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Physiological and biochemical responses of *Brassica napus* L. cultivars exposed to Cd stress

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Abstract: Four different rapeseed (*Brassica napus* L.) cultivars CZL, Benefit, Iwas and ZhongZhuang9, were used to analyse physiological responses to higher doses of cadmium (Cd) and their ability of Cd accumulation. Plants were exposed for 30 days to different Cd dosages (0, 50, 100, and 150 mg Cd/kg) in soil under greenhouse conditions. Cadmium was accumulated more in roots than in shoots of all tested cultivars, with the highest Cd in ZhongZhuang9. After the lowest Cd dose, the highest translocation factor was for CZL (0.8) and Benefit (0.6). The content of both K and Ca varied between treatments and cultivars. The chlorophyll fluorescence parameters and relative chlorophyll content were affected minimally. High constant levels of salicylic acid and a sharp increase in proline content were found mostly in Iwas; therefore, it may be considered as a more tolerant cultivar.

Keywords: rapeseed; heavy metal; proline; salicylic acid; phytoremediation

Cadmium (Cd) is one of the most toxic heavy metals present in the environment. Soil Cd pollution results from both anthropogenic and geogenic sources (Liu et al. 2013). As not biodegradable, Cd persists in the soil, and because of its high mobility, its impact on all living organisms is not negligible. Besides Asia, soil contamination is also an important issue across Europe, where approximately 3.5 million growing field sites were estimated to be potentially contaminated (Mahar et al. 2016).

In plants, high Cd concentrations can lead to severely reduced plant growth, a decrease in the chlorophyll content resulted in leaf chlorosis, changes in amino acid, salicylic acid (SA) and proline (Pro) content, reduction of mineral uptake and transport as well as induction of the reactive oxygen species (ROS) production (Nazar et al. 2012, Muradoglu et al. 2015, Šimek et al. 2016). In order to minimise ROS formation, plants have developed several defence strategies, including vacuolar compartmentalisation (Sharma et al. 2016), chelation by organic molecules and activation of antioxidants (Jozefczak et al. 2014). Several studies showed Pro as an effective antioxidant based on its ROS scavenging properties (Szabados and Savouré 2010). Salicylic acid is a ubiquitous plant phenolic compound functioning as a natural signalling molecule, the regulator of plant growth (Metwally et al. 2003), scavenger of ROS (Mateo et al. 2006) and enhancer of the antioxidant system (Guo et al. 2013). In many plant species, exogenously applied SA alleviates the negative impact of Cd on photosynthesis (Ahmad et al. 2018) or modulates Cd uptake and distribution (Belkhadi et al. 2010). Although the protective role of exogenous SA is well known, the role of endogenous SA under heavy metal

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stress has not been fully explained. Zawoznik et al. (2007) suggest that endogenous SA may also serve as an important signalling molecule in the generation of Cd-induced oxidative stress. Recently, the endogenous SA was identified as the link with the biosynthesis of glutathione, a key cellular compound for Cd chelation (Guo et al. 2016).

Rapeseed (*Brassica napus* L.) is an economically important crop plant expected to be a Cd-tolerant and Cd-accumulating plant. Thanks to its fast growth and high biomass production, it should be a promising plant for phytoremediation. Screening of tolerant cultivars might be highly beneficial for the development of phytoremediation technology. For example, Cao et al. (2020) showed significant genotypic differences between 28 cultivars in the ability to accumulate and translocate Cd. Thus, our aim was to investigate the differences in Cd uptake and translocation of four cultivars of *B. napus* with different characteristics and eco-physiological parameters. To show various possible responses to the Cd exhibition of selected cultivars, the changes in the content of chlorophyll, mineral nutrient, SA and amino acid were monitored. Based on these data, the possible tolerance range of used cultivars was indicated.

MATERIAL AND METHODS

Plant cultivation. Seeds of four *Brassica napus* cultivars (Benefit, Iwas, CZL, ZhongZhuang9) were obtained from the Crop Research Institute in Prague, the Czech Republic; basic characteristics are shown in Table 1. Pot experiments were performed in a greenhouse with standard lightning conditions and optimum water intake. Plants were grown in 9.5 L plastic pots (3 pcs/pot) with 9.0 kg of alluvial soil, clay loamy in texture. Three different doses of Cd were applied once: 50 (50Cd), 100 (100Cd) and 150 (150Cd) mg/kg of $CdCl_2$ dissolved in distilled water. The soil chemical properties in Mehlich III leach were as follows: pH_{KCl} – 5.80; content of available P 91.4, K 203, Mg 202, Ca 1 840 mg/kg soil; the total heavy metal content: Cd < 0.1; As 1.0; Cr 12.0; Pb 7.0; Zn 38.0; Cu 0.7 mg/kg. After 30 days, plants were harvested and divided into root and shoot and dried separately at 65 °C for 48 h. For further analysis, 3 plants from each pot were pooled together to create one biological replicate.

Estimation of physiological characteristics. The relative chlorophyll content was measured using chlorophyll meter CCM 200 (OptiScience, Hudson, USA). Chlorophyll fluorescence was measured in dark-adapted plants (15 min) using chlorophyll fluorometer OS1p (Opti-Sciences, Hudson, USA). Results were expressed as F_v/F_m, determined as the maximal fluorescence (F_m) less the minimal fluorescence (F_0), divided by F_m, i.e. $F_v/F_m = (F_m – F_0)/F_m$. The data was recorded on fully expanded leaves of 5 individuals of each replicate, treatment and genotype (n = 15).

Quantification of Cd, K and Ca. Powdered dried samples were digested in 3 mL HNO_3 and 1 mL 30% H_2O_2 using a microwave oven (Speed wave Berghof MWS3, Eningen, Germany). Concentrations of Cd, K and Ca were determined using the Atomic Absorption Spectrometer (AAS SOLAAR M5, Waltham, USA). The translocation factor (TF) was calculated as the ratio of Cd concentrations in the shoots to the root. Bioconcentration factor (BCF) was calculated as the ratio of Cd concentration in harvested tissue to soil Cd concentration (Ali et al. 2013).

Estimation of salicylic acid. Salicylic acid was detected by HPLC Agilent 1260 on a column Kinetex C18 (150 × 4.6 mm ID) column heated at 25 °C. Dried powdered samples were mixed with 5 mL 80% methanol using the Multi Reax shaker (Heidolph, Schwabach, Germany) overnight, then centrifuged at 3 000 *g* for 15 min (MPW 223, Warsaw, Poland). Extracts were evaporated at 30 °C under nitrogen purity 4.0 (SIAD). The mobile phase composed of a mixture of acetonitrile/3.35% phosphoric acid (40/60) was in isocratic elution at a flowrate of 1.5 mL/min. Salicylic acid was detected in the UV region at 235 nm.

Estimation of free amino acids. Dried powdered samples were mixed with 70% ethanol and shaken for 15 min (Multi Reax shaker Heidolph, Schwabach,

Table 1. The basic characteristics of used *Brassica napus* cultivars

Genotype	Geographic origin	Cultivar type	Characteristics of the cultivar
Benefit	Czech Republic	winter-crop	different range of dehydrins
Iwas	Japan	spring-crop	different habitus, nontolerant to frost
CZL	China/Czech Republic	winter-crop	early cultivar, low frost tolerance
ZhongZhuang9	China	autumn/winter-crop	early cultivar, yellowish seeds, very low frost tolerance

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Germany) and heated for 10 min at 120 °C. After cooling and centrifugation for 15 min at 5 000 rpm, the supernatant was transferred into a clean tube. The pellet was resuspended with 70% ethanol, shaken for 15 min and again centrifuged. This process was repeated twice. The collected supernatant was evaporated at 60 °C under an oxygen atmosphere (NDK 200-2, Hangzhou MIU Instruments Co., Zhejiang, China). Selected amino acids were detected on the HPLC Agilent 1260 equipped with the Zorbax Eclipse Plus RRHT C18 column (4.6 × 50 mm, 1.8 µm). The Agilent HPLC method (5990-4547EN) was adapted for the selected system. Derivatisation reagents (Borate Buffer 5061-3339, OPA Reagent 5061-3337) and Amino Acid standards 1 nmol/µL (5061-3330) were purchased from Agilent.

Statistical analysis. The differences among treatments were analysed using a one-way analysis of variance (ANOVA) followed by Tukey's test at the significance level of $P < 0.05$ (GraphPad Prism 9 Software, San Diego, USA).

RESULTS AND DISCUSSION

Effect of Cd on physiological parameters. The negative effect of Cd on plants is usually reflected in growth inhibition or imbalances in photosynthesis. The leaf chlorosis observed in several studies with rapeseed exposed to high Cd concentration were probably caused by Cd inhibitory effects on enzymes involved in the pigment biosynthesis (Sun et al. 2017) or due to Mg substitution in chlorophyll (Parmar et al. 2013). So, chlorosis occurrence can be used as an index to evaluate possible heavy metal tolerance. Here, Cd-treated plants exhibited leaf chlorosis compared to control plants (Figure 1). However, these yellowish spots were not as much noticeable as it might be expected after the application of high Cd doses. These variously large yellowish areas around the leaf veins were more frequent in young leaves. The relative chlorophyll content slightly decreased in the presence of Cd, but only in cultivars Benefit and Iwas (Figure 2). In the remaining two cultivars, CZL and ZhongZhuang9, the relative chlorophyll content was lower compared to the other two cultivars, and during the treatment, it stayed relatively unchanged. Opposite to our results, the decrement in chlorophyll content was monitored after higher Cd concentration (500 µmol) or after a prolonged period of Cd stress (Alit et al. 2014, Yan et al. 2016). Due to no significant changes in the relative chlorophyll content in the presence of Cd, we can suggest that the used cultivars do not react so sensitively to our chosen Cd dosage.

The ratio F_v/F_m describes the potential yield of the photochemical reaction and is often used as a stress indicator. Here, the values of F_v/F_m in control plants were slightly lower than the optimum (0.83) set by Jones et al. (1999) and varied between 0.7–0.73. The ratio did not change in any of the tested cultivars, whereas the minimum fluorescence and maximum fluorescence significantly decreased in CZL and Benefit (Figure 3). Obviously, the Cd impact on both chlorophyll fluorescence characteristics was much more severe in the highest Cd dose in Benefit. Similarly, according to Benyas et al. (2013), Cd had no significant effect on chlorophyll fluorescence and pigment content in oilseed rape.

Content of Cd, K, and Ca. Rapeseed tolerates relatively high concentrations of heavy metals in the soil, those it is a promising candidate for phytoextraction (Wang et al. 2009). In our experiment, increasing soil Cd concentrations led to the accu-

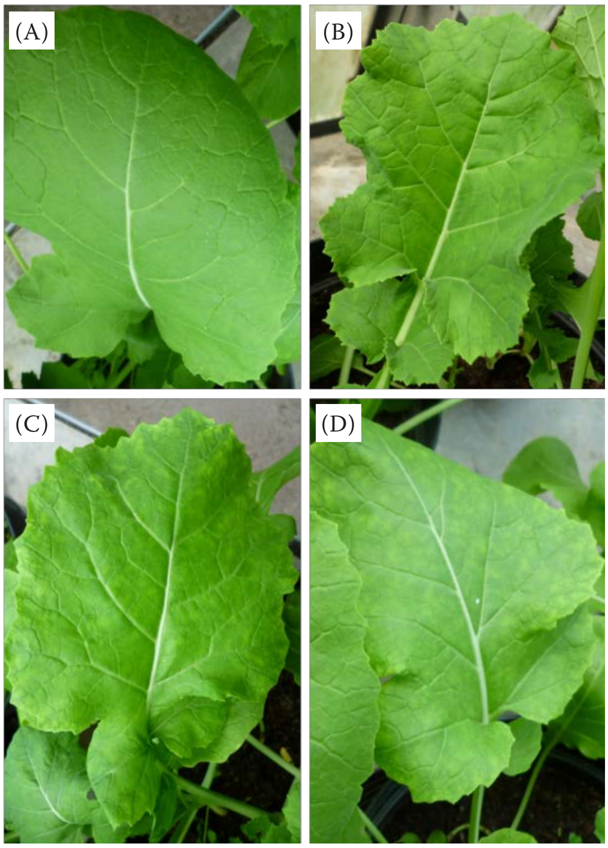

Figure 1. Leaf chlorosis of *Brassica napus* cv. Benefit grown 30 days in soil with different concentrations of $CdCl_2$, (A) 0 mg/kg of Cd, (B) 50 mg/kg of Cd, (C) 100 mg/kg of Cd, and (D) 150 mg/kg of Cd

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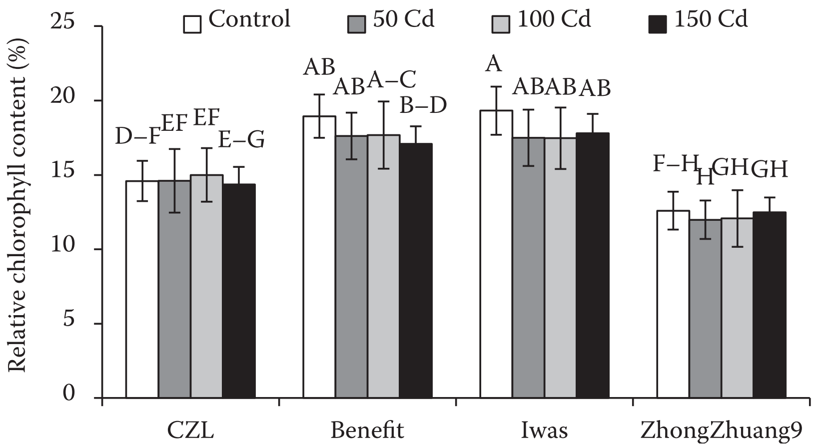

Figure 2. Relative chlorophyll content in leaves of *Brassica napus* cvs. CZL, Benefit, Iwas and ZhongZhuang9 grown for 30 days in soil with different concentrations of $CdCl_2$ (50, 100 and 150 mg/kg of Cd). Data are mean ± standard deviation (n = 15). Values within the column, followed by the same letter(s), are not significantly different according to Tukey's test (P < 0.05)

mulation of Cd in the roots and leaves (Figure 4). An exception is the cv. Iwas, where the highest Cd content occurred in variant 100Cd. High mobility of Cd from soil to roots was indicated in cvs. Iwas and especially in ZhongZhuang9, where the root and shoot Cd concentrations were much greater than those of the other two cultivars, on average 1.5 to 3.3-times. Surprisingly, in all tested cultivars,

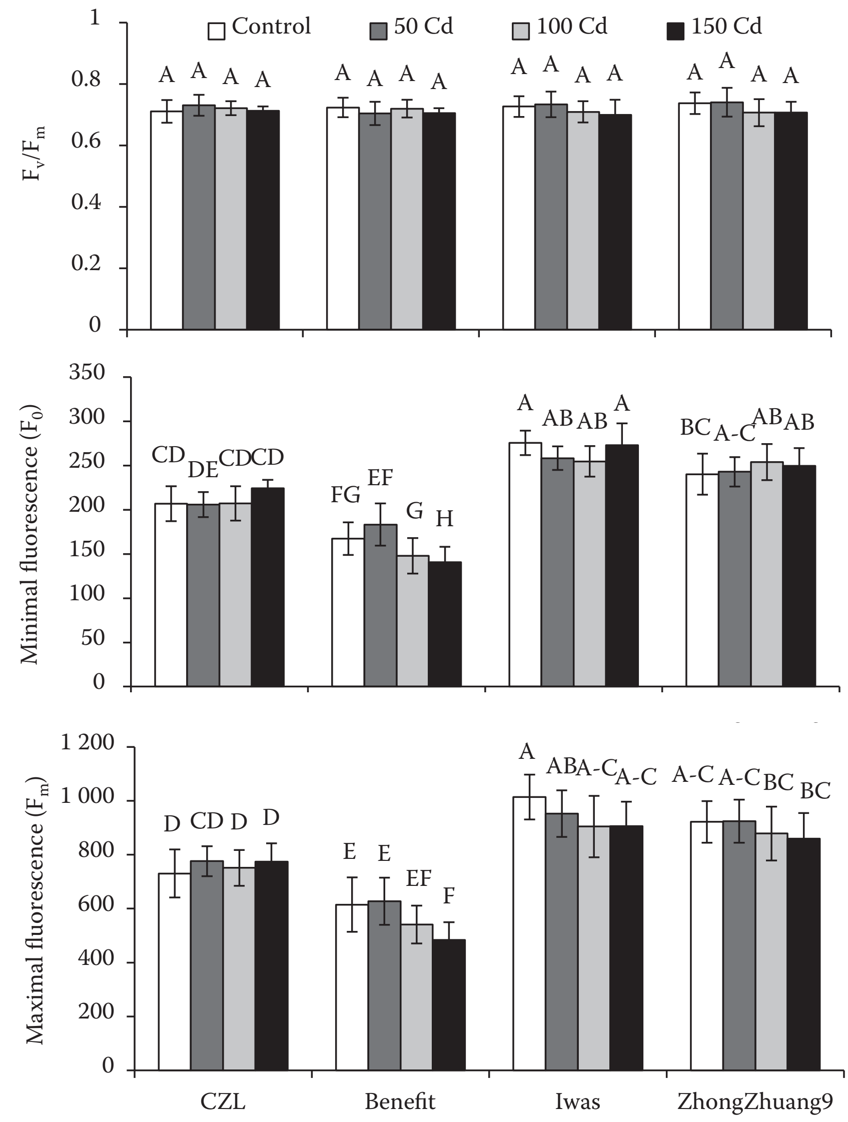

Figure 3. Chlorophyll fluorescence parameters determined in leaves of *Brassica napus* grown 30 days in soil with different concentrations of $CdCl_2$ (50, 100 and 150 mg/kg of Cd). Data are mean ± standard deviation (n = 15). Values within the column, followed by the same letter(s), are not significantly different according to Tukey's test (P < 0.05)

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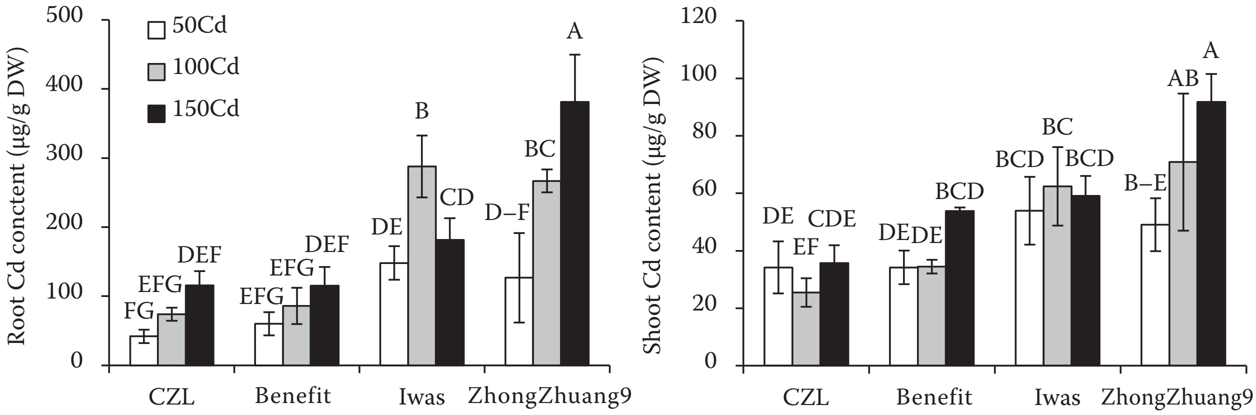

Figure 4. Content of cadmium (Cd) in roots and shoots (µg/g DW (dry weight)) in *Brassica napus* grown for 30 days in soil with different concentrations of $CdCl_2$ (50, 100 and 150 mg/kg of Cd). Data are mean ± standard deviation (n = 15). Values within the column, followed by the same letter(s), are not significantly different according to Tukey's test (P < 0.05)

the Cd content was higher in root than those in the shoot. Similar results were recorded by Herrero et al. (2003) and Farid et al. (2015), where, independently of used Cd concentration in the pot or hydroponic experiments, respectively, the accumulation of Cd was higher in roots than in the aboveground parts. On the contrary, Ghnaya et al. (2009) showed that Cd accumulation in selected organs varied according to the used cultivar. However, a field experiment with 28 rapeseed cultivars brought opposite results, and the Cd content decreased in the order stem > root > seed in all cultivars (Cao et al. 2020). This could be ascribed to genotypic variation, the used experimental system, and last but not least to soil properties which considerably influenced Cd uptake and translocation in plants. Several studies showed that the formation of complexes with Cl^- leads to increased soil Cd availability for plants and subsequently increased uptake, compared to SO_4^{2-} or NO_3^- ions. However, the subsequent translocation to the aboveground parts may already be slowed down (Šimek et al. 2016, Piršelová and Ondrušková 2021).

The overall evaluation and selection of plants for phytoremediation purposes entirely depend on the efficiency of a plant in accumulating a metal (BCF) and its consequent distribution in the plant (TF). The highest BCF was measured for the roots of cvs. Iwas and ZhnogZhuang9 (Table 2). However, for shoots, the values fell by more than half, which subsequently resulted in a relatively low TF, between 0.2–0.5. Low BCF values, both in the roots and shoots, were in cvs. CZL and Benefit. On the other hand, the greatest TF was found here, 0.827 for cv. CZL and 0.581 for cv. Benefit, but only in variants 50Cd. Higher Cd doses markedly reduced the TF values. According to Yoon et al. (2006), only plant species with both BCF and TF greater than 1 have the potential to be used for phytoextraction. These conditions are not met by any of the used cultivars. Even in the case of easy extraction of the root system from the soil, this fact could be a potential obstacle in selecting a suitable cultivar for phytoextraction. On the contrary, the application of various chelating agents to soil can optimise the metals absorption by roots, metal translocation to shoots and also the metal mobilisation. Farid et al. (2015) showed that the application of EDTA enhanced Cd accumulation in rapeseed roots, stems and leaves.

Table 2. Bioconcentration factor (BCF) and translocation factor (TF) in *Brassica napus* grown for 30 days in soil with different concentrations of $CdCl_2$ (50, 100 and 150 mg/kg of Cd)

Cd (mg/kg)	cv. CZL			cv. Benefit			cv. Iwas			cv. ZhongZhuang9		
	BCF		TF	BCF		TF	BCF		TF	BCF		TF
	root	shoot		root	shoot		root	shoot		root	shoot	
50Cd	0.837	0.684	0.827	1.200	0.684	0.581	2.962	1.078	0.376	2.535	0.980	0.551
100Cd	0.739	0.255	0.344	0.858	0.345	0.430	2.878	0.624	0.222	2.668	0.708	0.270
150Cd	0.770	0.238	0.310	0.767	0.359	0.484	1.208	0.394	0.329	2.541	0.612	0.243

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In the presence of Cd, the K content in roots showed different behaviour (Figure 5). Significantly higher K level was found in roots of cv. CZL, exposed to the highest Cd concentration. Contrary, in the cv. Iwas, higher Cd dosages led to a significant reduction in the K content. Surprisingly, a sharp drop was observed in cv. Benefit root in the 50Cd variant, whereas higher Cd dosages led to a significant increase. In shoots, the K content was slightly higher than in roots, and a slight but not significant increment was observed only in cvs. ZhongZhuang9 50Cd and CZL 150Cd variants. These findings are partly inconsistent with some previous studies, where Cd was reported to reduce the uptake of K in plants (Liu et al. 2012, Hédiji et al. 2015). It has been shown that K plays an important role in the interaction with Cd in plants, and improving the K nutrition of plants under stress can minimise oxidative cell damage by improving the activity of the antioxidant system (Siddiqui et al. 2012). For this reason, it can be suggested that higher amounts of K in the roots of the Cd-treated plants might serve as protection from heavy metal stress.

Generally, a huge variability in Ca content was observed in the roots of all tested cultivars after the Cd treatment; however, the differences were not significant (Figure 5). Considerably higher Ca levels were found in shoots than in roots. In shoots of the cvs. CZL and ZhongZhuang9, slightly lower contents of Ca were found in controls and in Cd-treated plants compared to cvs. Benefit and Iwas. Ca and Cd ions are known to have a highly analogous structure and Ca can regulate Cd-induced physiological and metabolic changes in plants together with alleviation of growth inhibition or oxidative stress caused by the Cd stress (Dayod et al. 2010, Huang et al. 2017). Similar Ca contents in our plants may point to a higher ability to tolerate Cd stress *via* enhanced Ca-dependent protective mechanisms.

Salicylic acid content. Salicylic acid plays an important role in eliciting specific responses to abiotic as well as biotic stresses. It can alleviate Cd toxicity by reducing Cd uptake and accumulation in plant organs (Horváth et al. 2007). Much higher SA contents were found in roots, where the greater part of Cd is accumulated (Figure 6). In roots of cv. Benefit, and especially in cv. ZhongZhuang9, the SA content was lower, but not significantly, compared to cvs. CZL and Iwas. However, no pronounced effect of different Cd treatments was observed in any tested cultivar. The shoot's highest SA level was in cv. Iwas, but same as in roots, Cd dosage almost did not alter its amount. The SA is known to bind Cd ions to inactivate it and so to prevent another important biomolecule against its toxic effects. Kovács et al. (2014) showed opposite results where a higher SA level was in the leaves and suggested that it may be due to the formation of its precursors in the roots and subsequent transport to the leaves. Exogenously added SA significantly improved photosynthesis, reduced oxidative stress or alleviated the Cd-induced ROS overproduction (Zhang and Chen 2011, Ahmad et al. 2018). Interestingly, there are no reports on whether exogenous and endogenous SA plays different or similar roles in response to oxidative stress. Zawoznik et al. (2007) suggest that endogenous SA may function rather as a signalling molecule essential for generating, maintaining or amplifying the Cd-induced oxidative stress. In the current study, the highest SA content in roots and shoots was observed, especially in cv. Iwas and partly in cv. CZL. Our data suggest that SA may protect the plant against Cd-induced damage by inactivation of Cd in the roots, which protect important photosynthetic aboveground parts, which are generally more susceptible to the toxic effects of heavy metals. It suggests that cv. Iwas might have a higher ability to produce and accumulate endogenous SA, which possibly allows a quicker response to Cd stress. This suggestion might be supported by a very low TF of this cultivar (on average 0.3), pointing to effective defence mechanisms in roots and shoots against the Cd stress. Moreover, cv. Iwas belongs to the spring sown crops, which are more often infected by viruses than winter sown crops. Considering this fact, higher SA content could be essential not only for effective resistance to viral pathogens but also for good tolerance to heavy metal stress.

Amino acid content. Proline has been reported to accumulate under heavy metal stress and thus acts as an indicator of environmental stresses (Ali and Hadi 2015). Plants accumulate proline by increasing its synthesis together with reduced catabolism under abiotic stress (Verbruggen and Hermans 2008). Different Cd treatments showed varying effects on proline contents, as shown in Figure 7. After Cd addition, the free proline content slightly increased in roots of cvs. CZL, Benefit and ZhongZhuang9. In cv. Iwas it significantly decreased in variants 100Cd and 150Cd; contrarily in shoots, Cd exposure induced its significant accumulation. Similar results were also found in shoots of cv. Benefit, where higher Cd doses led to proline increment. Higher proline contents here may be explained by an enhanced tolerance to metal stress or by a more efficient stress response.

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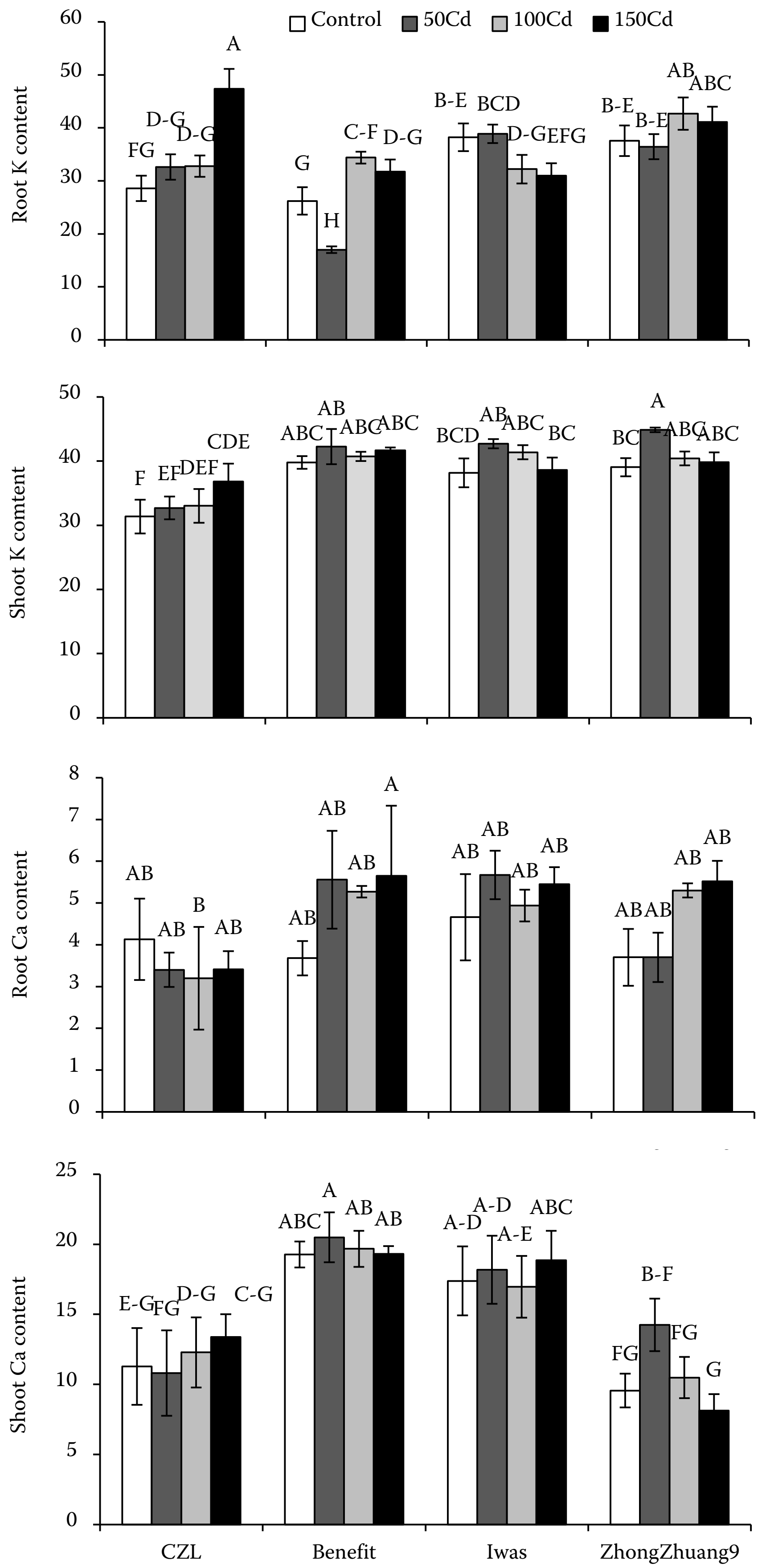

Figure 5. Content of K and Ca (mg/g DW (dry weight)) in *Brassica napus* grown for 30 days in soil with different concentrations of $CdCl_2$ (50, 100 and 150 mg/kg of Cd). Data are mean ± standard deviation (n = 15). Values within the column, followed by the same letter(s), are not significantly different according to Tukey's test (P < 0.05)

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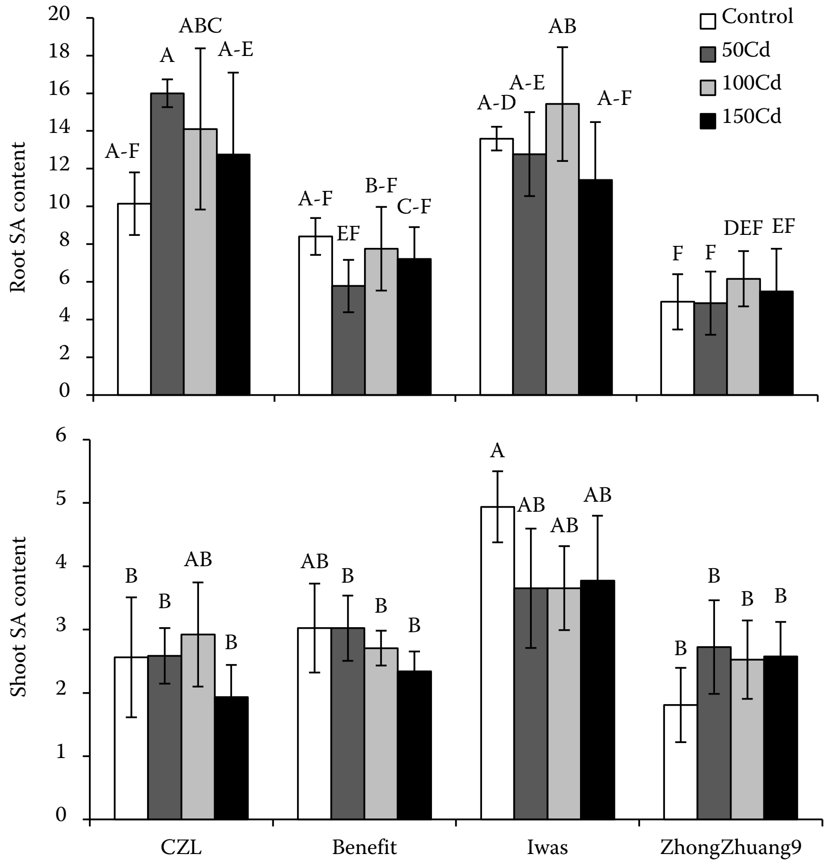

Figure 6. Content of salicylic acid (SA) in roots (A) and leaves (B) (µg/g DW (dry weight)) in *Brassica napus* grown for 30 days in soil with different concentrations of $CdCl_2$ (50, 100 and 150 mg/kg of Cd). Data are mean ± standard deviation (n = 15). Values within the column, followed by the same letter(s), are not significantly different according to Tukey's test (P < 0.05)

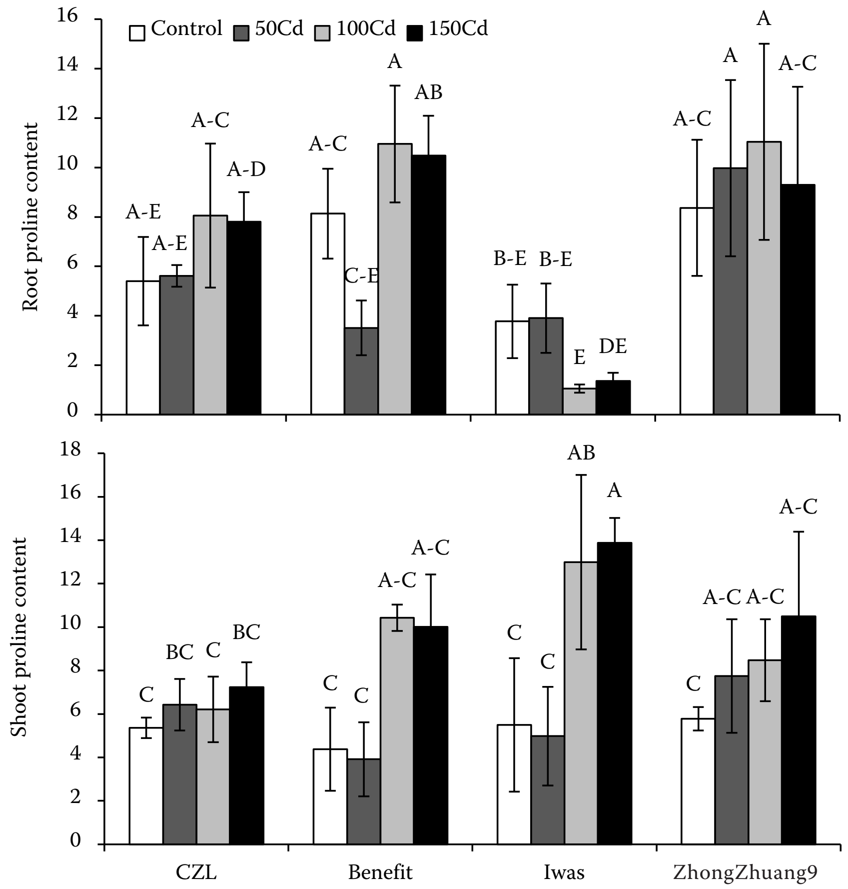

Figure 7. Content of proline in roots (A) and leaves (B) (µmol/g DW (dry weight)) in *Brassica napus* rown 30 days in soil with different concentrations of $CdCl_2$ (50, 100 and 150 mg/kg of Cd). Data are mean ± standard deviation (n = 15). Values within the column, followed by the same letter(s), are not significantly different according to Tukey's test (P < 0.05)

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Table 3. Correlation coefficient according to linear regression analysis between cadmium (Cd) concentrations and amino acids contents in *Brassica napus* grown 30 days in soil with different concentrations of $CdCl_2$. The critical value of the correlation coefficient was set as 0.520

	cv. CZL	cv. Benefit	cv. Iwas	cv. ZhongZhuang9
Root				
Arg	0.692	–	0.741	–
Ala	0.612	–	0.777	–0.976
Met	0.528	–	–	–
Lys	0.524	–	0.788	–0.961
Pro	0.863	–	–0.789	–
Shoot				
Arg	0.766	–	0.770	0.786
Ala	0.705	0.706	0.445	–
Met	0.704	–	0.766	0.581
Lys	0.854	–	–	0.612
Pro	0.907	0.645	0.624	0.974

Under heavy metal stress, enhanced accumulation of amino acids is due to the degradation of certain proteins or by *de novo* synthesis (Sharma and Dietz 2006). They serve as important intermediates in the metabolism of osmolytes or antioxidants; however, in the present study, their composition showed a lack of parallelism in Cd responses (data not shown). According to linear regression analysis between Cd dosage and the amino acid contents in roots and shoots separately, only five amino acids were found to have a significant correlation (Table 3). Arginine, alanine, methionine and lysine were strongly influenced by treatment in shoots of cvs. CZL and Iwas.

In conclusion, oilseed rape was found to be highly tolerant to Cd stress, according to chlorophyll content and fluorescence. Considering the low TF in almost all tested cultivars, it is an obstacle to select a suitable cultivar for phytoextraction. Cadmium also showed a huge variable effect on mineral and SA uptake in all tested *B. napus* cultivars. However, higher constant levels of SA and an increase in proline content in cv. Iwas were observed. Iwas was the only cultivar which belongs to spring sown crops. It is known that a large number of viruses cause more damage to spring-sown crops than to winter-sown crops (Zheng et al. 2019). Thus, spring sown crops with potentially higher constant levels of different stress signalling metabolites, including SA or proline, could also be successfully grown on Cd-contaminated soils.

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REFERENCES

Ahmad B., Jaleel H., Sadiq Y., Khan M.M.A., Shabbir A. (2018): Response of exogenous salicylic acid on cadmium induced photosynthetic damage, antioxidant metabolism and essential oil production in peppermint. Plant Growth Regulation, 86: 273–286.

Ali H., Khan E., Sajad M.A. (2013): Phytoremediation of heavy metals – concepts and applications. Chemosphere, 91: 869–881.

Ali B., Qian P., Jin R., Ali S., Khan M., Aziz R., Zhou W. (2014): Physiological and ultra-structural changes in *Brassica napus* seedlings induced by cadmium stress. Biologia Plantarum, 58: 131–138.

Ali N., Hadi F. (2015): Phytoremediation of cadmium improved with the high production of endogenous phenolics and free proline contents in *Parthenium hysterophorus* plant treated exogenously with plant growth regulator and chelating agent. Environmental Science and Pollution Research, 22: 13305–13318.

Belkhadi A., Hediji H., Abbes Z., Nouairi I., Barhoumi Z., Zarrouk M., Chaïbi W., Djebali W. (2010): Effects of exogenous salicylic acid pretreatment on cadmium toxicity and leaf lipid content in *Linum usitatissimum* L. Ecotoxicology and Environmental Safety, 73: 1004–1011.

Benyas E., Nassab A.D.M., Oustan S. (2013): Effects of cadmium on some morphological and physiological traits of amaranth (*Amaranthus caudatus* L.) and oilseed rape (*Brassica napus* L.). International Journal of Biosciences, 3: 17–26.

Cao X.R., Wang X.Z., Tong W.B., Gurajala H.K., He Z.L., Yang X.O. (2020): Accumulation and distribution of cadmium and lead in 28 oilseed rape cultivars grown in a contaminated field. Environmental Science and Pollution Research, 27: 2400–2411.

Dayod M., Tyerman S.D., Leigh R.A., Gilliham M. (2010): Calcium storage in plants and the implications for calcium biofortification. Protoplasma, 247: 215–231.

Farid M., Ali S., Ishaque W., Shakoor M.B., Niazi N.K., Bibi I., Dawood M., Gill R.A., Abbas F. (2015): Exogenous application of ethylenediamminetetraacetic acid enhanced phytoremediation of cadmium by *Brassica napus* L. International Journal of Environmental Science and Technology, 12: 3981–3992.

Ghnaya A.B., Charles G., Hourmant A., Hamida J.B., Branchard M. (2009): Physiological behaviour of four rapeseed cultivar (*Brassica napus* L.) submitted to metal stress. Comptes Rendus Biologies, 332: 363–370.

Guo B., Liu C., Li H., Yi K.K., Ding N.F., Li N.Y., Lin Y.C., Fu Q.L. (2016): Endogenous salicylic acid is required for promoting cadmium tolerance of *Arabidopsis* by modulating glutathione metabolisms. Journal of Hazardous Materials, 316: 77–86.

Guo Q., Meng L., Mao P.C., Jia Y.Q., Shi Y.J. (2013): Role of exogenous salicylic acid in alleviating cadmium-induced toxicity in Kentucky bluegrass. Biochemical Systematics and Ecology, 50: 269–276.

Hédiji H., Djebali W., Belkadhi A., Cabasson C., Moing A., Rolin D., Brouquisse R., Gallusci P., Chaïbi W. (2015): Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: consequences on fruit production. South African Journal of Botany, 97: 176–181.

Herrero E.M., López-Gonzálvez A., Ruiz M.A., Lucas-García J.A., Barbas C. (2003): Uptake and distribution of zinc, cadmium, lead and copper in *Brassica napus* var. *oleífera* and *Helianthus annus* grown in contaminated soils. International Journal of Phytoremediation, 5: 153–167.

Horváth E., Szalai G., Janda T. (2007): Induction of abiotic stress tolerance by salicylic acid signaling. Journal of Plant Growth Regulation, 26: 290–300.

Huang D.L., Gong X.M., Liu Y.G., Zeng G.M., Lai C., Bashir H., Zhou L., Wang D.F., Xu P., Cheng M., Wan J. (2017): Effects of calcium at toxic concentrations of cadmium in plants. Planta, 245: 863–873.

Jones R.J., Kildea T., Hoegh-guldberg O. (1999): PAM chlorophyll fluorometry: a new *in situ* technique for stress assessment in scleractinian corals, used to examine the effects of cyanide from cyanide fishing. Marine Pollution Bulletin, 38: 864–874.

Jozefczak M., Keunen E., Schat H., Bliek M., Hernández L.E., Carleer R., Remans T., Bohler S., Vangronsveld J., Cuypers A. (2014): Differential response of *Arabidopsis* leaves and roots to cadmium: glutathione-related chelating capacity vs antioxidant capacity. Plant Physiology and Biochemistry, 83: 1–9.

Kovács V., Gondor O.K., Szalai G., Darkó É., Majláth I., Janda T., Pál M. (2014): Synthesis and role of salicylic acid in wheat varieties with different levels of cadmium tolerance. Journal of Hazardous Materials, 280: 12–19.

Liu C.-H., Huang W.-D., Kao C.H. (2012): The decline in potassium concentration is associated with cadmium toxicity of rice seedlings. Acta Physiologiae Plantarum, 34: 495–502.

Liu Y.Z., Xiao T.F., Ning Z.P., Li H.J., Tang J., Zhou G.Z. (2013): High cadmium concentration in soil in the Three Gorges region: geogenic source and potential bioavailability. Applied Geochemistry, 37: 149–156.

Mahar A., Wang P., Ali A., Awasthi M.K., Lahori A.H., Wang Q., Li R.H., Zhang Z.Q. (2016): Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicology and Environmental Safety, 126: 111–121.

Mateo A., Funck D., Mühlenbock P., Kular B., Mullineaux P.M., Karpinski S. (2006): Controlled levels of salicylic acid are required for optimal photosynthesis and redox homeostasis. Journal of Experimental Botany, 57: 1795–1807.

Metwally A., Finkemeier I., Georgi M., Dietz K.-J. (2003): Salicylic acid alleviates the cadmium toxicity in barley seedlings. Plant Physiology, 132: 272–281.

Muradoglu F., Gundogdu M., Ercisli S., Encu T., Balta F., Jaafar H.Z.E., Zia-Ul-Haq M. (2015): Cadmium toxicity affects chlorophyll *a* and *b* content, antioxidant enzyme activities and mineral nutrient accumulation in strawberry. Biological Research, 48: 11.

Nazar R., Iqbal N., Masood A., Khan M.I.R., Szeed S., Khan N.A. (2012): Cadmium toxicity in plants and role of mineral nutrients in its alleviation. American Journal of Plant Sciences, 3: 1476–1489.

Parmar P., Kumari N., Sharma V. (2013): Structural and functional alterations in photosynthetic apparatus of plants under cadmium stress. Botanical Studies, 54: 45.

Piršelová B., Ondrušková E. (2021). Effect of cadmium chloride and cadmium nitrate on growth and mineral nutrient content in the root of fava bean (*Vicia faba* L.). Plants (Basel), 10: 1007.

Sharma S.S., Dietz K.-J. (2006): The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany, 57: 711–726.

Sharma S.S., Dietz K.-J., Mimura T. (2016): Vacuolar compartmentalization as indispensable component of heavy metal detoxification in plants. Plant, Cell and Environment, 39: 1112–1126.

Siddiqui M.H., Al-Whaibi M.H., Sakran A.M., Basalah M.O., Ali H.M. (2012): Effect of calcium and potassium on antioxidant system of *Vicia faba* L. under cadmium stress. International Journal of Molecular Sciences, 13: 6604–6619.

Šimek J., Tůma J., Dohnal V., Musil K., Ducaiová Z. (2016): Salicylic acid and phenolic compounds under cadmium stress in cucumber plants (*Cucumis sativus* L.). Acta Physiologiae Plantarum, 38: 172.

Sun H.Y., Wang X., Shang L., Zhou Z., Wang R. (2017): Cadmium accumulation and its effects on nutrient uptake and photosynthetic performance in cucumber (*Cucumis sativus* L.). Philippine Agricultural Scientist, 100: 263–270.

Szabados L., Savouré A. (2010): Proline: a multifunctional amino acid. Trends in Plant Science, 15: 89–97.

Verbruggen N., Hermans C. (2008): Proline accumulation in plants: a review. Amino Acids, 35: 753–759.

Wang B., Liu L.X., Gao Y.D., Chen J. (2009): Improved phytoremediation of oilseed rape (*Brassica napus*) by *Trichoderma* mutant constructed by restriction enzyme-mediated integration (REMI) in cadmium polluted soil. Chemosphere, 74: 1400–1403.

Yan H., Filardo F., Hu X.T., Zhao X.M., Fu D.H. (2016): Cadmium stress alters the redox reaction and hormone balance in oilseed rape (*Brassica napus* L.) leaves. Environmental Science and Pollution Research International, 23: 3758–3769.

Yoon J., Cao X.D., Zhou Q.X., Ma L.Q. (2006): Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of The Total Environment, 368: 456–464.

Zawoznik M.S., Groppa M.D., Tomaro M.L., Benavides M.P. (2007): Endogenous salicylic acid potentiates cadmium-induced oxidative stress in *Arabidopsis thaliana*. Plant Science, 173: 190–197.

Zhang W.N., Chen W.L. (2011): Role of salicylic acid in alleviating photochemical damage and autophagic cell death induction of cadmium stress in *Arabidopsis thaliana*. Photochemical and Photobiological Sciences, 10: 947–955.

Zheng X.R., Lopisso D.T., Eseola A.B., Koopmann B., von Tiedemann A. (2019): Potential for seed transmission of *Verticillium longisporumin* in oilseed rape (*Brassica napus*). Plant Disease, 103: 1843–1849.

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