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The effects of cadmium on the AsA-GSH cycle and antioxidant compounds in different cultivars of perennial ryegrass

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Abstract: Cadmium (Cd) is a common heavy metal contaminant in agricultural soils; its high bioaccumulation potential and toxicity mean it enters the human body *via* the food chain, posing a health risk. Perennial ryegrass (*Lolium perenne* L.) is renowned for its high tolerance to heavy metal toxicity and is frequently used in phytoremediation. This study examined growth morphology, activities of ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), glutathione peroxidase (GPX), and glutathione reductase (GR), as well as non-enzymatic antioxidant contents in two perennial ryegrass cultivars: WNS (Venus, low Cd accumulation) and YY (Excellent, high Cd accumulation), under Cd treatments of 0, 3, 6, and 12 mg/kg Cd^{2+}. Under Cd stress, WNS exhibited significantly higher plant height, leaf length, tiller number, GPX activity, and glutathione (GSH) content compared with YY. At 12 mg/kg Cd, GR activity in WNS was also significantly higher than in YY ($P < 0.05$). In contrast, at each Cd level, YY showed significantly higher leaf Cd accumulation, APX and DHAR activities, and ascorbic acid (AsA) content than WNS ($P < 0.05$), accompanied by elevated oxidised glutathione (GSSG) content. The comprehensive response index of YY under 3, 6, and 12 mg/kg Cd was 2.77, 2.91, and 2.94 times that of WNS, respectively. These findings suggest that WNS mitigates Cd toxicity and sustains growth homeostasis by maintaining higher GPX and GR activities and higher GSH content within the GSH-GSSG cycle. This study provides a theoretical basis for elucidating the differential Cd tolerance mechanisms among ryegrass cultivars with contrasting Cd accumulation capacities and offers insights to refine phytoremediation strategies in Cd-contaminated soils.

Keywords: heavy metal; cultivars comparison; accumulation capacity; soil pollution; antioxidant system

Rapid industrialisation has led to a marked increase in the extent and severity of heavy metal contamination in soils. Even trace amounts of cadmium (Cd) present in industrial emissions can severely inhibit plant growth and development (Lane et al. 2005, Elhakem 2024). In China, Cd is the predominant pollutant in agricultural soils, contaminating approximately 278 000 hectares of arable land (Riaz et al. 2021). Cd-contaminated farmland exhibits a series of phytotoxic responses, including inhibited growth, metabolic dysfunction, and even plant mortality (Hasan et al. 2009). Phytoremediation, an environmentally friendly and cost-effective remediation strategy, is frequently employed to remove Cd from contaminated soils. This approach utilises fast-growing, high-biomass, and easily harvestable

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plant species to mitigate environmental toxicity by immobilising or extracting pollutants (Mesnoua et al. 2016, Boysan Canal et al. 2023).

Cd toxicity can cause oxidative damage to plants, altering cell membrane permeability and generating reactive oxygen species (ROS) on organelles (Anjum et al. 2015). These ROS include hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH), which are the primary causes of denaturation in biomacromolecules such as proteins and nucleic acids, as well as lipid peroxidation in membranes, thereby inhibiting plant growth (Farooq et al. 2016). The ascorbate-glutathione (AsA-GSH) cycle is a key antioxidant pathway responsible for scavenging ROS in plant cells. Its principal enzymes include ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), and dehydroascorbate reductase (DHAR) (Hasanuzzaman et al. 2017). Ascorbic acid (AsA) and glutathione (GSH) are among the most abundant low-molecular-weight antioxidants in plants (Flores-Cáceres et al. 2015). APX uses AsA as an electron donor to reduce H_2O_2, thereby maintaining intracellular ROS homeostasis (Gao et al. 2024). DHAR links the GSH-GSSG and AsA-DHA systems by reducing DHA to AsA while concurrently oxidising GSH to GSSG. Together, GR and DHAR sustain the metabolic balance between the GSH and AsA pools (Deutsch 2000). GSH functions as a "first line of defence" against heavy metal stress; when DHA is reduced back to AsA, GSH is oxidised to GSSG, but GR subsequently restores GSH using electrons supplied by NADPH (Cobbett and Goldsbrough 2002, Mir et al. 2022).

Perennial ryegrass (*Lolium perenne* L.), a perennial species in the Poaceae family, is characterised by high aboveground biomass, rapid growth, and tolerance to repeated cutting. It exhibits strong resistance to toxic chemicals and can tolerate relatively high Cd levels (Wang et al. 2020, Song et al. 2024). Perennial ryegrass can absorb and transport Cd *via* its aboveground parts and roots, making it a suitable species for the bioremediation of Cd-contaminated soil (Li et al. 2020). Although numerous studies have examined plant physiological responses and adaptive mechanisms under heavy metal stress – for example, Liu et al. (2007) investigation of Cd effects on different rice cultivars and Dai et al. (2022). comparison of Cd accumulation in wild *versus* cultivated eggplant – most of this work has focused on major field crops and vegetables. Limited information is available regarding the physiological responses to Cd stress among forage cultivars with contrasting Cd accumulation capacities, and the variation in AsA-GSH antioxidant mechanisms among such cultivars remains poorly understood. Preliminary screening of 31 perennial ryegrass cultivars identified significant differences in Cd accumulation (Jiang et al. 2022), leading to the selection of WNS (low Cd accumulation) and YY (high Cd accumulation). Therefore, this study aims to systematically characterise the differential ROS-scavenging efficiency, key enzyme activities, and antioxidant profiles within the AsA-GSH cycle of these contrasting cultivars under Cd stress, thereby elucidating the regulatory mechanisms underlying Cd tolerance in perennial ryegrass. The findings provide a theoretical foundation and scientific support for understanding cultivar-specific Cd resistance strategies and for optimising phytoremediation approaches in Cd-contaminated soils.

MATERIAL AND METHODS

Planting material and treatments. Test materials: based on the results of the preliminary screening study of 31 cultivars, Venus (low Cd accumulation) and Excellent (high Cd accumulation) were selected as experimental materials (seeds purchased from Beijing Rytway Seed Co., Ltd., Beijing, China). The origins and accumulation characteristics of both cultivars are summarised in Table 1.

The potting experiment was conducted using greenhouse potting soil cultivated in containers. The soil was sourced from the Horticultural Research Base located on the hillside behind Yunnan Agricultural University in Panlong District, Kunming City (25°8'N, 102°45'E). The soil types and textures were red soil and loamy clay, respectively. The soil pH, cation exchange capacity, organic carbon content, total nitrogen, total phosphorus, and Cd background values were 4.99, 9.29 mmol/100 g, 7.73 g/kg, 0.39 g/kg, 1.37 g/kg, and 0.26 mg/kg. The soil used was air-

Table 1. The different cumulative characteristics in perennial ryegrass cultivars

Cultivar	Cd accumulation	Latin name	Source	Source of seeds
Excellent (YY)	high	*Lolium perenne* L.cv. Excellent	American	Beijing Rytway Seed Co., Ltd. (China)
Venus (WNS)	low	*Lolium Perenne* L.cv. Venus	Denmark	

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dried, sifted, and thoroughly mixed (through a 2 mm sieve), with 6 kg of soil (45 cm high and 40 cm inner diameter) placed in each plastic tub. A control group (0 mg/kg Cd^{2+}) and three treatment groups were designed based on Cd concentration limits defined in GB15618-2018, the national standard for soil environmental quality in cultivated land. The treatment concentrations were 3, 6, and 12 mg/kg Cd^{2+}, respectively, and $CdCl_2 \cdot 2.5\ H_2O$ (AR 99%, Enox, Suzhou, China) was added to the soil as the Cd source. After 30 days of soil ageing and equilibration, seeds were sown. Finally, fifty seedlings of the same cultivar of perennial ryegrass were retained in each pot for the formal experiment. A completely randomised design was used for three repeated experiments, with a total of 24 pots. During the experiment, deionised water was used to water once every 2 days, 1 L per pot. At the same time, to prevent Cd loss from watering, place a plastic tray under each pot and pour any collected water back into the pot.

Measurement of plant height, leaf length, and tiller number. From the 30th day after emergence until the 44th day, the plant height, leaf length, and number of tillers of 15 randomly selected seedlings in each pot were measured at 7-day intervals; this measurement was carried out on three occasions in total.

Determination of Cd content in leaves. Harvest plant leaves 45 days after emergence; the same applies below. Rinse leaf surfaces with ultrapure water. Take 2.5 g of leaves, blanch them in a 105 °C oven (DHG-9245A, Shanghai YIHENG Technical Co., Ltd., Shanghai, China) for 30 min, then reduce the temperature to 80 °C and dry to constant weight. Cool and weigh the dry mass. Grind the dried sample into powder (1000C, Dongguan Fangtai Electric Appliance Co., Ltd., Dongguan, China), place it in a digestion vessel, add 6 mL HNO_3 and 2 mL H_2O_2, and perform microwave digestion. Dilute to 25 mL with deionised water for later use. Measurements were conducted using flame atomic absorption spectroscopy (Thermo ICE 3000 series, Waltham, USA).

Ascorbate peroxidase (APX) activity. The determination of APX enzyme activity followed the method of Al-Huqail et al. (2025). Fresh leaf samples (0.2 g) were homogenised at 4 °C in phosphate buffer (0.05 mol/L, pH 7.8) containing 5 mmol/L EDTA-Na_2 (AR, 99%; Biosharp, Hefei, China) and 2 mmol/L ascorbic acid (AsA) using a tissue homogeniser (KZ-III-FP; Wuhan Servicebio Technology Co., Ltd., Wuhan, China). The homogenate was then centrifuged at 12 000 rpm for 20 min at 4 °C using a refrigerated centrifuge (3H20RI; Hunan Herexi Instrument and Equipment Co., Ltd., Changsha, China), and the supernatant was collected as the crude enzyme extract. Finally, the absorbance change at 290 nm was recorded every 10 s for 40 s using a UV-Vis spectrophotometer (X-7; Shanghai Metash Instruments Co., Ltd., Shanghai, China).

Dehydroascorbate reductase (DHAR) activity. The assay was performed strictly according to the manufacturer's instructions provided with the commercial kit (G0212F; Suzhou Grace Biotechnology Co., Ltd., Suzhou, China). Briefly, fresh leaf tissue samples (approximately 0.1 g) were homogenised on ice in 1 mL of extraction buffer and centrifuged at 12 000 rpm for 10 min at 4 °C. The supernatant was collected, and the change in absorbance at 265 nm was recorded every 10 s for 3 min using a UV spectrophotometer.

Glutathione peroxidase (GPX) activity. GPX enzyme activity was measured using the guaiacol method. 0.2 g of fresh plant leaves were added to the enzyme extract (containing 0.05 mol/L pH 7.0 Tris-HCl buffer, 0.001 mol/L EDTA, 1% polyvinylpyrrolidone, and 0.005 mol/L $MgCl_2$) at 4 °C. The enzyme extract was ground, centrifuged at 4 °C and 12 000 rpm for 20 min, and the crude enzyme solution was extracted. Finally, the OD values for the change every 30 s over 3 min were recorded using the spectrophotometer at 470 nm.

Glutathione reductase (GR) activity. GR enzyme activity was measured according to the method of Bashir et al. (2025).

Determination of AsA, GSH and GSSG contents. The contents of ascorbic acid (AsA) and oxidised glutathione (GSSG) were determined according to the methods described by Ali et al. (2023, 2025). Reduced glutathione (GSH) content was determined strictly according to the manufacturer's instructions provided with a commercial assay kit (G0206F; Suzhou Grace Biotechnology Co., Ltd., Suzhou, China). Briefly, fresh leaf tissue samples (approximately 0.1 g) were homogenised on ice in 1 mL of extraction buffer and centrifuged at 12 000 rpm for 15 min at 4 °C. The supernatant was collected, and the absorbance was measured at 412 nm using a spectrophotometer.

Calculation of the comprehensive response index. Following the method of Jiang et al. (2022), the comprehensive response index (RI) is calculated using the following formula:

$$\text{Response index (RI)} = \sum_{i=1}^{7} \frac{(\text{indicator}_t)_i - (\text{indicator}_c)_i}{(\text{indicator}_c)_i} \times 100$$

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In the formula, RI denotes the integrated response index, with indicator and indicatorc representing the treatment and control groups, respectively. When *i* range from 1 to 7, the indicators include APX activity, DHAR activity, GPX activity, GR activity, AsA content, GSH content, and GSSG content.

Statistical analysis. Data are presented as the mean ± standard error (SE) of three replicate experiments. The data in Figures 1–4 were analysed using SPSS 27.0 (Chicago, USA) to perform a one-way analysis of variance (ANOVA) and *LSD* (least significant difference) multiple comparisons, with means compared at a 5% significance level ($P < 0.05$). Where the assumption of homogeneity of variances was not met, the data were analysed using the non-parametric Kruskal-Wallis test. Create charts using OriginPro 2024 software (Northampton,USA).

RESULTS

Morphological differences between two perennial ryegrass cultivars under different Cd concentrations. Under exposure to 12 mg/kg Cd, plant height, leaf length, and tiller number in WNS were consistently lower than those of the control between 30 and 44 days after emergence (Figure 1). In YY, all three traits were reduced relative to the control across all Cd treatments during the same period. The maximum plant height growth rate in WNS reached 72.34% under 6 mg/kg Cd during days 37–44, whereas that in YY peaked at 25.28% under control conditions during days 30–37. The highest leaf length growth rate in WNS (42.12%) occurred under 6 mg/kg Cd during days 37–44, whereas YY reached a maximum of 16.41% under 3 mg/kg Cd during days 30–37. The maximum tiller growth rate in WNS (13.34%) occurred under 3 mg/kg Cd during days 37–44, compared with 5.49% in YY under control conditions during days 30–37.

Cd content in leaves of two perennial ryegrasses under different Cd concentrations. Cd exposure resulted in a significant, dose-dependent increase in leaf Cd concentrations in both perennial ryegrass cultivars ($P < 0.05$) (Figure 2). Significant differences were also detected among Cd treatment groups within each cultivar ($P < 0.05$). Moreover, at Cd concentra-

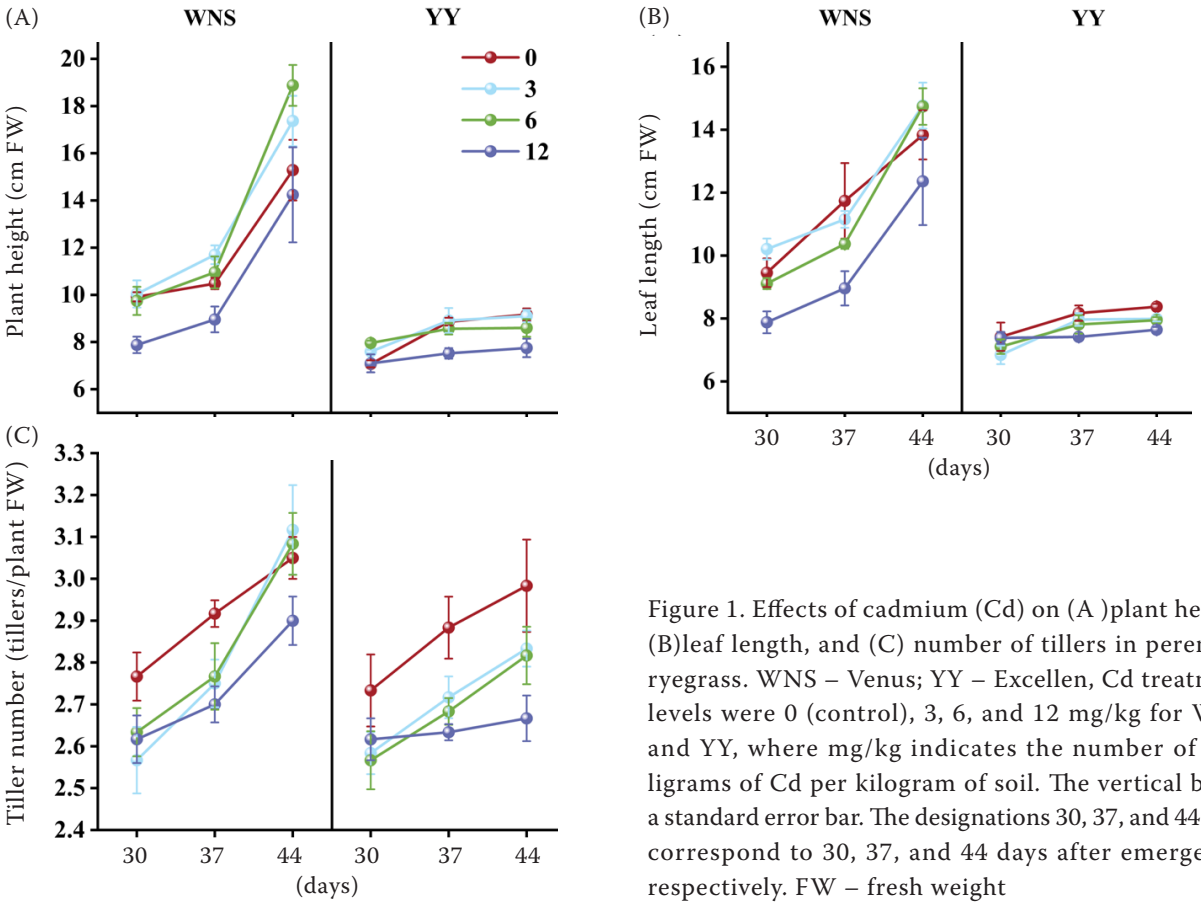

Figure 1. Effects of cadmium (Cd) on (A)plant height; (B)leaf length, and (C) number of tillers in perennial ryegrass. WNS – Venus; YY – Excellen, Cd treatment levels were 0 (control), 3, 6, and 12 mg/kg for WNS and YY, where mg/kg indicates the number of milligrams of Cd per kilogram of soil. The vertical bar is a standard error bar. The designations 30, 37, and 44 days correspond to 30, 37, and 44 days after emergence, respectively. FW – fresh weight

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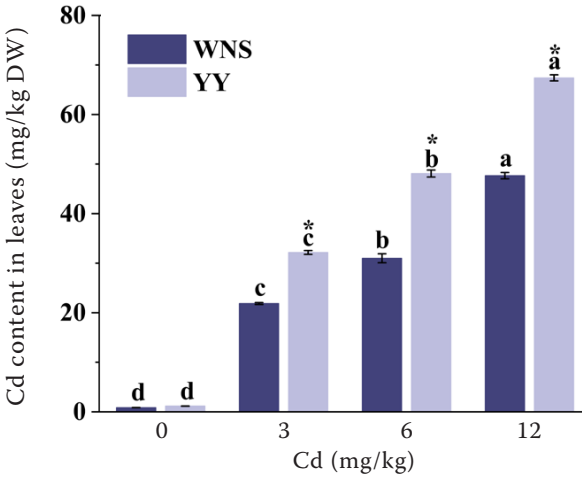

Figure 2. Cadmium (Cd) content in leaves of two perennial ryegrass cultivars. WNS – Venus; YY – Excellent; Values represent the mean ± standard error (n = 3). Different letters within vertical columns indicate significant differences among the same cultivar at different Cd concentrations, while * denotes significant differences between two perennial ryegrass cultivars at a specific Cd concentration, $P < 0.05$. The results of Cd content measurements in leaves are expressed on a dry weight basis (DW)

tions of 3, 6, and 12 mg/kg, leaf Cd accumulation in cultivar YY was significantly higher than that in WNS ($P < 0.05$), with increases of 1.47-, 1.55-, and 1.41-fold, respectively.

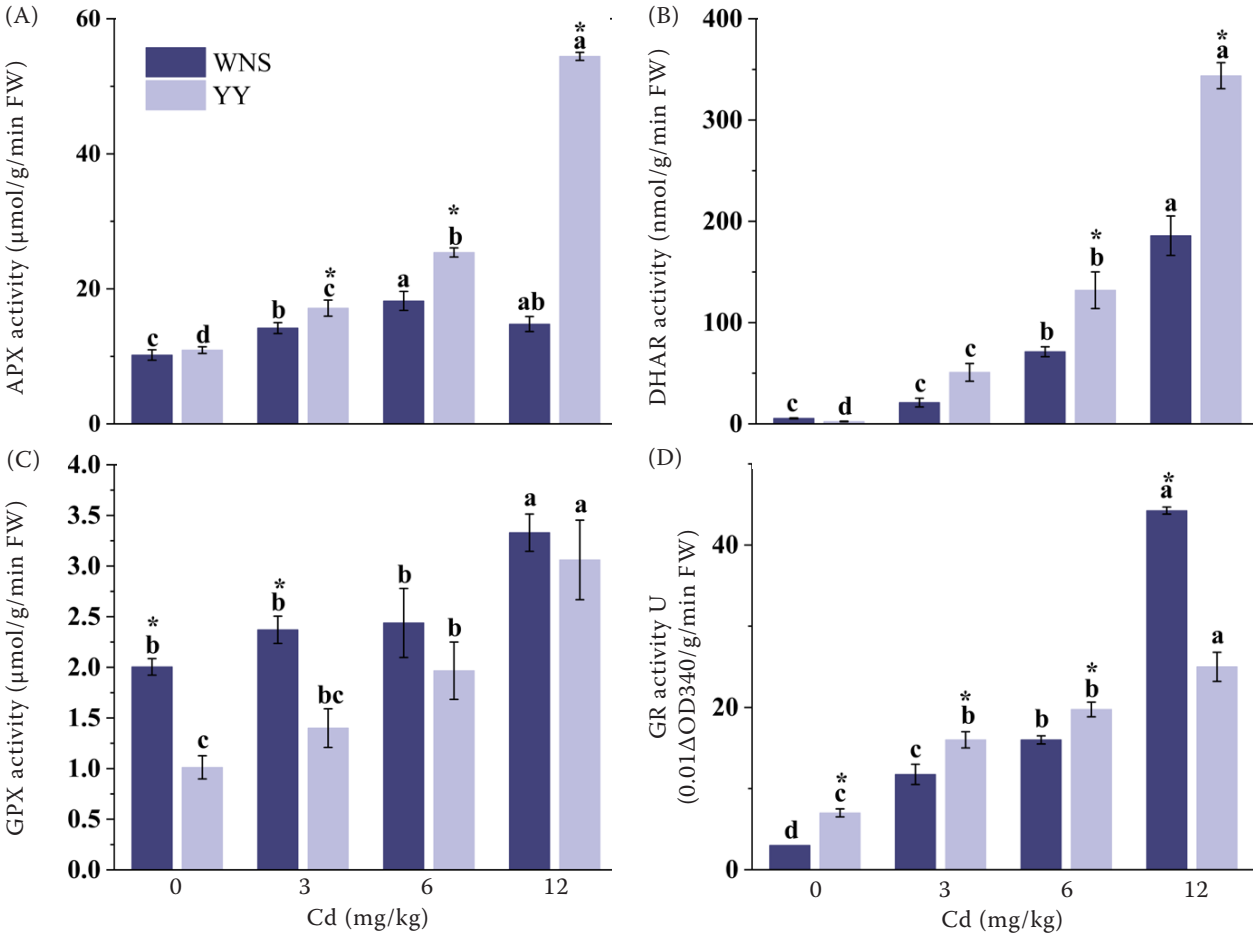

Figure 3. Leaf (A) ascorbate peroxidase (APX) activity; (B) dehydroascorbate reductase (DHAR) activity; (C) glutathione peroxidase (GPX) activity; (D) glutathione reductase (GR) activity in two perennial ryegrass cultivars. WNS – Venus; YY – Excellent; Values represent the mean ± standard error (n = 3). Different letters within vertical columns indicate significant differences among the same cultivar at different Cd concentrations, while * denotes significant differences between two perennial ryegrass cultivars at a specific Cd concentration, $P < 0.05$. The results were all measured based on fresh leaf weight (FW)

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AsA-GSH-related enzyme activities of two perennial ryegrasses under different Cd concentrations. Cd exposure induced a concentration-dependent increase in APX, DHAR, GPX, and GR activities in both WNS and YY cultivars (Figure 3). APX and GR activities in both cultivars were significantly higher than in the control ($P < 0.05$). At 3, 6, and 12 mg/kg Cd, APX activity in YY was significantly higher than in WNS ($P < 0.05$), with increases of 20.76, 39.41, and 268.10%, respectively. At 0, 3, and 6 mg/kg Cd, GR activity in YY was significantly higher than in WNS ($P < 0.05$), increasing by 133.33, 36.17, and 23.44%, respectively (Figure 3A, D). However, at 12 mg/kg Cd, GR activity in WNS was significantly higher than in YY ($P < 0.05$), by 77%. DHAR activity in YY was consistently higher than the control across Cd treatments, whereas WNS showed significant increases only at 6 and 12 mg/kg Cd ($P < 0.05$). At 6 and 12 mg/kg Cd, DHAR activity in YY was significantly higher than in WNS ($P < 0.05$), by 85.27% and 84.50%, respectively. GPX activity in WNS was consistently higher than in YY, although significant differences were observed only at 0 and 3 mg/kg Cd ($P < 0.05$), with increases of 97.97% and 69.36%, respectively.

The content of small-molecule antioxidant substances in two perennial ryegrasses under different Cd concentrations. Cd stress significantly reduced AsA and GSH contents in both WNS and YY, while markedly increasing GSSG levels compared with the control ($P < 0.05$) (Figure 4A, B). AsA and GSH levels decreased progressively with increasing Cd concentration, whereas GSSG exhibited an opposite, concentration-dependent increase (Figure 4C). Across Cd treatments, AsA content in YY was significantly higher than in WNS, whereas GSH content was consistently higher in WNS ($P < 0.05$). GSSG content in YY was higher than in WNS, although significant differences were detected only under 0 mg/kg Cd ($P < 0.05$). Under 0, 3, 6, and 12 mg/kg Cd, AsA levels in YY were 1.93-, 2.11-, 2.16-, and 2.11-fold those in WNS, respectively, while GSSG levels were 3.41-, 1.21-, 1.07-, and 1.06-fold higher. Conversely, GSH content in WNS was 1.46-, 1.22-, 2.26-, and 1.31-fold higher than in YY, respectively.

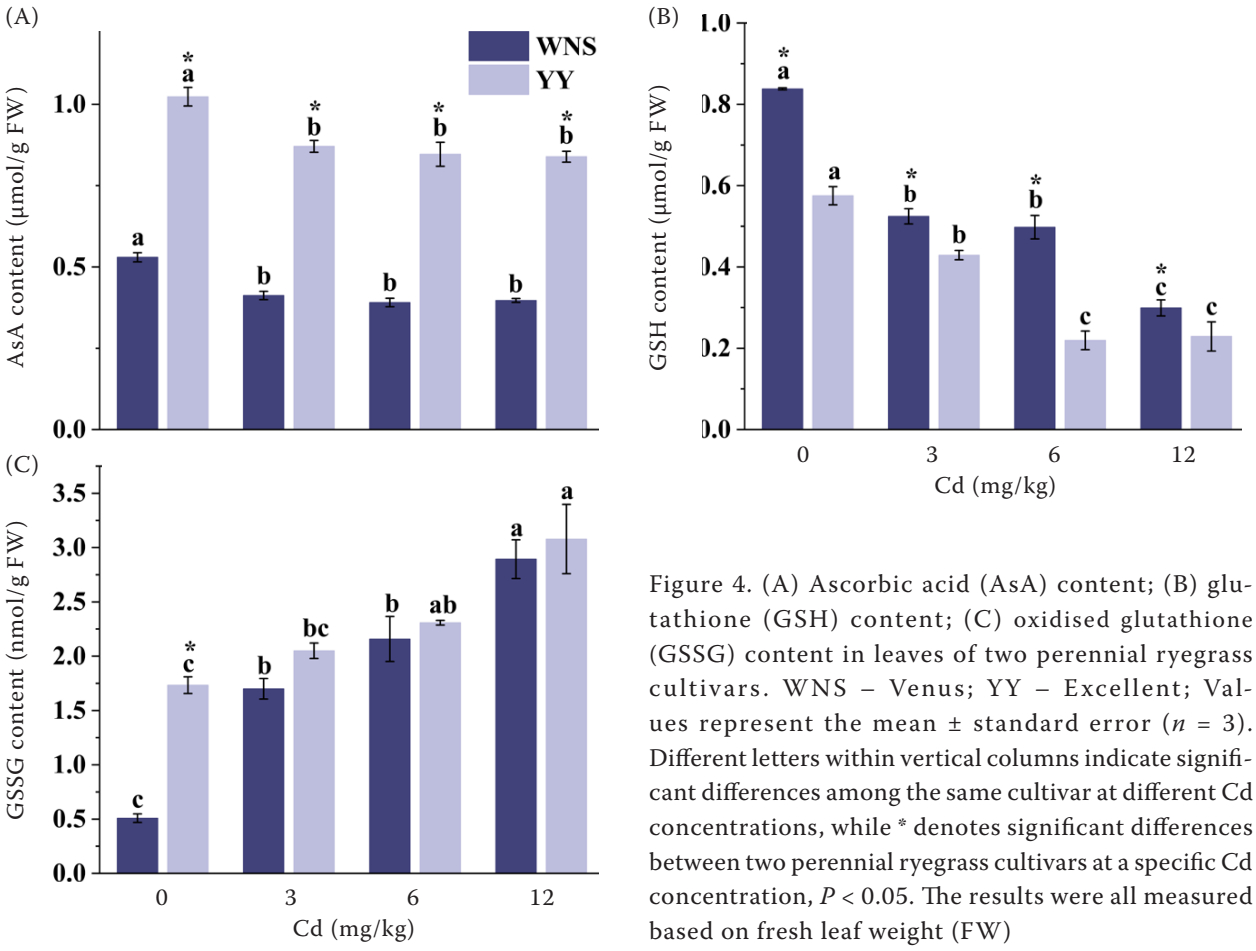

Figure 4. (A) Ascorbic acid (AsA) content; (B) glutathione (GSH) content; (C) oxidised glutathione (GSSG) content in leaves of two perennial ryegrass cultivars. WNS – Venus; YY – Excellent; Values represent the mean ± standard error ($n = 3$). Different letters within vertical columns indicate significant differences among the same cultivar at different Cd concentrations, while * denotes significant differences between two perennial ryegrass cultivars at a specific Cd concentration, $P < 0.05$. The results were all measured based on fresh leaf weight (FW)

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Table 2. Comprehensive response index of different indexes of 2 cultivars under different treatments

Cultivar	3 mg/kg Cd		6 mg/kg Cd		12 mg/kg Cd	
	WNS	YY	WNS	YY	WNS	YY
APX activity	39.11	56.86	78.48	132.35	44.88	398.04
DHAR activity	285.05	2 039.66	1 206.77	5 451.72	3 308.27	14 358.62
GPX activity	18.30	38.27	21.64	94.26	66.13	202.33
GR activity	291.67	128.57	433.33	182.14	1 375	257.14
AsA contents	–22.20	–14.92	–26.25	–17.30	–25.08	–18.01
GSH contents	–37.40	–25.41	–40.61	–61.85	–64.29	–60.16
GSSG contents	234.43	18.27	324.60	33.17	469.26	77.64
RI	808.96	2 241.30	1 997.96	5 814.49	5 174.17	15 215.60

The results were all measured based on fresh leaf weight (FW). APX – ascorbate peroxidase; DHAR – dehydroascorbate reductase; GPX – glutathione peroxidase; GR – glutathione reductase; AsA – ascorbic acid; GSH – glutathione; GSSG – oxidised glutathione; RI – response index; WNS – Venus; YY – Excellent

Comprehensive response index of two perennial ryegrass cultivars under different Cd concentrations. Across Cd treatments (3, 6, and 12 mg/kg), the integrated response index (RI) of YY was consistently higher than that of WNS (Table 2), reaching 2.77- and 2.91-fold that of WNS at 3 and 6 mg/kg Cd, respectively. At 12 mg/kg Cd, RI in YY reached a maximum value of 15 215.6, approximately 2.94-fold that of WNS. Among individual indicators, DHAR activity showed the strongest response to Cd stress, followed by GSSG content. Under identical Cd treatments, RI values for GR activity and GSSG content were higher in WNS than in YY.

DISCUSSION

Phenotypic differences between two perennial ryegrass cultivars under Cd stress. Cd is among the most phytotoxic heavy metals (Wang et al. 2007), and growth retardation is one of the most prominent symptoms in plants exposed to Cd stress (Bai et al. 2024, Saeed et al. 2024). In this study, the two perennial ryegrass cultivars with contrasting Cd accumulation capacities showed decreased plant height under the 12 mg/kg Cd treatment. This observation is consistent with Bibi et al. (2025), as this relatively high Cd concentration exerted prominent growth-inhibitory effects on both cultivars. This finding indicates that elevated Cd concentrations disrupt plant metabolism through multiple physiological pathways, thereby suppressing plant growth (Genchi et al. 2020). Interestingly, WNS showed significant increases in plant height, leaf length and tiller number relative to its own 0 mg/kg Cd control at 37 days after treatment under 3 and 6 mg/kg Cd, demonstrating a typical hormetic effect triggered by low Cd concentrations (Mengdi et al. 2021). Fan et al. (2025) likewise observed elevated biomass accumulation in tobacco under low Cd exposure, whereas YY failed to exhibit such growth-stimulating responses. This phenomenon may be related to the inherently weak growth potential of cultivar YY; the mild growth stimulation induced by low-concentration Cd cannot offset its innate growth defects. In addition, YY possesses strong Cd absorption and translocation capacity. Even at Cd treatments of 3 mg/kg and 6 mg/kg, continuous accumulation of intracellular Cd causes irreversible damage to the photosynthetic apparatus and consumes abundant antioxidants involved in the ASA-GSH cycle. These adverse physiological impacts completely counteract the hormetic effect triggered by low Cd concentrations.

From 37 to 44 days after emergence, the high Cd-accumulating cultivar YY exhibited lower plant height, leaf length, and tiller number than the control under Cd stress, along with reduced growth rates. This is consistent with the findings of previous studies, which have shown that Cd reduces plant height, leaf biomass, and the number of tillers (Li et al. 2022, Jia et al. 2025). The maximum growth rates of plant height and leaf length for WNS occurred in the late experimental stage from day 37 to 44, while the growth peak of YY only appeared in the early stage from day 30 to 37. Accordingly, we infer that this temporal difference stems from the time-sequential effect whereby Cd accumulation intensifies continuously as growth duration increases. As the treatment time prolonged, Cd accumulated progressively in YY

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plants, and Cd-induced growth inhibition was further aggravated between days 37 and 44. In contrast, WNS could rely on its cell walls and cell membranes to block Cd uptake over a long period, and massive Cd accumulation would not occur as stress time increased. For this reason, WNS maintained superior growth performance compared with YY after 37 days of continuous Cd exposure. This also represents an advantage of low Cd-accumulating plants.

AsA-GSH cycle responses of two perennial ryegrass cultivars under Cd stress. Our research group has completed extensive cultivar screening in prior work, and the present study was conducted based on the screening outcomes reported by Jiang et al. (2022). The Cd concentration in the leaves of YY was markedly higher than that of WNS. Overall, the leaf Cd content of both cultivars increased with the elevation of Cd stress concentrations, and leaf Cd levels under all treatments were significantly higher than those of the control group. This result is consistent with Dai et al. (2022), which demonstrates a positive correlation between soil Cd concentrations and foliar Cd accumulation in plants (Dai et al. 2022). Cd accumulation induces oxidative damage and disrupts the AsA-GSH cycle, thereby perturbing plant growth (Jung et al. 2021). Within this cycle, APX initiates H_2O_2 detoxification (Ekmekçi et al. 2008), and DHAR, GPX, and GR also play key roles in ROS scavenging. In the present study, the activities of APX, DHAR, GPX, and GR increased progressively with Cd exposure, indicating a pronounced effect of Cd on antioxidant enzyme systems (Zhang et al. 2023). This trend is consistent with observations by Lou et al. (2017) in both Cd-sensitive and Cd-resistant Chinese cabbage cultivars, suggesting that perennial ryegrass may enhance antioxidant enzyme activities to mitigate Cd-induced oxidative stress and maintain cellular homeostasis.

Except for GPX, YY exhibited significantly higher APX, DHAR, and GR activities than WNS under Cd stress (Figure 3), consistent with findings in rapeseed cultivars differing in Cd accumulation capacity (Wu et al. 2015). The comprehensive response index of YY was also consistently higher, likely reflecting its greater Cd uptake and the consequent need for enhanced ROS detoxification. Higher Cd accumulation typically intensifies ROS production and toxic injury, thereby requiring greater enzymatic activity to sustain redox balance. It is well established that Cd disrupts intracellular ion homeostasis once internalised, competing with essential cations (e.g., Fe, Zn) at enzyme active sites and impairing antioxidant function (Cao et al. 2024). This competition may explain the lower GPX activity across all Cd treatments and reduced GR activity at 12 mg/kg Cd in YY relative to WNS, as excessive Cd likely impeded enzyme function in the high-accumulating cultivars.

AsA, GSH, and GSSG constitute key non-enzymatic antioxidants within the AsA-GSH cycle and play critical roles in ROS detoxification (Chen et al. 2024, Wu et al. 2024). In both cultivars, AsA and GSH contents declined as Cd concentrations increased, whereas GSSG levels increased (Figure 4). This pattern accords with the findings of Tan et al. (2022) in Cd-stressed Chinese cabbage, although their treatment effects were less differentiated, possibly due to differences in Cd gradient design. The observed decline in AsA and GSH reflects their consumption in response to ROS production during oxidative stress. Concurrently, rising GSSG indicates the oxidation of GSH *via* GPX-mediated reactions – a trend similar to that reported by Ortega-Villasante et al. (2005) in Cd-treated alfalfa.

Under Cd stress, the AsA content in WNS was consistently lower than that in the YY cultivar. This is consistent with the findings of a study by Zhu et al. (2024), who, in their research on two rice cultivars under Cd stress, noted that the AsA content in the high-accumulation rice cultivar was higher than that in the low-accumulation rice cultivar. However, in the present study, the GSH content of the low-accumulation cultivar WNS was higher than that of the high-accumulation cultivar YY. This contrasts with the findings of studies by Zhu et al. (2024) and Wu et al. (2015), which indicated that GSH levels in high-accumulation cultivars were consistently higher than in low-accumulation cultivars; this discrepancy may reflect species- or cultivar-specific differences. In the present study, the comprehensive response index indicated that GSH plays a particularly prominent role in detoxification. Under Cd treatment, the low-accumulating WNS maintained significantly higher GSH levels and exhibited superior growth compared with YY.

Overall, the high Cd-accumulating cultivar YY displayed more sensitive responses of antioxidant components under Cd stress, which rendered its growth and physiological homeostasis susceptible to damage. In contrast, the low Cd-accumulating cultivar WNS maintained higher glutathione-related enzyme activities and antioxidant contents, thereby sustaining superior growth performance under Cd exposure.

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A comprehensive evaluation of growth traits, enzyme activities, and antioxidant indicators revealed that the two perennial ryegrass cultivars adopted distinct mechanisms to tolerate Cd stress. Under low and moderate Cd concentrations, the low Cd-accumulating cultivar maintained higher growth rates with milder decreases in plant height, leaf length, and tiller number, while the high Cd-accumulating cultivar suffered more severe growth inhibition at identical Cd levels. Meanwhile, it exhibited greater increases in ascorbate peroxidase and dehydroascorbate reductase activities, and in AsA content, demonstrating higher sensitivity to Cd-induced oxidative stress and a heavy reliance on AsA-dominated detoxification pathways. Although both cultivars activated the AsA-GSH cycle, WNS alleviated Cd toxicity *via* the GSH-dependent branch by elevating the activities of glutathione peroxidase and glutathione reductase and promoting GSH accumulation. These differences show that the two cultivars use different ways to remove reactive oxygen species. They also have genotype-specific regulatory patterns in their ROS-scavenging networks. Future studies can further explore these underlying mechanisms in detail.

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