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Physio-Biochemical Indexes as Indicators of Cadmium Tolerance in *Brassica napus* L. Cultivars

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Abstract: Cadmium (Cd) is a non-essential heavy metal and a pervasive pollutant in agricultural soils. Despite numerous studies investigating Cd accumulation and tolerance in plants, there is a lack of systematic analysis of how various physio-biochemical indexes respond to Cd toxicity, particularly their indicative role in plant tolerance mechanisms. A pot experiment was conducted in greenhouse to assess the differences in Cd accumulation and tolerance among three *Brassica napus* L. cultivars ('Hanyou 2', 'Hanyou 3', and 'Hanyou 16') under the treatments of CK (0.18 mg kg^{-1} in soil), T1 (2.18 mg kg^{-1} in soil), T2 (4.18 mg kg^{-1} in soil), and T3 (8.18 mg kg^{-1} in soil). All three cultivars exhibited high tolerance indexes (TIs) and strong Cd tolerance when exposed to a Cd concentration of 2.18 mg kg^{-1} in soil (T1). There were significant positive correlations between TI and chlorophyll a (Chla), chlorophyll b (Chlb), carotenoids (Car), net photosynthetic rate (Pn), transpiration rate (Tr), and activities of antioxidant enzymes and non-enzymatic antioxidants such as superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione (GSH), and ascorbic acid (ASA), while negatively correlating with intercellular CO_2 concentration (Ci) and malondialdehyde (MDA) content. These findings underscore the significant indicative role of these physio-biochemical indexes in elucidating Cd tolerance mechanisms in *B. napus* and may be used in breeding programs to develop cultivars with a high Cd-tolerance but low Cd uptake profile. However, this was a pot experiment only. Field experiments might be more useful in the future.

Keywords: *Brassica napus* L.; cadmium tolerance; physiological indexes; indicative role

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1. Introduction

Cadmium (Cd) is a heavy metal showing high bio-toxicity, and even at low concentrations, exogenous Cd deposited in soil is readily absorbed by plants [1]. Currently, there are varying degrees of heavy metal pollution across different regions in China. Among these, soil Cd pollution is the most severe. Cd is one of the primary heavy metal pollutants [2]. Human activities are a significant source of Cd pollution, with areas such as metal mining

regions and industrial zones being particularly concentrated in cadmium pollution [3]. In these regions, Cd can enter agricultural soils through atmospheric deposition, rivers, groundwater, and other pathways [4], and is transmitted to humans through the food chain, leading to a range of related diseases. This poses a severe threat to both the ecosystem and public health [5]. Plants growing in Cd-polluted soils may also experience growth and development damage, which impacts their yield [6,7].

During the plant growth cycle, Cd stress can negatively affect various fundamental functions of plants at the physiological and biochemical levels [8]. On the one hand, Cd stress can reduce plant photosynthesis by inhibiting chlorophyll synthesis, damaging photosynthetic organs, and inducing the production of photo-inhibition pigments. On the other hand, Cd stress can lead to an imbalance in reactive oxygen species (ROS), which can directly or indirectly cause damage to the plant, promote lipid peroxidation, damage cell membranes, and affect various metabolic activities in the plant [9,10]. To protect themselves from ROS, plants employ an antioxidant system to combat oxidative stress induced by Cd. The antioxidant system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as non-enzyme antioxidants like glutathione (GSH) and ascorbic acid (ASA) [11]. These components work together to reduce excess ROS within the plant, thereby minimizing oxidative damage. Ci et al. [12] reported that tolerant wheat cultivars accumulated less Cd, and both SOD and CAT activities were higher in Cd-tolerant wheat compared to Cd-sensitive wheat. This indicates the importance of these enzymes for Cd tolerance [13]. To safely utilize Cd-contaminated soils, using Cd hyperaccumulator plants for soil remediation and planting Cd-safe crops in Cd-polluted soils are two environmentally friendly methods with good economic potential [14]. Therefore, plants used in Cd-contaminated soils need to have strong Cd tolerance [15,16]. Studies have found that rapeseed has a relatively strong tolerance to cadmium, and its cadmium accumulation is higher than that of other species, making it an ideal plant for the remediation of Cd-contaminated soils [17].

Brassica napus L. in the Brassicaceae family is an important oilseed crop grown worldwide. It serves as a major source of edible plant oil, vegetables, animal feed, green manure, and biodiesel, and is widely cultivated in China. It can also be used as green manure or even as an ornamental crop [18]. The Cd tolerance of *B. napus* depends on the rhizosphere soil and the variety of *B. napus* [19]. Screening *B. napus* cultivars for enhanced Cd tolerance is crucial for improving growth in Cd-contaminated soils. Wang et al. [20] identified two high-yielding *B. napus* cultivars ('72A' and '47') with low Cd accumulation. When grown in soils containing 1.57 mg kg^{-1} of Cd, these cultivars accumulated less than 0.30 mg kg^{-1} of Cd in their seeds, well below the food safety standard of 0.50 mg kg^{-1}. At the same time, they also identified three *B. napus* cultivars ('Nanchongjie', 'Pengzhoubai', and 'J-25') with high biomass and Cd content. In general, *B. napus* cultivars with strong Cd tolerance and high Cd accumulation can be used for soil remediation in Cd-contaminated soils, while those with strong Cd tolerance but low Cd accumulation are suitable for crop cultivation in Cd-polluted soils [21]. However, there is a lack of studies on the relationship between the tolerant physiological characteristics of *B. napus* and Cd, especially among its different cultivars [22,23]. We hypothesized that the Cd tolerance of *B. napus* will be inversely correlated with foliar Cd concentration. Furthermore, we hypothesized that a Cd-induced reduction in growth would occur with reduced ROS and decreased SOD, CAT, and APX. We aimed to quantify the Cd uptake and enzyme activity of *B. napus* in soil containing Cd concentrations from 0.18 to 8.18 mg kg^{-1}.

2. Materials and Methods

2.1. Experimental Materials and Design

Soil (yellow-brown) was collected from the plow layer (0–20 cm) of the experiment field of Shaanxi University of Technology. The pH was 6.5, the total nitrogen content was 1.71 g kg^{-1}, the organic matter content was 26.8 g kg^{-1}, the available potassium content was 17.3 mg kg^{-1}, the available phosphorus content was 16.8 mg kg^{-1}, and Cd content was 0.18 mg kg^{-1} [24]. Following the National Standard of the People's Republic of China, specifically the Soil Environmental Quality Standard for Soil Pollution Risk Control of Agricultural Land (Trial), the soil was classified as clean [25]. The pot experiment was a two-factor experiment (factor a: soil treatment with 4 graduations; factor b: genotype with 3 graduations), with final Cd concentrations in the soil amounting to 0.18 mg kg^{-1} (CK), 2.18 mg kg^{-1} (T1), 4.18 mg kg^{-1} (T2). and 8.18 mg kg^{-1} (T3). Superior pure grade $CdCl_2$ 2.5 H_2O reagent was spiked to soil samples to obtain target Cd concentrations, and the final values were confirmed through measurements [24]. According to the general situation of Cd contamination in fields of China, the soil in CK is quite clean with low-grade pollution in T1, T2 and T3 are heavily polluted. The Cd concentration gradient in soils might better reflect the tolerance of plants [24]. After a two-month equilibration period, seeds of different rapeseed cultivars were sown into 2 kg of soil per pot under the respective treatments.

The *B. napus* cultivars 'Hanyou 2', 'Hanyou 3', and 'Hanyou 16' were provided by the Hanzhong Institute of Agricultural Sciences. The seeds were sterilized with 5% NaClO and soaked in ultrapure water for 1 day then sown into a tray containing the substrate soil under greenhouse conditions (25 °C, 15,000 lx, 10/14 D/L) to wait for their germination. The seedlings were transplanted to Cd-containing soil when they germinated with the length of the radicle ca. 30 mm (one week). Each pot was planted with five seedlings. Pots were arranged in a randomized block design. There were three replicates of each treatment. Pots were watered with tap water to maintain the water holding capacity (WHC) at 75% of the field water capacity. The average cycle of the three *B. napus* cultivars was 150 days and plants were harvested at the seedling stage (65 days, near the great vegetative stage without flowering) after translocation. Samples used to measure physio-biochemical indexes were collected at the same time. There was no specific phenological stage or time period for leaf collection for the determinations made.

2.2. Measurement of Samples

2.2.1. Biomass and Cd Content Determination

Plant samples were divided into roots and leaves, washed thoroughly with ultrapure water, dried in an oven at 105 °C for 5 min first, and then dried at 75 °C until a constant weight was obtained. The dry weights of the leaves and roots of the plants were recorded [24].

Based on the biomass, a tolerance index (TI) was calculated following Equation (1) [19]:

$$\text{TI (\%)} = \text{Dry weight of treated plants/Dry weight of the CK} \times 100\% \quad (1)$$

Cd concentration in plants was determined in the digestion solution using an atomic absorption spectrophotometer (Hitachi 180-80, Tokyo, Japan). Before the determination, the dried plant samples were ground into a fine powder. Digestion was performed using a mixture of concentrated nitric acid and perchloric acid in a 9:3 ratio. Quality control and evaluation were conducted using the reference material GBW07405 (GSS-5) [24].

2.2.2. Chlorophyll Content Determination

Chlorophyll content was determined using the ethanol–acetone mixture extraction method [26]. Fresh, fully expanded leaves were weighed and immersed in acetone–anhydrous ethanol solution (1:1, *v*/*v*, Shanghai, China) in the dark for 24 h. Absorbance was measured at OD470, OD645, and OD663. The contents of chlorophyll a (Ch1a), chlorophyll b (Ch1b), and carotenoids (Car) were calculated according to the method of Dai et al. [27].

2.2.3. Measurement of Photosynthetic Parameter

The measurements were taken between 9:00 a.m. and 11:00 a.m., with a temperature of approximately 20–25 °C, light intensity ranging from 1200 to 2000 lx, and relative humidity between 60 and 80%. The net photosynthetic rate (Pn), transpiration rate (Tr), and intercellular CO_2 concentration (Ci) of *B. napus* leaves were measured by a CI-340 portable photosynthesizer (CID, Camas, WA, USA), following the protocols of measurement [28].

2.2.4. Antioxidant Enzyme Activity Determination

An enzyme solution was prepared for the determination of SOD, APX, and MDA. Fresh *B. napus* leaves (1 g) were weighed out and 5 mL of pre-cooled 50 mM phosphate buffer (pH = 7.8, Hangzhou, China) was added. The mixture was ground in an ice bath and the solution made up to 10 mL. Following the centrifugation at 12,000× *g* at 4 °C for 20 min, the supernatant was decanted and stored for analyses [24].

SOD activity determination: 100 µL of the enzyme solution was mixed with 2 mL of 39 mM methionine (Met, Shanghai, China), 2 mL of 0.225 mmol/L nitro-blue tetrazolium (NBT, Ningbo, China), 1 mL of 0.6 mmol/L EDTA-Na2 (Guangzhou, China), and 1 mL of 0.012 mM riboflavin (Handan, China). The mixture was shaken well and placed in an artificial incubator at 30 °C under a 4000 lx fluorescent lamp for 20 min and terminated in the dark; the reaction was then terminated in the dark. Absorbance was measured at OD560 using a UV spectrophotometer. The control used for calibration was an illuminated tube without enzyme solution, and the zeroing was performed using a sample with no illumination and no enzyme solution [29].

APX activity determination: 100 µL of the enzyme solution, and 5 µL of 9 mM H_2O_2 solution (Baoding, China) were sequentially added to 3 mL of 50 mM PBS (pH = 7.0, Hangzhou, China) containing 0.1 mM EDTA-Na2 and 0.3 mM ASA. The reaction solution was mixed homogeneously, and the change in absorbance was measured over time at OD290 using a UV spectrophotometer (Shimadzu, Kyoto, Japan). The APX activity of the samples was then calculated according to the formula described by Zhang et al. [30].

MDA content determination: 2 mL of enzyme solution was mixed with 2 mL of 0.5% thiobarbituric acid solution (dissolved in 10% trichloroacetic acid (Zibo, China), Wuhan, China). The reaction mixture was heated at 95 °C for 30 min, cooled rapidly, and centrifuged at 10,000× *g* for 10 min. Absorbance was measured at OD532, OD600 and OD450. The control was prepared by replacing the enzyme solution with 2 mL of water and adding thiobarbituric acid solution. The MDA content was calculated according to the formula provided by Dhindsa and Matowe [31].

2.2.5. Determination of Non-Enzymatic Components

For determining the reduced GSH content, 0.2 g of *B. napus* leaves was ground with 10 mL of pre-cooled 5% HPO_3 solution (Shanghai, China). The mixture was then centrifuged at 4 °C for 10 min. The resulting supernatant was used as the sample solution. Absorption at OD412 was measured using the DTNB (5,5′-dithiobis (2-nitrobenzoic acid), Zhenjiang, China) method [28].

To determine the ASA content, 0.2 mL of the stock solution was mixed with 1.4 mL of 75 mM NaH_2PO_4 solution (pH = 7.4, Yangzhou, China). Next, 0.4 mL of 10% HPO_3, 0.4 mL of 44% H_3PO_4 (Kunming, China), 0.4 mL of 4% 2,2-dipyridine (Wuhan, China), and 0.2 mL of 3% $FeCl_3$ (Langfang, China) were added to the mixture and incubated at 37 °C for 1 h. Afterward, the absorbance at OD525 was measured, and the ASA content was calculated using the standard curve equation [32].

2.3. Data Processing and Statistical Analysis

The mean value, standard deviation (SD), and figures were calculated or generated using Microsoft Excel 2021 and Origin 2021. The statistical software SPSS 27 was used to analyze the difference significance of the data among different cultivars and treatments by the LSD method at levels of $p < 0.05$, $p < 0.01$, or $p < 0.001$.

3. Results

3.1. Cd Accumulation and Tolerance Differences of Three B. napus Cultivars

As shown in Figure 1, from CK to T1-T3, the Cd concentration in leaves (a) and roots (b) of three *B. napus* cultivars were significantly increased ($p < 0.05$), indicating the significant role of the Cd concentration gradient in soils. In treatments of T1–T3, the levels of this heavy metal in the leaves of three cultivars were largely lower than in the roots, but there were no huge differences between leaves and roots in the CK treatment. Among different cultivars, Cd concentrations in the leaves and roots of the cultivars Hayou 16 and Hanyou 2 were comparable, but significantly higher than those observed in Hanyou 3 ($p < 0.05$).

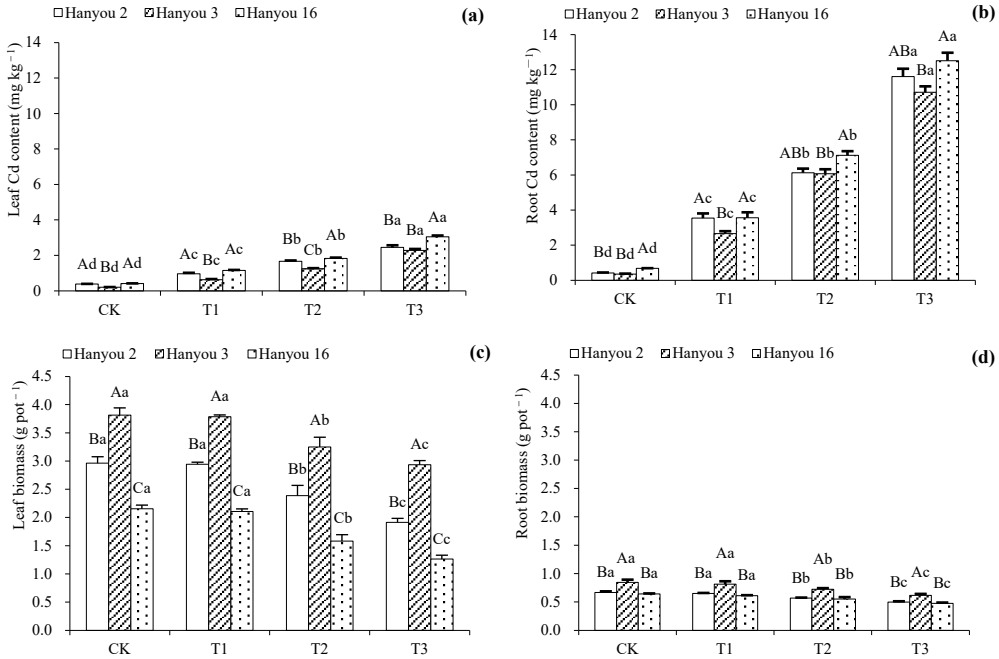

Figure 1. Leaf Cd content (**a**), root Cd content (**b**), leaf biomass (**c**) and root biomass (**d**) of three *B. napus* cultivars. Different capital letters over columns between different cultivars indicate significant differences and different lowercase letters in different treatments of same cultivar indicate significant differences at $p < 0.05$.

The biomasses of leaves and roots of three *B. napus* cultivars under study generally decreased with increasing Cd concentrations (from CK and T1 to T2 and T3) in soils ($p < 0.05$) as shown in Figure 1c,d. Leaf and root biomasses in the T2 and T3 treatments were significantly lower compared to the CK treatment, while no significant reduction was observed in T1 ($p < 0.05$). Significant differences in leaf biomass were observed among the

cultivars ($p < 0.05$). The root biomass of Hanyou 3 was significantly higher than that of Hanyou 2 and Hanyou 16 ($p < 0.05$). Hanyou 2 showed higher biomass than Hanyou 16, and Hanyou 3 had the highest biomass overall.

Table 1 shows the TIs of three *B. napus* cultivars. Generally, TIs in T1 treatments were the highest and gradually decreased from T2 to T3 ($p < 0.05$). The differences in TIs among the three cultivars were not significant in the T1 treatment. However, the TIs of Hanyou 3 were obviously the highest in T2 and T3 (with Hanyou 2 in the middle), indicating Hanyou 3's stronger tolerance to Cd.

Table 1. Tolerance indexes (TIs) of three *B. napus* cultivars.

Treatment	Cultivars	TI
T1	Hanyou 2	99 ± 0.04 Aa
	Hanyou 3	99 ± 0.03 Aa
	Hanyou 16	97 ± 0.03 Aa
T2	Hanyou 2	81 ± 0.04 Bb
	Hanyou 3	85 ± 0.02 Ab
	Hanyou 16	76 ± 0.06 Cb
T3	Hanyou 2	66 ± 0.03 Bc
	Hanyou 3	76 ± 0.01 Ac
	Hanyou 16	62 ± 0.04 Cc

Note: Different uppercase letters indicate significant differences in TIs among cultivars under the same treatment. Different lowercase letters indicate significant differences among treatments within the same cultivar ($p < 0.05$).

3.2. Chlorophyll Differences Among Three B. napus Cultivars

The chlorophyll a (Chla), chlorophyll b (Chlb), and carotenoid (Car) contents of the three *B. napus* cultivars gradually decreased with increasing Cd concentration (T1–T3) (Figure 2). However, compared to the CK, the Chla, Chlb, and Car contents in the T1 treatment were not significantly decreased ($p < 0.05$). Among the cultivars, Hanyou 3 consistently contained the highest Chla, Chlb, and Car contents, followed by Hanyou 2 and Hanyou 16.

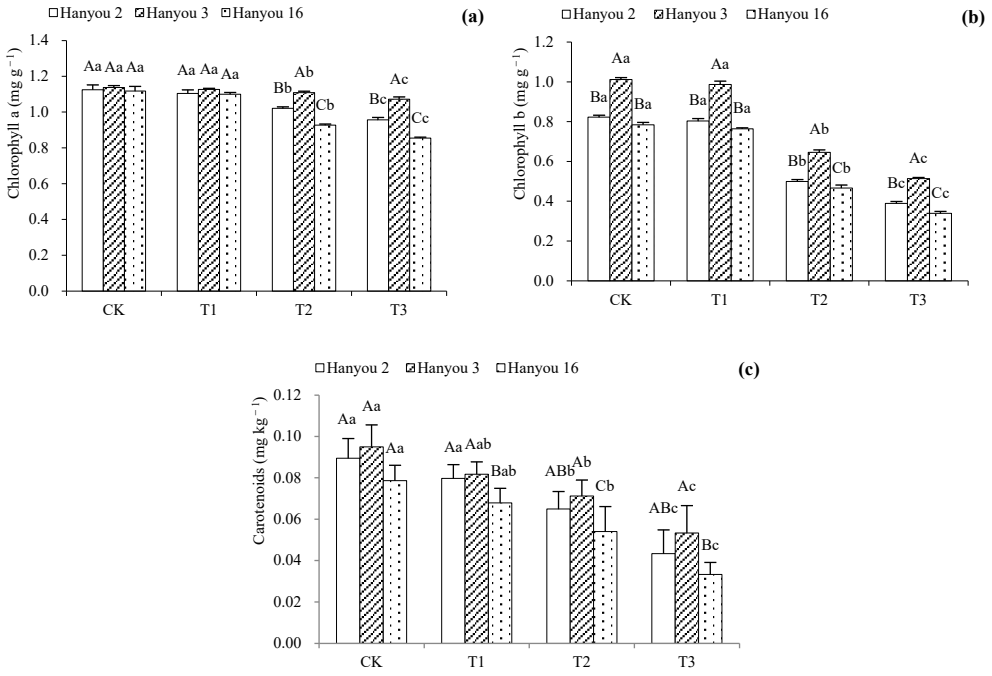

Figure 2. Chlorophyll a (**a**), Chlorophyll b (**b**) and Carotenoids (**c**) of three *B. napus* cultivars. Different capital letters over columns between different cultivars indicate significant differences and different lowercase letters in different treatments of same cultivar indicate significant differences at $p < 0.05$.

3.3. Differences in Photosynthetic Parameter Among Three B. napus Cultivars

Figure 3a illustrates that the net photosynthetic rate (Pn) showed a decreasing trend with increasing Cd concentrations. Compared to the CK treatment, Pn values for the three cultivars were not significantly reduced in T1, but significant declines were recorded in treatments T2 and T3 ($p < 0.05$). Hanyou 3 was significantly higher than Hanyou 2 and Hanyou 16 under the T2 treatment, but there was no significant difference under T3 ($p < 0.05$), with Hanyou 3 showing the highest Pn, while Hanyou 2 and Hanyou 16 reached similar values.

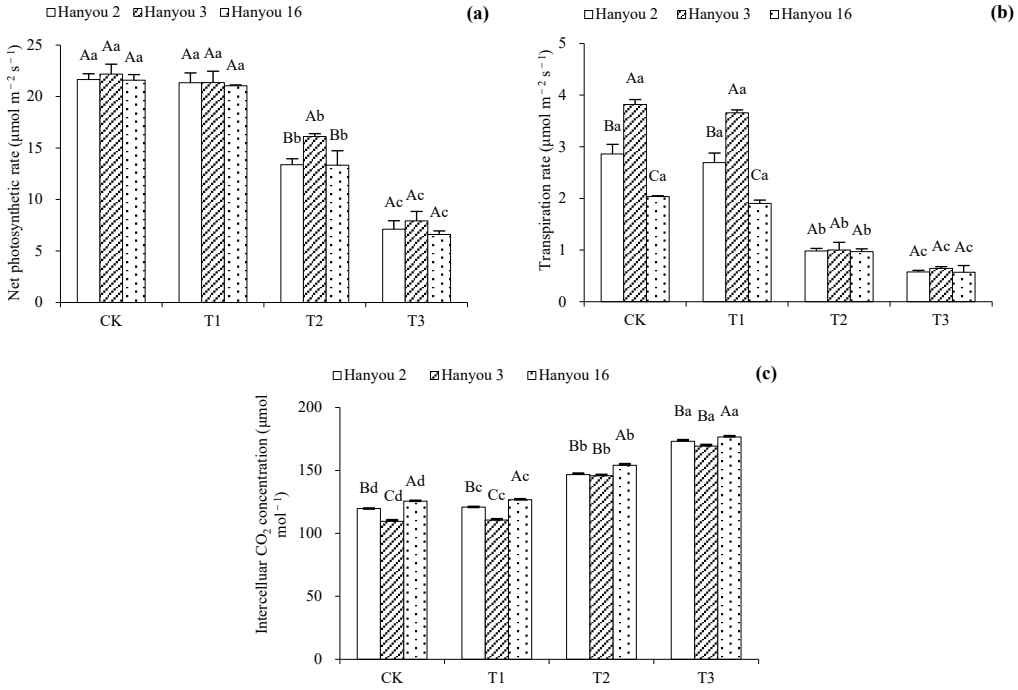

Figure 3. Net photosynthetic rate (**a**), transpiration rate (**b**), and intercellular CO_2 concentration (**c**) of three *B. napus* cultivars. Different capital letters over columns between different cultivars indicate significant differences and different lowercase letters in different treatments of same cultivar indicate significant differences at $p < 0.05$.

The transpiration rate (Tr) followed a similar trend to Pn (Figure 3b). In T1, no significant reduction in Tr was observed compared to the CK treatment, but marked decreases were measured in T2 and T3 in all three cultivars ($p < 0.05$). The Tr of Hanyou 3 was the highest in the CK and T1 treatments, while no significant differences were detected among the three cultivars in treatments T2 and T3 ($p < 0.05$).

The intercellular CO_2 concentration (Ci) showed an opposite trend to that of Pn and Tr (Figure 3c). The Ci of all three cultivars was significantly increased with the Cd concentration enhanced in soils (T1–T3) ($p < 0.05$). Among the cultivars, Hanyou 3 had the lowest Ci values, while higher but similar values were obtained for Hanyou 2 and Hanyou 16 ($p < 0.05$).

3.4. MDA Content Differences Among Three B. napus Cultivars

Figure 4 illustrates that the MDA content in the leaves and roots of the three cultivars increased with the rising Cd concentration in the soils. Compared to the CK treatment, the MDA content in the T1 treatment did not change significantly, whereas significant increases were observed in T2 and T3 ($p < 0.05$). The MDA content of Hanyou 16 was the highest across all treatments, and Hanyou 3 and Hanyou 2 were the second and third, respectively ($p < 0.05$).

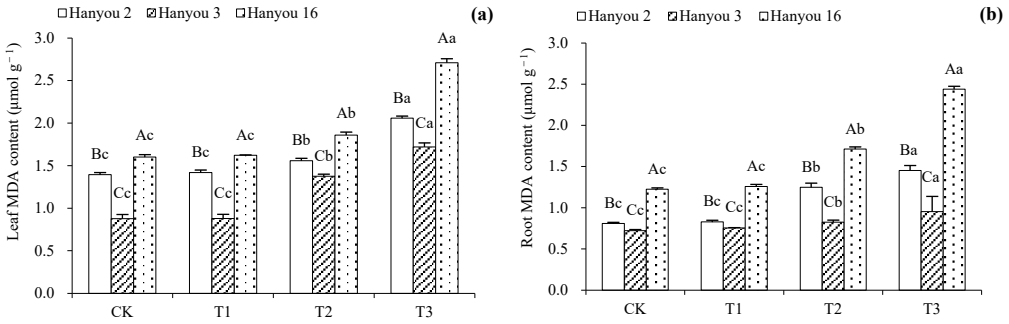

Figure 4. Leaf MDA content (**a**) and root MDA content (**b**) of three *B. napus* cultivars (different capital letters over column between different cultivars indicate significant differences and different lower case letters in different treatments of same cultivar indicate significant differences at $p < 0.05$).

3.5. Antioxidant Enzyme Activity Differences Among B. napus Cultivars

The activities of SOD and APX in the leaves and roots of the three cultivars followed similar trends in all treatments (CK, T1-T3; Figure 5). Compared to the CK treatment, SOD, and APX activities in leaves and roots were not significantly reduced in the T1 treatment but showed significant decreases in the T2 and T3 treatments ($p < 0.05$). Among the cultivars, Hanyou 3 demonstrated the highest SOD and APX activities in both leaves and roots, followed by Hanyou 2 and Hanyou 16, respectively ($p < 0.05$).

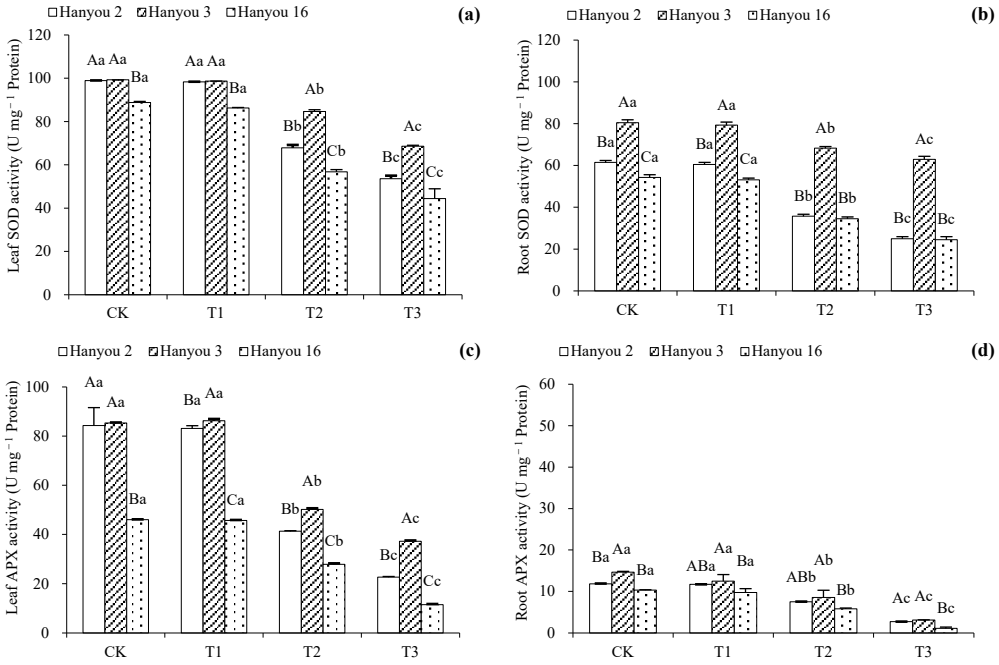

Figure 5. Leaf SOD activity (**a**), root SOD activity (**b**), leaf APX activity (**c**) and root APX activity (**d**) activities of three *B. napus* cultivars. Different capital letters over columns between different cultivars indicate significant differences and different lowercase letters in different treatments of same cultivar indicate significant differences at $p < 0.05$.

3.6. Non-Enzymatic Antioxidants in the Three B. napus Cultivars

The contents of GSH and ASA in the leaves and roots of the three cultivars (Figure 6) showed similar trends to the SOD and APX activities (Figure 5). There were no significant differences recorded between the CK and T1 treatments for GSH and ASA contents; however, these markers were significantly decreased in T2 and T3 compared to the CK treatment ($p < 0.05$). Hanyou 3 had the highest levels of GSH and ASA, followed by Hanyou 2 and Hanyou 16 ($p < 0.05$).

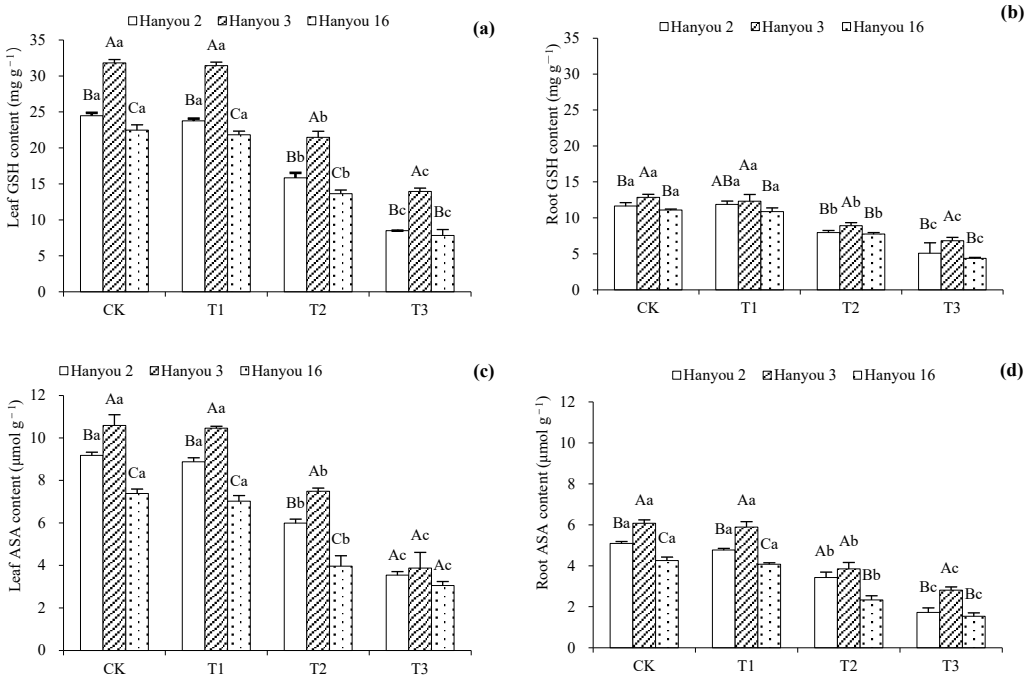

Figure 6. Leaf GSH content (**a**), root GSH content (**b**), leaf ASA content (**c**) and root ASA content (**d**) of three *B. napus* cultivars. Different capital letters over columns between different cultivars indicate significant differences and different lowercase letters in different treatments of same cultivar indicate significant differences at $p < 0.05$.

3.7. Pearson Correlation Analysis of Cd Concentration and Biomass with Physiological Indicators of Three B. napus Cultivars

Figure 7 shows Pearson's correlation analysis between TI (tolerance index), Cd concentration, biomass, and physiological indicators of the three cultivars in all treatments. The TI of all three cultivars demonstrated significant positive correlations with Chla, Chlb, Car, Tr, SOD, APX, GSH, and ASA. Conversely, it showed significant negative correlations with Ci and MDA at $p < 0.05$, $p < 0.01$ or $p < 0.001$. Additionally, TI was significantly positively correlated with both leaf and root biomasses ($p < 0.05$ or $p < 0.01$, respectively).

The Cd content in both leaves and roots showed significant negative correlations with their biomasses, Chla, Chlb, Car, Tr, SOD, APX, GSH, ASA, and TI. In contrast, we observed significant positive correlations between Ci and MDA ($p < 0.05$, $p < 0.01$ or $p < 0.001$). The relationship between leaf and root biomasses with Cd content and these physiological indices followed the same pattern as that of TI (Figure 7).

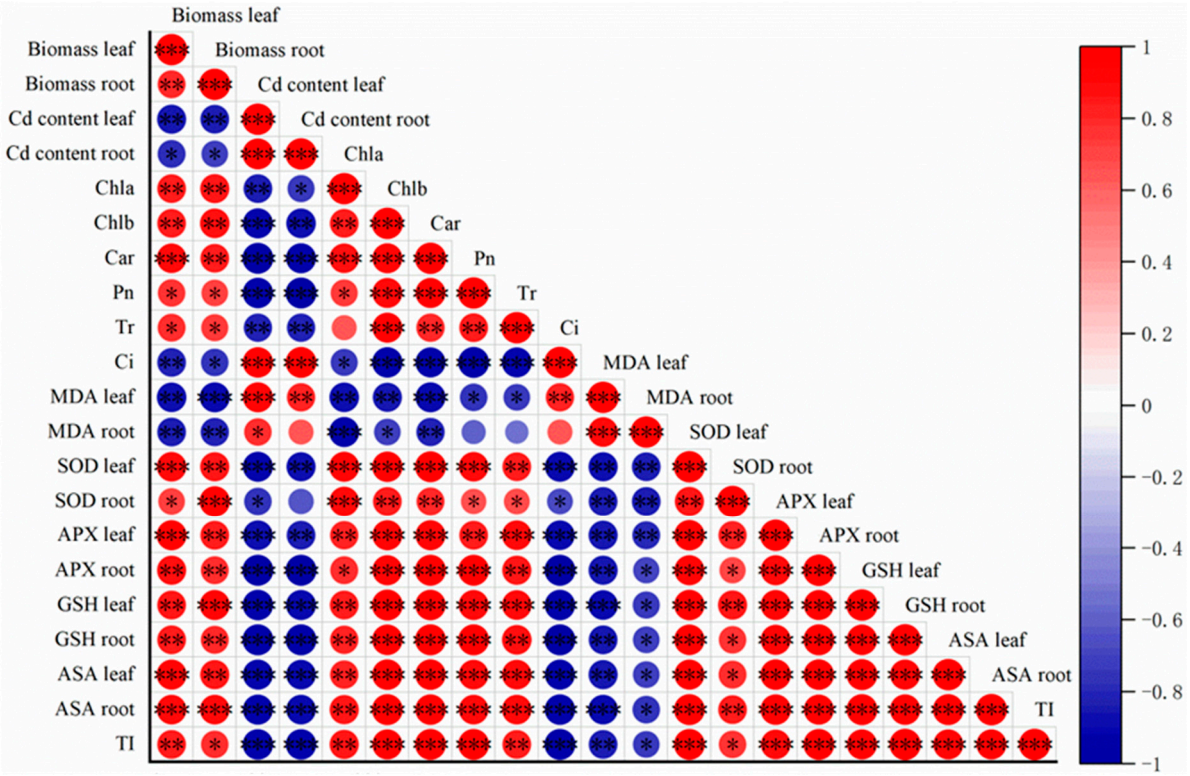

Figure 7. Pearson correlation of tolerance index (TI) with Cd concentration, biomass, and physiological indicators of three cultivars. Red indicates positive correlation and blue indicates negative correlation. * means significance level at * $p < 0.05$, ** at $p < 0.01$ and *** at $p < 0.001$.

4. Discussion

The three cultivars' biomasses in the T1 treatment were not significantly decreased compared to the CK treatment, and all exhibited high Cd tolerance indexes, indicating they were Cd-tolerant cultivars under such conditions [33]. However, the Cd concentration in the leaves exceeded the limit of pollutants in food (0.1 mg kg^{-1}, fresh matter) [34]. Therefore, they cannot be classified as low Cd-accumulating cultivars when soil Cd concentration is below 2 mg kg^{-1} (T1) [33]. While Hanyou 3 exhibited the highest biomass, all three cultivars followed a similar trend in all treatments (T1–T3), i.e., plant biomass remained stable under T1 but declined significantly under T2 and T3 conditions compared to the CK treatment (Figure 1), indicating tolerance to low Cd concentrations (T1) [33]. The TI treatment more directly reflected the level of tolerance as it showed a positive correlation with biomass (Figure 7).

Chlorophyll content, photosynthesis, respiration, and transpiration are significantly affected by Cd stress. Photosynthetic pigments play a crucial role in absorbing and transferring light energy during plant photosynthesis, and their levels indicate external stresses [35]. Under Cd stress, chlorophyll a (Chla), chlorophyll b (Chlb), and carotenoid (Car) contents in *Brassica* and strawberry leaves significantly decrease, leading to a marked reduction in net photosynthesis rate (Pn) and transpiration rate (Tr), while the internal CO_2 concentration (Ci) increases [23,36]. In this study, compared to the CK, Pn, Tr, Chla, Chlb, and Car of the three *B. napus* cultivars did not significantly decrease under T1 conditions, but significantly decreased under T2 and T3 conditions. Conversely, Ci did not significantly increase under T1 conditions but significantly increased under T2 and T3 conditions (Figures 2 and 3). These findings were consistent with the responses of *Robinia pseudoacacia* and castor bean, which showed strong tolerance to Cd [37,38]. Cd impacts photosynthesis by disrupting electron transfer and damaging chloroplast integrity [39]. As Cd concentration in plants

increases, chlorophyll content decreases significantly [40]. Beyond altering chloroplast structure, Cd stress reduces chlorophyll content by inhibiting the expression of key enzymes involved in its synthesis. Heavy metals, including Cd, exacerbate carotenoid degradation by increasing the expression of degradative enzymes [41], and Cd also replaces Mg^{2+} in chlorophyll and Ca^{2+} in the Ca/Mn cluster of the PSII complex [42]. Li et al. [43] suggested that the Ci was a key indicator for distinguishing between stomatal and non-stomatal limitations. When Pn and Ci all decreased simultaneously, photosynthesis was primarily limited by stomata. Conversely, if Pn decreased while Ci significantly increased, the limitation to photosynthesis was non-stomatal. In this study, Cd treatments at different concentrations reduced Pn and increased Ci in the three *B. napus* cultivars. This indicated that the limitation of photosynthesis under Cd stress was due to non-stomatal factors, primarily through damage to the photosynthetic apparatus and inhibition of enzyme activity during the dark reaction, thereby reducing photosynthetic efficiency.

Beyond affecting photosynthesis, Cd stress significantly increases the MDA content in *B. napus*. Under T1 conditions, compared to the CK, the MDA content of this variety did not show a significant increase (Figure 4), indicating a certain level of Cd tolerance. Cd stress can damage plant cell membranes, increasing cell membrane permeability, the extravasation of intracellular soluble substances, the destruction of intracellular enzymes, and metabolic action in the original region. MDA content reflects the strength of lipid peroxidation [44]. Damage to the cell membrane can lead to a dysfunction of the balance between membrane-bound enzymes and the intracellular membrane, allowing a large number of substances to extravasate and toxic substances to enter the cell freely, leading to a series of disturbances in the physiological and biochemical processes of the cell [45]. However, plants can increase their tolerance to Cd through avoidance and detoxification strategies [46]. SOD, APX, GSH, and ASA can scavenge reactive oxygen species from plants and reduce Cd damage by regulating the antioxidant system, indicative of Cd tolerance to some extent [47]. In the study by Zhang et al. [48], the Cd-tolerant cultivar castor Zibo No. 8 had higher GSH and SOD activities than Zibo No. 5. In this experiment, the activities of antioxidant enzymes and non-enzymes of Hanyou 3, which exhibited high Cd tolerance in T1, were higher than those of the other two *B. napus* cultivars. This antioxidant enzyme and non-enzymatic indices of Hanyou 3 in T1 treatment were not significantly altered compared to the CK, significantly positively correlated with the Cd tolerance index, and had similar tolerance to Cd. This may be a response by the plant to protect itself from reactive oxygen species (ROS) damage, triggering a series of complex antioxidant enzyme defense mechanisms to avoid or reduce oxidative damage caused by cadmium. The enzyme protection system includes antioxidant enzymes such as SOD and APX. SOD is an important protective enzyme in the enzymatic defense system against ROS in plants, while APX plays a key role in maintaining a balanced redox state, enhancing the stability of the GSH-ASA cycle, and maintaining high levels of GSH and ASA to counteract the potential problems caused by oxidative damage [49]. These physio-biochemical indexes play an indicative role in the *B. napus* cultivars' response to Cd stress.

5. Conclusions

There were significant differences in the Cd tolerance between the Hanyou 2, 3, and 16 cultivars, which showed high tolerance indexes (TIs) and strong Cd tolerance at a Cd concentration of 2.18 mg kg^{-1} (T1) in soil. Among them, Hanyou 3 demonstrated the highest tolerance index (TI) and the least biomass reduction under Cd stress, indicating its superior adaptability to Cd-contaminated environments. The physiological responses of the cultivars were closely linked to their Cd tolerance. There was a significant positive correlation between TI and various physiological indicators, including chlorophyll content,

net photosynthetic rate, transpiration rate, and antioxidant enzyme activities, underscoring the importance of these parameters in conferring Cd tolerance. Notably, Hanyou 3 showed higher activities of SOD, APX, GSH, and ASA, indicating a robust antioxidant defense system that contributes to its enhanced Cd tolerance. The study highlights the need for further genetic studies to identify the specific genes or gene networks responsible for Cd tolerance, particularly those influencing antioxidant enzyme activities and chlorophyll synthesis. Additionally, this was a pot experiment only. Considering that plant–element interactions could differ significantly when compared to field conditions, particularly regarding the accumulation and concentration of Cd in the leaves, a field experiment might be needed for further confirmation.

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