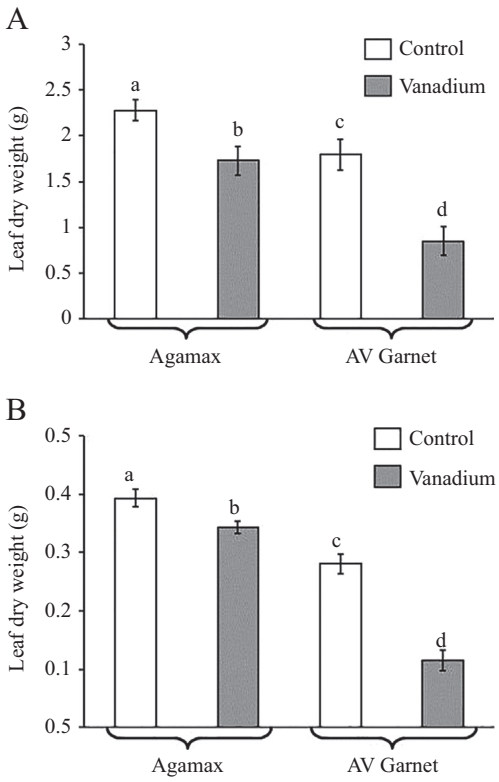

Fig. 2. The effects of control or 350 μM Vanadium treatments on two *B. napus* cultivar leaf (A) fresh weight and (B) dry weight. All measurements were performed immediately at the end of 21 days. Means (± SE) represent data of three replicates from three independent experiments and the same letter do not differ significantly at $P < .05$ according to the Tukey–Kramer test.

3.2. Effect of Vanadium on chlorophyll content

Vanadium treatment had no significant effect on chlorophyll a content of Agamax when compared to the control. However, a 43% reduction was observed in chlorophyll a content of AV Garnet following Vanadium treatment as compared to the control (Table 1). Furthermore, Vanadium treatment had no significant effect on chlorophyll b content of Agamax when compared to the control but a 27% reduction was observed in chlorophyll b content of AV Garnet following Vanadium treatment as compared to the control (Table 1). Vanadium treatment had no significant effect on total chlorophyll content of Agamax when compared to the control. Conversely, a 39% reduction was observed in total chlorophyll content of AV Garnet following Vanadium treatment as compared to the control (Table 1).

3.3. Effect of Vanadium on leaf superoxide content

Vanadium treatment induced an increase in O_2^- content in leaves of both *B. napus* cultivars when compared to respective controls. Furthermore, Vanadium treatment induced an increase of 23% in Agamax leaves when comparing O_2^- content to the control. A significant increase in O_2^- of 71% was observed in AV Garnet leaves as compared to the control (Fig. 3A).

3.4. Effect of Vanadium on leaf superoxide dismutase enzymatic activity

Vanadium treatment induced a decrease in leaf SOD enzymatic activity of both *B. napus* cultivars when compared to respective controls. Moreover, Vanadium treatment led to a decrease of 13% in Agamax leaves when comparing the SOD activity to the control. We observed a decrease in SOD activity of 35% in AV Garnet leaves as compared to the control (Fig. 3B).

3.5. Effect of Vanadium on leaf lipid peroxidation

Vanadium treatment induced an increase in MDA content in leaves of both *B. napus* cultivars when individually compared to the controls. Furthermore, Vanadium treatment induced an increase of 28% in Agamax leaves when comparing MDA content to the control. In addition, we observed a significant increase in MDA content of 177% in AV Garnet leaves as compared to the control (Fig. 4A).

3.6. Effect of Vanadium on leaf cell viability

Vanadium treatment led to a decrease in cell viability in leaves of both *B. napus* cultivars when compared to the respective controls. This is shown by the increase in Evans blue uptake in leaf tissue of both cultivars (Fig. 4B). Furthermore, Vanadium treatment induced an increase of Evans blue uptake of 32% in Agamax leaves when compared to the control. A drastic increase in Evans blue uptake of 137% was observed in AV Garnet leaves as compared to the control (Fig. 4B).

3.7. Classification of superoxide dismutase isoforms

SOD in-gel analysis on Agamax control leaves using inhibitors of the various types of SOD isoforms; suggested the existence of two manganese SOD (MnSOD) isoforms, two copper-zinc SOD (Cu/ZnSOD) isoforms and three iron SOD (FeSOD) isoforms (Fig. 5). In Agamax leaves, the activity of the first two (numbering from the top of the gel) SOD isoforms 1 and 2 were resistant to both H_2O_2 and KCN but slightly sensitive to SDS, which suggests that they are MnSOD isoforms. The SOD activity of isoforms 3 and 4 (numbering from the top of the gel) were resistant to SDS but inhibited by both H_2O_2 and KCN, suggesting that they are Cu/ZnSOD isoforms; hence they were named Cu/ZnSOD 1 and Cu/ZnSOD 2, respectively. The SOD activity of isoforms 5, 6 and 7 (numbering from the top of the gel) were sensitive to H_2O_2, resistant to KCN and sensitive to SDS, thus identifying them as FeSOD isoforms and hence referred to as FeSOD 1, FeSOD 2 and FeSOD 3, respectively (Fig. 5).

SOD in-gel analysis on AV Garnet control leaves using the various inhibitors suggested the existence of one MnSOD isoform, two Cu/ZnSOD isoforms and three FeSOD isoforms (Fig. 6). In AV Garnet leaves, the activity of the first (numbering from the top of the gel) SOD isoform (isoform 1) was resistant to both H_2O_2 and KCN but sensitive to SDS, which suggests that it is a MnSOD isoform. When compared to the SOD profile of Agamax leaves (Fig. 5), this isoform corresponds to MnSOD 2 and was therefore named as such. The SOD activity of isoforms 2 and 3

Table 1
The effects of control or 350 μM Vanadium treatments on chlorophyll a and b content ($\mu g.g^{-1}$) of two *B. napus* cultivar leaves. Means (± SE) of three replicates from three independent experiments are represented and the same letter per row do not differ significantly at $P < .05$ (Tukey–Kramer test).

Chlorophyll content	Agamax		AV Garnet	
	Control	Vanadium	Control	Vanadium
Chlorophyll a	0.489 ± 0.04a	0.495 ± 0.03a	0.498 ± 0.05a	0.284 ± 0.02b
Chlorophyll b	0.184 ± 0.04a	0.177 ± 0.03a	0.173 ± 0.03a	0.127 ± 0.02b
Chl a + b	0.673 ± 0.04a	0.672 ± 0.03a	0.671 ± 0.04a	0.411 ± 0.02b

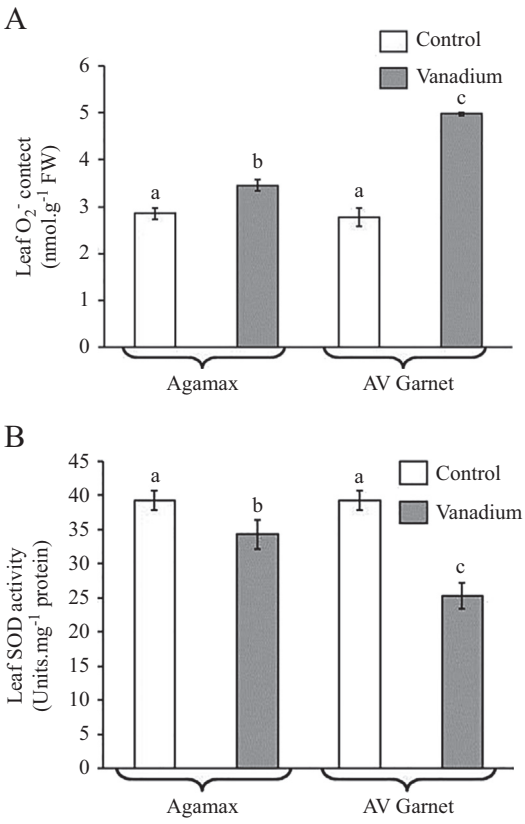

Fig. 3. (A) Superoxide content and (B) superoxide dismutase activity of two *B. napus* cultivar leaves after control or 350 μM Vanadium treatments. Bars represent means (± SE) of three replicates from three independent experiments and different letters differ significantly at $P < .05$ according to the Tukey–Kramer test.

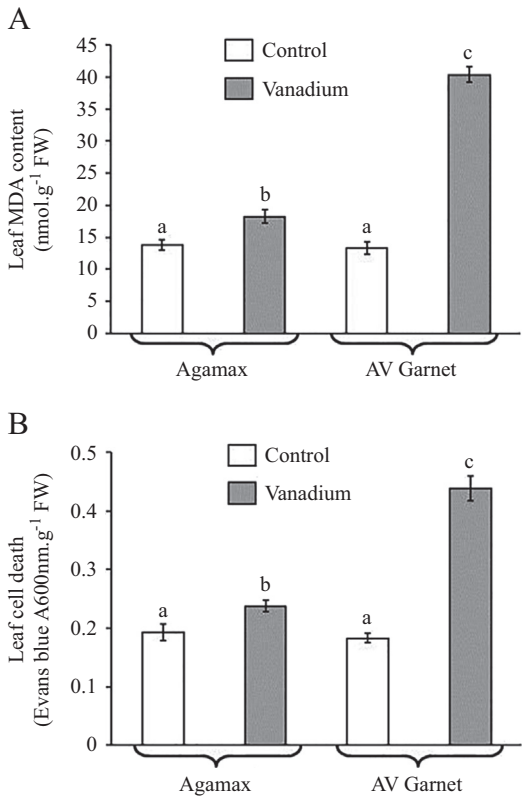

Fig. 4. Two *B. napus* cultivar leaf (A) MDA content and (B) Evans blue uptake (cell death) after control or 350 μM Vanadium treatments. All the values are mean (± SE) of three replicates from three independent experiments. Different letters differ significantly at $P < .05$ according to the Tukey–Kramer test.

(numbering from the top of the gel) were resistant to SDS but inhibited by both H_2O_2 and KCN, suggesting that they are Cu/ZnSOD isoforms and therefore, they were named Cu/ZnSOD 1 and Cu/ZnSOD 2, respectively. The SOD activity of isoforms 4, 5 and 6 (numbering from the top of the gel) were sensitive to H_2O_2, resistant to KCN and sensitive to SDS, thus identifying them as FeSOD isoforms and therefore, referred to as FeSOD 1, FeSOD 2 and FeSOD 3, respectively (Fig. 6).

3.8. Effect of Vanadium on superoxide dismutase isoforms

Densitometry analysis on Fig. 7 revealed that Vanadium treatment did not alter the leaf MnSOD 1 isoform of Agamax when compared to the control (Table 2). Similarly, Vanadium treatment did not alter the leaf MnSOD 2 activity of Agamax when compared to the control. Vanadium treatment also did not alter the leaf MnSOD 2 activity of AV Garnet when compared to the control. Furthermore, Vanadium treatment did not alter the leaf Cu/ZnSOD 1 activity of both Agamax and AV Garnet when compared to respective controls. Likewise, Vanadium treatment did not alter the leaf Cu/ZnSOD 2 activity of both Agamax and AV Garnet when compared to respective controls. Vanadium treatment induced an increase of 19% in the leaf FeSOD 1 activity of Agamax when compared to the control. Contrastingly, Vanadium treatment led to a decrease of 7% in the leaf FeSOD 1 activity of AV Garnet when compared to the control. Moreover, Vanadium treatment decreased the leaf FeSOD 2 activity of both Agamax and AV Garnet by 75 and 78%, respectively when compared to individual controls. Vanadium treatment induced an increase in the leaf FeSOD 3 activity of Agamax by 6% when compared to the control. However, contrastingly, Vanadium treatment led to a 6% decrease in the leaf FeSOD 3 activity of AV Garnet when compared to the control (Table 2).

3.9. Effect of exogenous Vanadium treatment on total Vanadium content in roots and leaves

AAS analysis revealed that exogenous application of Vanadium induced an increase in root Vanadium content in both *B. napus* cultivars (Agamax and AV Garnet) when individually compared to controls (Table 3). Furthermore, AAS analysis showed that Agamax root Vanadium content increased by 108% when compared to the control after Vanadium treatment. Similarly, following Vanadium treatment, AAS analysis showed that AV Garnet root Vanadium content increased by 50% when compared to the control. ICP-OES analysis also revealed that exogenous application of Vanadium induced an increase in root Vanadium content in both Agamax and AV Garnet. Moreover, ICP-OES analysis illustrated that Agamax root Vanadium content increased by 106% when compared to the control after Vanadium treatment. Similarly, following Vanadium treatment, ICP-OES analysis showed that AV Garnet root Vanadium content increased by 51% when compared to the control (Table 3).

AAS analysis also revealed that exogenous application of Vanadium induced an increase in leaf Vanadium content in both *B. napus* cultivars when compared to respective controls (Table 4). Furthermore, AAS analysis showed that Agamax leaf Vanadium content increased by 80% when compared to the control after Vanadium treatment. Likewise, following Vanadium treatment, AAS analysis showed that AV Garnet leaf Vanadium content increased by 218% when compared to the control. ICP-OES analysis also illustrated that exogenous application of Vanadium induced an increase in leaf Vanadium content in both Agamax and AV Garnet. Moreover, ICP-OES analysis revealed that Agamax leaf Vanadium content increased by 66% when compared to the control, after Vanadium treatment. Similarly, following Vanadium treatment, ICP-OES analysis showed

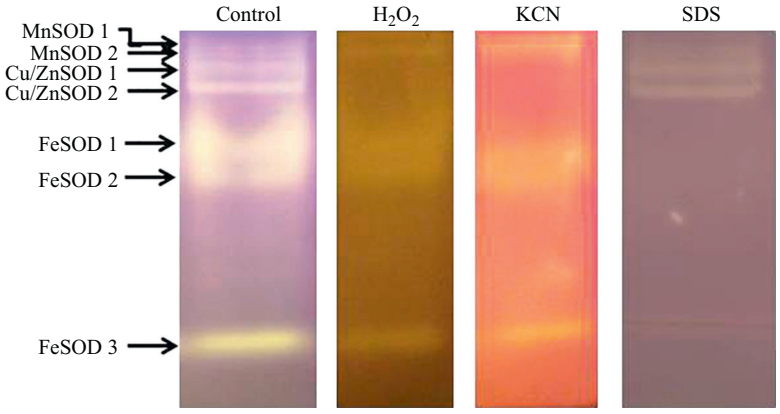

Fig. 5. Classification of SOD isoforms in Agamax leaves on the basis of resistance or sensitivity to 5 mM H_2O_2, 5 mM KCN and 2% (m/v) SDS. Representative images show SOD isoforms separated on 13% (v/v) resolving native-PAGE using 200 μg of protein per lane.

that AV Garnet leaf Vanadium content increased by 192% when compared to the control (Table 4).

3.10. Translocation of total Vanadium from roots to leaves

The TF from AAS analysis revealed that exogenous application of Vanadium led to a 11% decrease in Vanadium translocation from root to leaf in Agamax when compared to controls (Table 5). Furthermore, contrastingly, the TF from AAS analysis showed that AV Garnet increased the translocation of Vanadium from root to leaf by 124% when compared to the control following Vanadium treatment (Table 5).

The TF from ICP-OES analysis showed that exogenous application of Vanadium led to a 19% decrease in Vanadium translocation from root to leaf in Agamax when compared to controls (Table 5). Contrastingly, the TF from ICP-OES analysis revealed that AV Garnet increased the translocation of Vanadium from root to leaf by 103% when compared to the control after exogenous Vanadium treatment (Table 5).

4. Discussion

Prior to initiating this study, we conducted an identical pot experiment to evaluate the impact of different concentrations of Vanadium ($NaVO_3$ at 0 μM, 150 μM, 250 μM, 350 μM, 450 μM and 550 μM) on leaf biomass (dry weights) of two *B. napus* cultivars namely Agamax and AV Garnet in order to identify a suitable Vanadium concentration for future studies (data not shown). In that study, we identified 350 μM of Vanadium (as $NaVO_3$) to be the most suitable concentration for plant physiology and biochemistry studies on the two *B. napus* cultivars (data not shown). In general, plant biomass is often considered a highly sensitive plant response parameter to any stress and can be used to measure plant tolerance to metal stress (Imtiaz et al. 2015b). In addition, under heavy metal stress, leaf yellowing is often a sign of possible chlorophyll degradation in plants (Shen et al. 2017). In the present study, we observed morphological changes in leaves (the second youngest) of the two *B. napus* cultivars (Agamax and AV Garnet) following Vanadium treatment. We observed a leaf size reduction in both Agamax and AV Garnet in response to Vanadium. In addition, AV Garnet leaves displayed yellowing where Agamax leaves did not display any visible leaf yellowing in response to Vanadium treatment. This result suggests that Agamax is less sensitive to Vanadium treatment and AV Garnet is more sensitive to Vanadium treatment. To further strengthen the observation of Vanadium tolerance and sensitivity, we measured the leaf fresh weights after Vanadium treatment. Our results showed that Vanadium treatment decreased the leaf fresh weight of AV Garnet more drastically then the leaf fresh weight of Agamax. Furthermore, we also measured the leaf dry weights after Vanadium treatment. Similar to the leaf dry weight result, Vanadium decreased the dry weight of AV Garnet much more than the leaf dry weight of Agamax. Our results are in agreement with that observed by Saco et al. (2013) which showed that Vanadium concentrations above 240 μM decreased the biomass of leaves and roots of *Phaseolus vulgaris*. Furthermore, our results are also in agreement with results obtained by Imtiaz et al. (2015b) which observed decreases in *Cicer arietinum* leaf biomass and the authors could also positively correlate less biomass reduction after Vanadium treatment to *C. arietinum* genotype tolerance and sensitivity to Vanadium stress.

According to Muradoglu et al. (2015) chlorophyll pigments seem to be one of the main molecules which influence heavy metal injury in

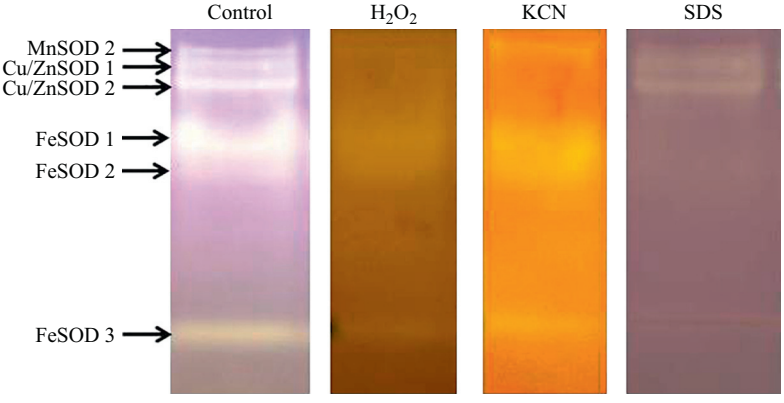

Fig. 6. AV Garnet leaf SOD isoform classification on the basis of resistance or sensitivity to 5 mM H_2O_2, 5 mM KCN and 2% (m/v) SDS. Images are from representative gels separated on 13% (v/v) resolving native-PAGE using 200 μg of protein per lane.

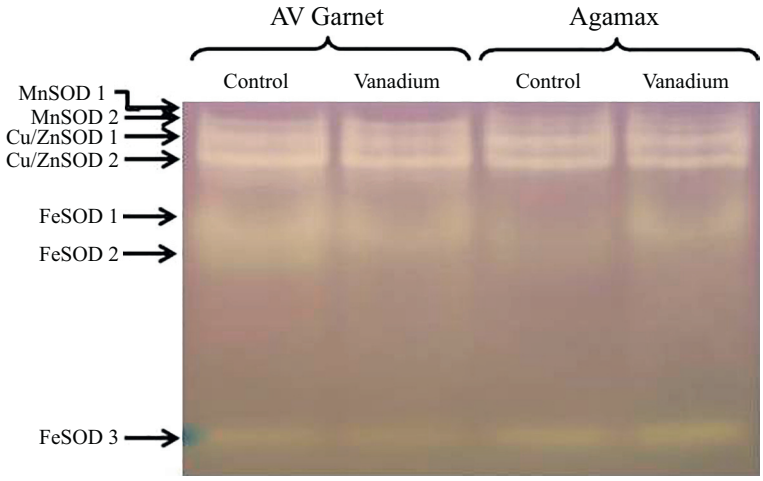

Fig. 7. Zymogram of SOD isoforms in two *B. napus* cultivar leaves in response to control or 350 μM Vanadium treatments. The representative image was separated on a 13% (v/v) resolving native-PAGE using 200 μg of protein per lane.

plants. Indeed, Qian et al. (2009) observed that cadmium (Cd) induced a declining effect on chlorophyll and carotenoid contents in *Chlorella vulgaris*. Furthermore, Hörtensteiner and Kräutler (2011) stated that chlorophyll breakdown, i.e. yellowing or bleaching leads to cell death when plants are exposed to stress. Therefore, in our study, after observing the yellowing of AV Garnet leaves, we measured the chlorophyll a and b content in both Agamax and AV Garnet leaves after Vanadium treatment. We observed that Vanadium treatment had no effect on chlorophyll a, chlorophyll b as well as total chlorophyll content of Agamax leaves. However, Vanadium treatment reduced the chlorophyll a, chlorophyll b and total chlorophyll content of AV Garnet leaves. This result strengthened our hypothesis that chlorophyll loss led to yellowing of AV Garnet leaves. Our result is in agreement with observations by Kumar and Rao (2008) which treated two Mulberry cultivars with fluoride. These authors observed a decrease in total chlorophyll content in cultivar S54 but did not observed any changes in total chlorophyll content in cultivar M5 at 2.1 $\mu g.g^{-1}$ fluoride when compared to the no-flouride control. Kumar and Rao (2008) therefore concluded that M5 was tolerant to fluoride stress and S54 was sensitive to fluoride stress.

Superoxide (O_2^-) is the first ROS which is produced in the Foyer-Halliwell-Asada pathway in plants (Yadav 2010). Furthermore, heavy metal toxicity leads to the generation of toxic O_2^- radicals which ultimately leads to plant cell death (Jain et al., 2010). Merzlyak et al. (1991) showed that O_2^- directly interacts with chlorophyll a therefore ultimately leading to chlorophyll degradation. Therefore, in our study we measured O_2^- content in both Agamax and AV Garnet leaves to understand whether O_2^- content could contribute to Vanadium induced chlorophyll degradation and ultimately sensitivity and tolerance to Vanadium stress. Thus, in our study we observed that Vanadium treatment increased O_2^- content in both Agamax and AV Garnet leaves but that the increase was more drastic in AV Garnet. Our data suggest that the increase in O_2^- in Agamax does not lead to chlorophyll degradation but that the increase (71%) in O_2^- content in AV Garnet when treated with Vanadium (in comparison to the control) does lead to chlorophyll degradation. This O_2^- toxicity makes AV Garnet more sensitive to Vanadium stress. Our result is in agreement with the O_2^- content result observed by Keyster et al. (2013). In that study, the authors observed a 50% increase in O_2^- content in *Zea mays* leaves of the salt sensitive cultivar (SK) and an approximate 24% increase in O_2^- content in the salt tolerant cultivar (GR). Keyster et al. (2013) therefore concluded that the capacity to control O_2^- content under salt stress is key in determining salt stress sensitivity and tolerance in *Zea mays*.

SOD enzymatic activity is one of the major paths for the detoxification of O_2^- in plants (Foyer and Noctor 2005). Therefore, in our study, we measured SOD enzymatic activity after observing differences in O_2^- content in Agamax and AV Garnet leaves after Vanadium treatment. We observed a decrease in leaf SOD enzymatic activity in response to Vanadium treatment in the two *B. napus* cultivars. However, we observed a smaller decrease in Agamax leaf SOD activity after Vanadium treatment than in AV Garnet when compared to the controls. This result explains why we observed much more O_2^- content in AV Garnet leaves when compared to the Agamax leaf O_2^- content because the much more inhibition of SOD activity in AV Garnet is responsible for the 71% increase in O_2^- content in AV Garnet leaves. Our result is in agreement with observations of Gallego et al. (1996) that observed that excess iron (Fe) and Cd, respectively caused a decrease in SOD activity in Sunflower leaves. Contrastingly, our SOD activity results differ from the results observed in Imtiaz et al. (2015b). In that study, the authors observed an increase in SOD activity in all chickpea genotypes in response to Vanadium treatments. Therefore, we hypothesise that the differential SOD activity responses to Vanadium stress in our study and in the study of Imtiaz et al. (2015b) could be plant species specific. We postulate that *B. napus* and Chickpea respond differently to Vanadium stress which ultimately leads to differential regulation of enzymes such as SOD. Our hypothesis is supported by results observed by Nouairi et al. (2009) which observed differences in leaf SOD activity between *B. juncea* and *B. napus* at 25 μM and 50 μM Cd. In that study, the authors observed no changes in SOD activity in *B. juncea* between 25 and 50 μM Cd but contrastingly observed a decrease in SOD activity in *B. napus* between 25 and 50 μM Cd. Therefore, Nouairi et al. (2009) concluded that the antioxidative defence systems are highly dependent on the plant species.

Table 2
Densitometry analysis [average values (AVG's)] of the SOD zymogram (Fig. 7) of two *B. napus* cultivars after control or 350 μM Vanadium treatments. Means (± SE) represent three replicates from three independent experiments and different letters per row differ significantly at $P < .05$ (Tukey–Kramer test).

SOD isoform	Agamax		AV Garnet	
	Control	Vanadium	Control	Vanadium
MnSOD 1	50 ± 1.26a	49 ± 1.31a	N/A	N/A
MnSOD 2	48 ± 1.31a	49 ± 1.25a	66 ± 1.41b	66 ± 1.37b
Cu/ZnSOD 1	83 ± 1.50a	82 ± 1.33a	85 ± 1.29a	84 ± 1.55a
Cu/ZnSOD 2	90 ± 1.33a	91 ± 1.39a	92 ± 1.49a	92 ± 1.51a
FeSOD 1	51 ± 1.57a	61 ± 1.49b	56 ± 1.59c	52 ± 1.52a
FeSOD 2	51 ± 1.27a	13 ± 1.35b	55 ± 1.41c	12 ± 1.29b
FeSOD 3	70 ± 1.57a	74 ± 1.49b	64 ± 1.54c	60 ± 1.56d

Table 3
Root total Vanadium content (μg.g^{-1}) after control or 350 μM Vanadium treatments of two *B. napus* cultivars. Means (± SE) of three replicates from three independent experiments are represented and different letters per row differ significantly at $P < .05$ (Tukey–Kramer test).

Detection method	Agamax		AV Garnet	
	Control	Vanadium	Control	Vanadium
AAS	35.22 ± 1.11a	73.25 ± 1.21b	35.19 ± 1.17a	50.12 ± 1.13c
ICP-OES	36.14 ± 0.79a	74.45 ± 0.99b	35.29 ± 0.96a	51.29 ± 0.95c

Failure of SOD to sufficiently scavenge O_2^- can lead to extensive lipid damage due to direct and indirect interaction of O_2^- with lipids in biological membranes (Shah et al. 2001). Therefore, measurement of MDA content is routinely used as an index of lipid peroxidation under stressful conditions (Lin and Kao 2000). Hence, in our study, we measured MDA content after observing that the inhibition of SOD activity led to an increase in leaf O_2^- content in Agamax and AV Garnet, respectively, following Vanadium treatment. In our study, we observed that Vanadium treatment increased MDA content in both Agamax and AV Garnet leaves but that the increase (±two-fold) was more severe in AV Garnet. Furthermore, we observed a correlation between O_2^- content and MDA content in the two cultivars following Vanadium treatment. We therefore hypothesise that the increase in O_2^- content leads to lipid peroxidation in both cultivars after Vanadium treatment. Furthermore, we postulate that the more severe increase in leaf MDA content in AV Garnet, makes AV Garnet more sensitive to Vanadium stress than Agamax. Our results are in agreement with results obtained by Shah et al. (2001) which observed that Cd treatments led to an increase in O_2^- content in two rice cultivars (Ratna and Jaya). In that study, the authors concluded that the increase in O_2^- is responsible for an increase in MDA content which ultimately led to the cultivar Jaya being more sensitive to Cd than Ratna.

Continuous lipid peroxidation induces uninterrupted cell wall and plasma membrane injuries which will subsequently lead to a decrease in cell viability (Liu et al., 2016). Therefore, cell viability or cell death is an important variable to monitor in plant stress experiments (Baker and Mock 1994). For this purpose, Evans blue dye is often used to measure membrane integrity. Plant cells with a damaged membrane cannot exclude the dye and are stained blue (Mergemann and Sauter 2000). Therefore, in our study, we measured Evans blue uptake in the leaves of Agamax and AV Garnet following Vanadium treatment in order to assess the level of cellular damage as a result of the increase in lipid peroxidation. Our results show that Vanadium treatment increased Evans blue uptake in both Agamax and AV Garnet leaves. Furthermore, we observed an increase in Evans blue uptake of ±two-fold in AV Garnet leaves following Vanadium treatment. This increase was much more drastic compared to the Evans blue uptake increase in Agamax. Moreover, we observed a positive correlation between MDA content and Evans blue uptake in the two cultivars following Vanadium treatment. Therefore, we postulated that the increase in lipid peroxidation leads to cell viability loss in both cultivars but that cellular damage was more pronounced in the Vanadium sensitive cultivar AV Garnet. Results in our study, is in agreement with results obtained by Song et al. (2011) which observed that Zinc (Zn) treatments led to an increase in Evans blue uptake in two rice cultivars (TY-167 and FYY-326). In that study, the authors concluded that the increase in MDA content could be responsible for an increase in Evans blue uptake which eventually led to the classification of cultivar FYY-326 being more sensitive to Zn stress than TY-167.

After observing that Vanadium treatments decreased total SOD enzymatic activity in the leaves of both Agamax and AV Garnet, we measured the in-gel SOD activity (as isoforms) on non-denaturing polyacrylamide gel electrophoresis (native PAGE). Comparot et al. (2002) suggested that changes in the levels of particular SOD isoforms, rather than changes in total SOD activity, might give more insights into the plant's responses to environmental changes. Comparot et al. (2002) measured the SOD in-gel activity of *B. napus* leaves and concluded that there are six SOD isoforms (two MnSOD isoforms, two FeSOD isoforms and two Cu/ZnSOD isoforms). Contrastingly, Abedi and Pakniyat (2010) measured the SOD in-gel activity of ten *B. napus* cultivars and concluded that there are eight SOD isoforms in *B. napus* (three MnSOD isoforms and five Cu/ZnSOD isoforms and no FeSOD isoforms). Furthermore, and also contrastingly, Feigl et al. (2014) observed a different SOD activity profile in *B. napus* than observed by both Comparot et al. (2002) as well as Abedi and Pakniyat (2010). Feigl et al. (2014) concluded that there are five SOD isoforms in *B. napus* (one MnSOD isoform, one FeSOD isoform and three Cu/ZnSOD isoforms). Knowing that these various studies obtained different and contrasting in-gel SOD activity profiles for *B. napus* leaves, we measured the SOD in-gel activity in our study for leaves of both Agamax and AV Garnet. We observed that Agamax leaves contain seven SOD isoforms (two MnSOD isoforms, three FeSOD isoforms and two Cu/ZnSOD isoforms) and that AV Garnet leaves contain six SOD isoforms (one MnSOD isoforms, three FeSOD isoforms and two Cu/ZnSOD isoforms). Our result differ from that observed in Comparot et al. (2002), Abedi and Pakniyat (2010), and Feigl et al. (2014) because we performed a 13% separating gel native PAGE and not a 10% separating gel native PAGE as in the studies of Comparot et al. (2002), Abedi and Pakniyat (2010) and Feigl et al. (2014). Furthermore, we loaded much more protein onto our native PAGE gels (200 μg) compared to the studies of Comparot et al. (2002), Abedi and Pakniyat (2010) and Feigl et al. (2014), by employing a fused well strategy. In addition, we used SDS as described by Brou et al. (2007) to further distinguish between the various SOD isoforms. Indeed, Brou et al. (2007) stated that most SOD profiles cannot be clearly separated due to the co-migration of FeSOD and Cu/ZnSOD. Nevertheless, we observed that Agamax leaves contain two MnSOD isoforms where AV Garnet only contains one leaf MnSOD isoform.

After observing a difference in the in-gel SOD profile of Agamax and AV Garnet, we analysed the responses of the various leaf SOD isoforms to Vanadium stress using native PAGE and densitometry analysis. In our study, Vanadium treatment did not alter the leaf MnSOD isoforms of both Agamax and AV Garnet. Furthermore, Vanadium treatment

Table 4
Total Vanadium content (μg.g^{-1}) in two *B. napus* cultivar leaves after control or 350 μM Vanadium treatments. Means (± SE) of three replicates from three independent experiments are represented and different letters per row differ significantly at $P < .05$ (Tukey–Kramer test).

Detection method	Agamax		AV Garnet	
	Control	Vanadium	Control	Vanadium
AAS	10.02 ± 1.05a	18.05 ± 1.01b	10.11 ± 1.07a	32.14 ± 1.10c
ICP-OES	11.12 ± 0.84a	18.49 ± 0.94b	11.09 ± 0.85a	32.41 ± 0.74c

Table 5
Translocation factor (TF) of total Vanadium in two *B. napus* cultivars after control or 350 μM Vanadium treatments. Means (± SE) of three replicates from three independent experiments are represented and similar letters per row do not differ significantly at $P < .05$ (Tukey–Kramer test).

Detection method	Agamax		AV Garnet	
	Control	Vanadium	Control	Vanadium
AAS	0.28 ± 0.01a	0.25 ± 0.01b	0.29 ± 0.01a	0.65 ± 0.01c
ICP-OES	0.31 ± 0.02a	0.25 ± 0.02b	0.31 ± 0.01a	0.63 ± 0.02c

also did not alter the leaf Cu/ZnSOD isoforms of both Agamax and AV Garnet. However, we observed differential regulation of leaf FeSOD isoforms in individual cultivars following Vanadium stress. FeSOD 1 activity was upregulated in Agamax leaves in response to Vanadium treatment. Contrastingly, the FeSOD 1 activity in AV Garnet was slightly decreased in response to Vanadium treatment. We observed that the leaf FeSOD 2 activity was decreased in both Agamax and AV Garnet. We postulate that this isoform might be sensitive to Vanadium stress. Furthermore, we observed that FeSOD 3 activity was upregulated in Agamax leaves in response to Vanadium stress but that FeSOD 3 activity in AV Garnet was slightly decreased in response to Vanadium stress. Based on our findings, we hypothesise that FeSOD isoforms are crucial in regulating O_2^- produced by Vanadium stress. In addition, we postulate that the differential regulation of FeSOD isoforms in the two cultivars is responsible for the decrease in total SOD activity following Vanadium treatment. Differential regulation of FeSOD isoforms in our study contributes to classifying Agamax as Vanadium tolerant and AV Garnet as Vanadium sensitive. Differential regulation of leaf SOD isoforms was also observed by Brou et al. (2007) when cowpea cultivars (TN88–63 and B21) was exposed to water deficit. Brou et al. (2007) concluded that the differential regulation of SOD isoforms in the two cowpea cultivar leaves contributed to classifying B21 as water deficit tolerant and TN88–63 as water deficit sensitive.

In a study by Imtiaz et al. (2015b) it was observed that differences in Vanadium concentrations between roots and leaves of Chickpea genotypes following Vanadium treatment was crucial in identification of tolerant and sensitive genotypes. In another study, Yang et al. (2017) observed that an increase in exogenous Vanadium concentration as treatment led to a decrease in Soybean pod number and ultimately bean fresh weight. This detrimental observation was linked to an increase in Vanadium concentration in roots and leaves of treated Soybean plants (Yang et al. 2017). Vachirapatama et al. (2011) used HPLC and ICP-MS for detection of Vanadium content in roots and leaves of Chinese green mustard and stated that the two methods were necessary due to the complex nature of the plant digests. Therefore, in our study, we used AAS and ICP-OES to measure Vanadium content in roots and leaves of Agamax and AV Garnet following Vanadium treatment. We observed good agreement between the two detection methods with variability below 10% for root and leaf values. We observed more Vanadium content in the roots of both Agamax and AV Garnet than in the respective leaves following Vanadium treatment. Both methods revealed that Agamax root Vanadium content increased more drastically (± two-fold) than root Vanadium content of AV Garnet following Vanadium treatment. Furthermore, both detection methods revealed that AV Garnet leaf Vanadium content increased more drastically (± three-fold) than leaf Vanadium content of Agamax after Vanadium treatment. Our results suggest that Vanadium sensitivity of AV Garnet is linked to Vanadium uptake into the leaves following exogenous Vanadium treatment. Our results are in agreement with observations by Nowakowski (1993) who observed that the Vanadium sensitive *Pisum sativum* cultivar namely Opal accumulates more Vanadium in the shoots than the Vanadium tolerant *P. sativum* cultivar namely Laser.

After observing differences in Vanadium accumulation in roots and leaves of Agamax and AV Garnet following Vanadium treatment, we calculated the TF from the Vanadium concentrations (values) obtained from AAS and ICP-OES. Egbenda et al. (2015) stated that TF is the transfer of heavy metals from roots to leaves. Furthermore, El-Ghawi et al. (2005) stated that TF is one of the important considerations used to estimate accumulation of toxic heavy metals in plants. Nonetheless, our results show good agreement between the two detection methods (AAS and ICP-OES) with a variability below 10% for root and leaf TFs. Furthermore, we observed similar TFs for Agamax and AV Garnet following control treatments. However, when Vanadium was applied as a treatment, we observed a decrease in Vanadium TF from roots to leaves when compared to the control treatment. This result suggests that Agamax accumulate Vanadium in the roots and decrease the translocation of Vanadium into the leaves after Vanadium treatment. Contrastingly, we observed an increase in Vanadium TF (± two-fold) from roots to leaves in AV Garnet following Vanadium treatment when compared to the control treatment. This result suggests that AV Garnet actively translocate Vanadium from the roots into the leaves following Vanadium treatment. We hypothesise that the active translocation of Vanadium from roots to leaves in AV Garnet following Vanadium treatment is responsible for the over accumulation of Vanadium in the leaves and ultimately leading to more Vanadium toxicity. Furthermore, we postulate that active translocation of Vanadium by AV Garnet is contributing to Vanadium sensitivity. Our hypothesis is in agreement with the conclusion of Kaplan et al. (1990) who stated that failure to immobilise Vanadium in the roots is presumed to be the primary reason for plant sensitivity to large quantities of Vanadium.

In conclusion, we observed that Vanadium treatment led to leaf cell death, chlorophyll degradation and a decreased in biomass in both *B. napus* cultivars (Agamax and AV Garnet). However, the cell death, chlorophyll degradation and biomass loss was more pronounced in AV Garnet leaves than in the Agamax leaves following Vanadium treatment. Furthermore, the superoxide content was more in AV Garnet leaves than in the Agamax leaves after Vanadium treatment and the difference in superoxide content led to differences in lipid peroxidation. Indeed, the lipid peroxidation in AV Garnet leaves was more pronounced than in the Agamax leaves after Vanadium treatment. We postulated that superoxide toxicity led to an increased in lipid peroxidation which ultimately increased leaf cell death, chlorophyll degradation and biomass loss in both cultivars. Therefore, the regulation of superoxide content by SOD was crucial in order to prevent leaf damage. In summary, even though SOD activity was inhibited in both cultivars in response to Vanadium we observed higher SOD activity in Agamax leaves than in AV Garnet leaves following Vanadium treatment. The difference in SOD activity was attributed to differential regulation of the iron-SOD class in the two cultivars. In addition, we observed more Vanadium content in Agamax roots than in AV Garnet roots following Vanadium treatment but contrastingly we observed more Vanadium in AV Garnet leaves than in Agamax leaves. We postulated that Agamax immobilises the Vanadium in the roots and transport little Vanadium into the leaves and that AV Garnet transport the Vanadium more actively to the leaves. This hypothesis was confirmed by TF analysis which showed that AV Garnet had a higher Vanadium TF from roots to leaves after Vanadium treatment where Agamax after Vanadium treatment had a Vanadium TF lower than the control. We thus conclude that AV Garnet is sensitive to Vanadium stress and Agamax is more tolerant to Vanadium stress. The study highlights that Vanadium immobilisation to the roots and regulation of SOD activity are two key strategies or characteristics for combating Vanadium toxicity in *B. napus*. Future studies should address the impact of Vanadium toxicity on *B. napus* under field conditions (i.e. near mining sites). Furthermore, other mitigation mechanisms should be investigated such as hormonal and ionome regulation under Vanadium stress.

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Author contribution statement

AG did all the experiments presented in this article. LFC and AG conducted the AAS experiments. MK conceived, designed and supervised the research. All authors wrote, critically read and approved the final manuscript.

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