



# Nano-Priming with La<sub>2</sub>O<sub>3</sub> Improves Early Growth and Regulates Physio-Biochemical Mechanisms in Fragrant Rice Against Cadmium Toxicity

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## Abstract

**Purpose** Cadmium (Cd) is amongst the most toxic heavy metals which severely affects the plant growth. Application of nanoparticles (NPs) could be helpful to negate the deleterious impacts of Cd stress in crop plants.

**Methods** In this study, seed priming of two fragrant rice cultivars, i.e., Xiangyaxiangzhan and Yuxiangyouzhan, applied with four levels of Lanthanum oxide nanoparticles (La<sub>2</sub>O<sub>3</sub> NPs) i.e., 0, 50, 100, and 300 mg L<sup>-1</sup>, grown under three Cd levels i.e., 0, 50, and 100 mg L<sup>-1</sup>.

**Result** Results revealed that the nano-priming with La<sub>2</sub>O<sub>3</sub> significantly improved the seed germination rate under the Cd stress. Nano-priming exhibited substantial improvements in the total fresh weight, shoot length, root length, leaf sheath length and prophyll leaf length under the Cd stress. Moreover, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs substantially modulate the antioxidant activities i.e., superoxide dismutase, peroxidase, and catalase, and enhanced the α-amylase activity. Nano-priming further reduced the malondialdehyde, metallothionein, glutathione, proline, and soluble protein contents whilst enhanced the chlorophyll and carotenoids contents.

**Conclusions** Seed priming with La<sub>2</sub>O<sub>3</sub> NPs substantially enhanced the early growth of rice seedlings by improving morphological attributes and modulating the physiological and biochemical responses under Cd toxicity. The insights gained from this study may be of assistance to understand the effects of La<sub>2</sub>O<sub>3</sub> NPs on early growth of rice, and its potential applications in enhancing the Cd tolerance of crops.

**Keywords** La<sub>2</sub>O<sub>3</sub> NPs · Cadmium · Antioxidant enzymes · Plant growth · Fragrant rice

## Abbreviations

NPs Nanoparticles  
La Lanthanum  
Cd Cadmium

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La <sub>2</sub> O <sub>3</sub> NPs	Lanthanum oxide nanoparticles
LNP 0, LNP 50, LNP 100, and LNP 300	
0, 50, 100, and 300 mg L <sup>-1</sup> La <sub>2</sub> O <sub>3</sub> NPs	
DAS	Days after sowing
Cd 0, Cd 50, and Cd100	0, 50, and 100 mg L <sup>-1</sup> CdCl <sub>2</sub>
SOD	Superoxide dismutase
POD	Peroxidase
CAT	Catalase
MDA	Malondialdehyde
MT	Metallothionein
GSH	Glutathione
ANOVA	Analysis of variance
Ns	Nonsignificant
SEM	Scanning electron microscopy
V	Variety

## 1 Introduction

Rice is an essential food for more than four billion people worldwide which is grown all over the world under different climatic conditions (Li et al. 2021b). Among heavy metals, cadmium (Cd) is considered to be the most toxic and cause severe inhabitation of plant growth at high concentration (Kanu et al. 2019). Industrialization, excessive use of agrochemicals and other anthropogenic activities are major factors for arable land pollution with heavy metals (Rascio et al. 2008; Roberts 2014). Rice has a high bioaccumulation of numerous toxic metals, such as cadmium (Cd), lead (Pb), and arsenic (As) (Ashraf et al. 2015; Khanam et al. 2020) whereas consumption of foods of plant origin with high levels of toxic metals, especially Cd, poses a severe health risk to consumers (Clemens and Ma 2016). Rice accounted about half of the total Cd intake for those who consume rice as staple food (Wang et al. 2019). Therefore, it is essential to minimize the transfer of Cd from the environment to the plants, especially rice.

Although Cd is a non-essential element, it is readily absorbed via plant roots by competing with other divalent ions and accumulates in edible plant parts (Clemens 2001, 2006; Palmgren et al. 2008). Cd toxicity often inhibits the development of plants (Huang et al. 2020a; Rao et al. 2019). Cd could decrease rice germination, biomass, root-shoot ratio, as well as leaf growth (Ahsan et al. 2007; Song et al. 2015). Rice growth and biomass reduction is linked to Cd-toxicity-related alterations at molecular level (Lee et al. 2013; Srivastava et al. 2014). Moreover, Cd uptake in rice plants causes oxidative stress owing to excessive production of reactive oxygen species (ROS) and lipid peroxidation (Srivastava et al. 2014; Yu et al. 2013). Plants activate its antioxidant defense system to counteract the oxidative stress (Ashraf et al. 2018; Yu et al. 2013). Furthermore,

Cd toxicity significantly reduces the rice germination, seed vigor, the length of radicle, and amylase activities (He et al. 2008).

Currently, nanotechnology is becoming popular due to its wide scale application and potential benefits in agriculture and recognized as a convenient tool for increasing crop yields and ensuring food security (Hussain et al. 2019; Shang et al. 2019). Due to high surface area and reactive character, as well as potent adsorption ability, the nanoparticles (NPs) could negatively impact the transport of heavy metals, pesticides, and other toxic chemicals (Deng et al. 2017; Glomstad et al. 2016; Yang and Xing 2010; Zhang et al. 2019). The application of NPs has a substantial impact on the heavy metal uptake and accumulation in plants (Rizwan et al. 2019; Singh et al. 2018). No doubt, nano-materials are well popularized in the field of science, however nano-enabled agriculture is still in its infancy (Li et al. 2021b; Pulizzi 2019; Zahedi et al. 2020).

In addition, rare earth oxide nanoparticles (REO NPs) are quite efficient due to their excellent magnetic, optical, and electronic properties (Hwang et al. 2019). Lanthanum oxide nanoparticles (La<sub>2</sub>O<sub>3</sub> NPs) are one of the REO NPs used in agro-film, vehicle emissions cleaning, electrode materials, as well as optical fibers (Ma et al. 2011; Yue et al. 2017). Currently scientists have different opinions on whether NPs have a beneficial or harmful effect on plants, e.g., 10 mg L<sup>-1</sup> of La<sub>2</sub>O<sub>3</sub> NPs increased the root biomass of radish (Xiao et al. 2021). On the contrary, several studies have reported the negative impact of La<sub>2</sub>O<sub>3</sub> NPs in plants. The elongation of root was greatly inhibited by La<sub>2</sub>O<sub>3</sub> NPs in a variety of higher plants, including wheat, tomato, rape, lettuce, cucumber, and cabbage (Ma et al. 2010). La<sub>2</sub>O<sub>3</sub> NPs can significantly down-regulate photosynthesis-related genes in maize. (Liu et al. 2020). Thus, NPs could have beneficial or detrimental impacts on plants depending upon plant variety, growth stage, growing environment, treatment method, and application dose (Rastogi et al. 2017).

Nano-priming is thought to be a simple method to improve early plant growth under unfavorable conditions (Mahakham et al. 2017). Previous studies reported some biotransformation and potential adverse effects of La<sub>2</sub>O<sub>3</sub> in plants (Hwang et al. 2019), however, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs in fragrant rice under Cd stress have not yet been investigated. The present study was therefore conducted to assess the effectiveness of nano-priming with La<sub>2</sub>O<sub>3</sub> NPs on the early growth and the related physio-biochemical attributes of fragrant rice under Cd stress, with the hypothesis that the La<sub>2</sub>O<sub>3</sub> NPs application would alleviate Cd toxicity and improve the early growth of fragrant rice. This research may find potential applications in improving the resistance of rice against Cd toxicity.

## 2 Materials and Methods

### 2.1 Experimental Materials

Seed of two fragrant rice cultivars i.e., Xiangyaxiangzhan and Yuxiangyouzhan were obtained from College of Agriculture, South China Agricultural University, Guangzhou, China. These two varieties are commercially planted in south China and are highly popular (Gui et al. 2022). Based on the evaluation of shoot dry weight under  $100 \text{ mg L}^{-1}$  of Cd, Xiangyaxiangzhan and Yuxiangyouzhan are tolerant and sensitive to Cd toxicity, respectively (Li et al. 2021b). Qing et al. (2022) also reported that the  $100 \text{ mg L}^{-1}$  of Cd significantly reduced the total dry weight of Xiangyaxiangzhan and Yuxiangyouzhan. The  $\text{La}_2\text{O}_3$  NPs (purity: 99.99%, particle size: 50 nm) were purchased from Macklin Biochemical Co. Ltd., Shanghai, China. The structural properties of  $\text{La}_2\text{O}_3$  NPs were visualized under the scanning electron microscope (SEM, Sigma-500, Carl Zeiss AG, Germany). The  $\text{La}_2\text{O}_3$  NPs were ranging from 27.81 to 156.66 nm and an average SEM size of 72.14 nm (Fig. 1).

### 2.2 Experimental Details

The experiment was conducted in September 2021 at the College of Agriculture, South China Agricultural University, Guangzhou, China. Seeds were sterilized in 5% sodium hypochlorite solution for 15 min, and thoroughly washed with deionized water.  $\text{La}_2\text{O}_3$  NPs were stirred in deionized water (pH=6.5) for 5 min before being dispersed in deionized water using a water bath and ultrasonic treatment chamber (HZS-H, China) for 30 min (Huang et al. 2020b). Seeds were subsequently immersed in solutions of varying concentrations of  $\text{La}_2\text{O}_3$  NPs, i.e., 0, 50, 100, and 300  $\text{mg L}^{-1}$  over-night and denoted as the LNP0, LNP50, LNP100, and LNP300, respectively, and continuously aerated for 20 h at  $25^\circ\text{C}$ , and then rinsed with deionized water.

Rice seeds of uniform size were placed on filter paper in transparent culture box  $13.6 \times 8.6 \times 4.8 \text{ cm}$  in length, width and height, respectively, and treated with Kimura B nutrient solution (pH 4.7–4.9) as described previously (Chen et al. 2015), containing 0, 50 and  $100 \text{ mg L}^{-1}$  of  $\text{CdCl}_2$  denoted as Cd0, Cd50 and Cd100. The filter paper was rinsed daily with Kimura B nutrient solution containing the same concentration of  $\text{CdCl}_2$  to keep it moist and to keep the concentration of the treatment constant. The culture boxes were placed in an incubator (G&G Measurement Plant, Suzhou, Jiangsu, China) with 14/10 h light/dark period at  $28/25^\circ\text{C}$ , respectively for 7 days, and the relative humidity was set at 70%. Here, the concentration of Cd and  $\text{La}_2\text{O}_3$  NPs are based on previous studies (He et al. 2008; He et al. 2014; Li et al. 2021b; Xiao et al. 2021) which reported that low concentrations of lanthanum (20–100  $\text{mg L}^{-1}$ ) had a promotive effect on rice at the early growth stages, while Cd at  $100 \text{ mg L}^{-1}$  caused significant reduction in rice growth. The experiment was comprised of 24 treatments with seven replicates.

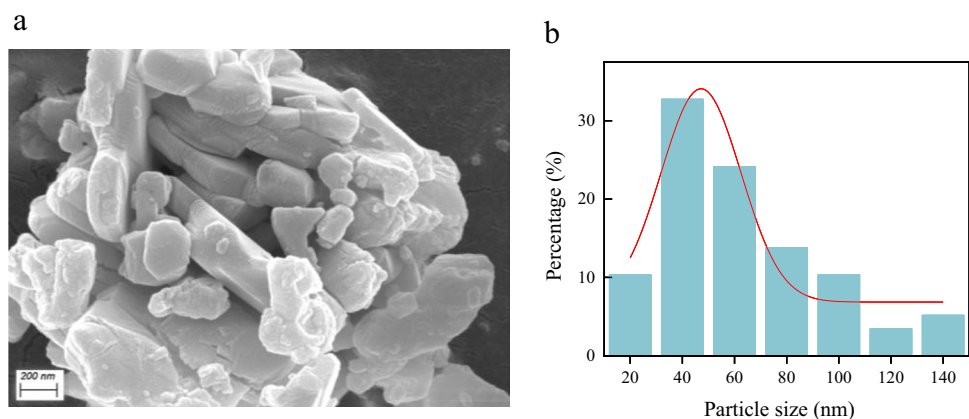
### 2.3 Sampling and Measurements

Germination was measured at every 2 days after sowing. Seedlings were harvested after 7 days of growth for morpho-physiological traits (Li et al. 2019). Four boxes of 60 rice seedlings were randomly selected from each treatment and the samples were immediately soaked in liquid nitrogen and stored in  $-80^\circ\text{C}$  for physio-biochemical assays.

### 2.4 Determination of Germination and Morphological traits

Rice seeds are considered to have germinated when the roots break through the seed coat and extend 2 mm. Germination rate is defined as the number of germinated seeds as a percentage of the number of seeds sown (Li et al. 2021b). The germination index (GI) of each treatment were measured as

**Fig. 1** Scanning electron microscopy (SEM) images of  $\text{La}_2\text{O}_3$  NPs (scale 50 nm) (a), particle size distributions of  $\text{La}_2\text{O}_3$  NPs were measured using the Nano Measurer 1.2 (b)



described previously (Fashui et al. 2000). The seed vigor index is defined as the germination index multiplied by the shoot length (Li et al. 2014). The shoot, root, coleoptile, leaf sheath, and prophyll leaf length of five seedlings from each of six randomly selected incubators were measured at 7 days after sowing. The fresh weight was determined by an electronic balance of five seedlings from each of six randomly selected incubators. After drying for three days at 60 °C, the dry weights of each plant part were measured by an electronic balance. The shoot-to-root ratio was defined as the ratio of the dry weight of the shoot to the dry weight of the root.

## 2.5 Determination of Antioxidant Enzyme Activities and MDA Contents

The activities of antioxidant enzymes were quantified according to previous method (Li et al. 2019). Fresh samples (0.3 g) were taken, homogenized in 3 ml of 100 mM phosphate buffered saline (PBS) solution (pH = 7.8) and centrifuged for 15 min at 14,000 rpm at 4 °C. PBS is a water-based salt solution generally used as a solvent and protective reagent whose main components are  $\text{Na}_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{NaCl}$ , and  $\text{KCl}$  (Drummond and Austin 2013). The supernatant was the crude enzyme extract, which was used for subsequent determination of antioxidant activities.

For superoxide dismutase (SOD) activity, the reagents were mixed in proportion (150  $\mu\text{L}$  of 50 mM PBS, 30  $\mu\text{L}$  of 130 mmol Met, 30  $\mu\text{L}$  of 750  $\mu\text{mol}$  NBT, and 30  $\mu\text{L}$  of 100  $\mu\text{mol}$  EDTA- $\text{Na}_2$ ). An aliquot of 5  $\mu\text{L}$  of enzyme extract was added with 240  $\mu\text{L}$  of the reaction mixture, mixed well and added with 30  $\mu\text{L}$  of 20  $\mu\text{mol}$  riboflavin. The reaction was carried out for 20 min under 4000 lx light and the absorbance was read at 560 nm, using 50% inhibition as a unit of enzyme activity. The enzyme solution was replaced by PBS as a control, one in the light as a blank control. The amount of enzyme required to inhibit 50% of the NBT photoreduction reaction was taken as one enzyme activity unit (U). To detect absorbance at 560 nm, SOD activity was calculated as  $\text{U g}^{-1}$  FW. For peroxidase (POD) activity, 100  $\mu\text{L}$  of mM PBS, 95  $\mu\text{L}$  of 0.2% guaiacol, 100  $\mu\text{L}$  of 0.3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), and 5  $\mu\text{L}$  enzyme solution were mixed and read at 470 nm. The absorbance was recorded every 30 s for 2.5 min. The absorbance increase of one unit per minute ( $\text{U g}^{-1}$  FW) was the unit of POD activity. For catalase (CAT) activity, 1.95 ml of 50 mM PBS, 1 ml of 0.3%  $\text{H}_2\text{O}_2$  solution and 0.05 ml of enzyme extract was added to the sample tube. The reaction was started immediately after the addition of the enzyme extract and the absorbance value was measured at 240 nm. A decrease in  $A_{240}$  of 0.01 per minute was used as a unit of enzyme activity (U). CAT activity was calculated as  $\text{U g}^{-1}$  FW.

The MDA was quantified according to previous method (Qing et al. 2022). Fresh samples (0.1 g) of the seedlings were homogenized with 2 ml of phosphate buffer (pH = 7.8) and centrifuged at 8000 rpm for 15 min at 4 °C. The supernatant was used as the sample extract. The reaction mixture was comprised of 0.4 ml of 0.5% thiobarbituric acid and 0.2 ml of sample extract. The absorbance was read at 532, 600 and 450 nm. The content of malondialdehyde was calculated as  $n(\mu\text{mol}) = 6.45(\text{OD}_{532} - \text{OD}_{600}) - 0.56\text{OD}_{450}$  and expressed as  $\mu\text{mol g}^{-1}$  FW.

## 2.6 Determination of Proline and Soluble Protein Content

The proline was quantified according to previous method (Bates et al. 1973). Fresh leaves samples (0.1 g) were homogenized with 2 ml of 3% sulfosalicylic acid solution. Supernatant (0.4 ml) was added to 0.4 ml of glacial acetic acid and 0.4 ml of 6.25 g ninhydrin dissolved in 150 ml glacial acetic acid and 100 ml of 6 mol  $\text{L}^{-1}$  PBS were placed in boiling water bath for to 30 min. After cooling, 0.8 ml toluene was mixed into the reaction mixture and the absorbance of the red chromophore in the toluene fraction was measured at 520 nm and the proline contents were expressed as  $\mu\text{g g}^{-1}$  FW.

The soluble protein was measured according to the method described previously (Qing et al. 2022). Fresh leaves (0.1 g) were homogenized with 2 ml of PBS and centrifuged at 4000 rpm for 20 min at 4°C. The reaction mixture contained 0.1 ml sample extract and 1 ml coomassie brilliant blue. After mixing the reaction mixture, the color development was set at 2 min at room temperature and then absorbance was read at 595 nm. The soluble protein content was expressed as  $\mu\text{g g}^{-1}$  FW.

## 2.7 Determination of Glutathione (GSH), Metallothionein (MT) Contents, and $\alpha$ -Amylase Enzyme Activity

The glutathione (GSH) content was measured according to the method described previously (Zhang et al. 2013). Fresh samples (0.1 g) were homogenized with 2 ml of 5% trichloroacetic acid and centrifuged at 13,000 rpm for 20 min at 4°C. The reaction mixture was comprised of 0.2 ml supernatant, 4.4 ml PBS, and 0.4 ml 0.04% 2-nitrobenzoic acid solution. The absorbance of the reaction mixture was read at 412 nm and the GSH contents were expressed as  $\mu\text{g g}^{-1}$  FW.

The metallothionein (MT) contents were measured according to the method described previously (Erk et al. 2002). Fresh samples (0.1 g) were homogenized with 0.5 ml of solution containing 20  $\mu\text{mol}$  Tris-HCl and 0.5  $\mu\text{mol}$  phenylmethylsulfonyl fluoride (pH = 8.6), and 1.5 ml of 0.01%  $\beta$ -mercaptoethanol and centrifuged at 13,000 rpm at 4 °C. The supernatant was heated in a water bath at 70 °C for 10 min and centrifuged. The reaction mixture for the determination of MT was comprised of 4.4 ml of

demetallic solution, 0.2 ml of extraction solution and 0.4 ml of 0.04% 2-nitrobenzoic acid solution containing 0.8 mmol NaCl. The reaction was carried out at 30 °C for 30 min and the absorbance was measured at 412 nm. The MT content was calculated by following the standard curve and expressed as  $\mu\text{mol g}^{-1}$  FW.

The  $\alpha$ -amylase activity was estimated according to the method described previously (Li et al. 2021a). Fresh samples (0.3 g) were homogenized with 3.75 ml of citric acid buffer (pH=5.6) and centrifuged at 4000 rpm for 10 min at 4 °C. The supernatant was mixed with distilled water to a final volume of 25 ml and the resultant solution was used as enzyme extract. 1 ml of enzyme extract was heated in a water bath at 70 °C for 15 min. 1 ml of citric acid buffer (pH=5.6) was added to the reaction mixture water bath at 40 °C for 15 min and then 4 ml of 0.4 mol L<sup>-1</sup> NaOH was quickly added to terminate the amylase reaction. 1 ml of the reaction mixture was added with 1 ml of 3,5-dinitrosalicylic acid reagent was read at 520 nm. The  $\alpha$ -amylase activity was expressed as  $\text{mg (g min)}^{-1}$ .

## 2.8 Determination of Chlorophyll and Carotenoid Contents

Fresh leaves (0.1 g) were added to 6 ml of 95% ethanol and placed in the dark at 4 °C. After 24 h, centrifugation was carried out at 5000 rpm for 10 min at 4 °C. The absorbance was recorded at 665, 649, and 470 nm, respectively (Arnon 1949). The chlorophyll and carotenoid contents were expressed as  $\text{mg kg}^{-1}$ .

## 2.9 Statistical Analyses

The data were recorded in Microsoft Excel 2019 and analyzed by Statistix version 8.0 for ANOVA and multiple comparisons. The means of each treatment were compared at the least significant difference LSD test at 5% probability. Pearson correlation analysis was performed on the data using the software IBM SPSS Statistics 21. Figures were created by Origin 2021. We kept one concentration of Cd for germination analysis and growth and physiological and biochemical parameters analysis in the main text. All the experimental data were presented in the supplementary file.

## 3 Results

### 3.1 The Germination, Germination Index, and Seed Vigor index

Seed germination was affected by V, Cd, La<sub>2</sub>O<sub>3</sub> NPs, V×La<sub>2</sub>O<sub>3</sub> NPs, and La<sub>2</sub>O<sub>3</sub> NPs×Cd significantly (Table 1). Cd treatments significantly reduced the germination rate by 2.41%–14.57% compared with Cd0 for both varieties (Fig. 2a, b). Nano-priming with La<sub>2</sub>O<sub>3</sub> NPs treatments

significantly improved the germination rate at 1 DAS by 13.44%, 8.60%, and 5.04% at LNP50, LNP100, and LNP300, respectively under the Cd100 treatment for Xiangyaxiangzhan (Fig. 2c). However, for Yuxiangyouzhan, all of La<sub>2</sub>O<sub>3</sub> NPs treatments significantly decreased the germination rate at 1 DAS under the Cd100 treatment (Fig. 2c). Similarly, seed germination index and seed vigor index were affected by V, Cd, La<sub>2</sub>O<sub>3</sub> NPs, V×La<sub>2</sub>O<sub>3</sub> NPs, and Cd×La<sub>2</sub>O<sub>3</sub> NPs significantly. Overall, seed priming with La<sub>2</sub>O<sub>3</sub> NPs (LPN50 and LPN100) significantly improved the germination index, however, germination index was significantly decreased at LNP300 for Yuxiangyouzhan (Fig. 2d). For Xiangyaxiangzhan, the LNP50 and LNP100 significantly increased the seed vigor index by 29.70% and 23.17%, respectively compared with the LNP0 under the Cd0. For Yuxiangyouzhan, the LNP100 significantly improved the seed vigor index by 22.19% under the Cd50 treatment (Fig. 2e, Additional file: Table S1).

### 3.2 Morphological Attributes

V, Cd, La<sub>2</sub>O<sub>3</sub> NPs, V×Cd, V×La<sub>2</sub>O<sub>3</sub> NPs, and La<sub>2</sub>O<sub>3</sub> NPs×Cd were significantly affected the fresh weight of shoot, root, and the total fresh weight, shoot length, root length, leaf sheath, coleoptile length, and prophyll length of rice seedlings, root and shoot dry weight, and shoot / root ratio (Table 1).

The total fresh weight significantly increased for all La<sub>2</sub>O<sub>3</sub> NPs treatments for Xiangyaxiangzhan, and at LNP300 for Yuxiangyouzhan compared with LNP0 under Cd0. For Xiangyaxiangzhan, nano-priming significantly improved the total fresh weight by 9.78%, 33.70%, and 27.17% at the LNP50, LNP100, and LNP300, respectively, compared with LNP0 under Cd50 (Fig. 3a). The shoot fresh weight of Xiangyaxiangzhan was significantly enhanced by 9.7%, 45.96%, and 27.17% at the LNP50, LNP100, and LNP300, respectively. For Yuxiangyouzhan, the shoot fresh weight was improved by 12.08% at the LNP100 compared with the LNP0 under Cd50 (Fig. 3b). Similarly, under the Cd0 treatment, the root fresh weight was significantly enhanced by 3.27% and 14.86% at LNP100 and LNP300 for Xiangyaxiangzhan. The Cd treatment significantly reduced the root fresh weight by 47.15% and 46.80% of Xiangyaxiangzhan and Yuxiangyouzhan in non-primed seeds. The root fresh weight substantially improved at LNP100 under Cd treatment for Xiangyaxiangzhan (Fig. 3c). The root fresh weight was enhanced in Yuxiangyouzhan at the LNP100 treatment compared with LNP0 under the Cd100 (Additional file: Table S2).

Moreover, the shoot length was significantly increased by 9.24% at LNP50 under Cd0 for Xiangyaxiangzhan. Under Cd50, the shoot length increased by 3.86% and 13.77% at LNP50 and LNP100, respectively, compared

**Table 1** Analysis of variance (ANOVA) of the investigated parameters

Parameter	V	Cd	V × Cd	La <sub>2</sub> O <sub>3</sub> NPs	V × La <sub>2</sub> O <sub>3</sub> NPs	Cd × La <sub>2</sub> O <sub>3</sub> NPs	V × Cd × La <sub>2</sub> O <sub>3</sub> NPs
Germination rate at 1 DAS	**	**	ns	**	**	**	**
Germination rate at 3 DAS	ns	**	ns	**	**	**	**
Germination rate at 5 DAS	*	*	ns	**	**	ns	*
Germination rate at 7 DAS	*	*	ns	**	**	ns	*
Germination Index	**	**	ns	**	**	**	**
Seed Vigor Index	**	**	ns	**	**	**	**
Shoot fresh weight	**	**	**	**	**	**	**
Root fresh weight	*	**	*	**	**	**	**
Total fresh weight	**	**	*	**	**	**	**
Coleoptile length	ns	ns	ns	**	**	**	**
First leaf length	ns	**	**	**	**	**	**
Leaf sheath length	**	**	**	**	**	**	**
Shoot length	**	**	**	**	**	**	**
Root length	**	**	**	**	**	**	**
Shoot dry weight	**	**	**	**	**	*	**
Root dry weight	ns	**	ns	ns	*	ns	**
Total dry weight	ns	**	ns	ns	**	ns	**
Shoot / root ratio	ns	*	ns	ns	*	*	*
SOD activity	**	**	**	**	**	**	**
POD activity	*	**	**	**	**	**	**
CAT activity	**	**	**	**	**	**	**
MDA content	**	**	ns	**	**	**	**
Proline content	**	**	**	**	**	**	**
GSH content	**	**	**	**	**	**	**
MT content	ns	**	**	**	**	**	**
α-amylase activity	*	**	**	**	**	**	**
Soluble protein content	**	ns	*	**	**	**	*
Chlorophyll a content	*	**	ns	**	**	**	**
Chlorophyll b content	*	**	ns	**	**	*	**
Carotenoids content	ns	**	**	**	**	**	**
Chlorophyll content	ns	**	ns	**	**	**	**
chlorophyll a: chlorophyll b	ns	**	*	**	**	**	**

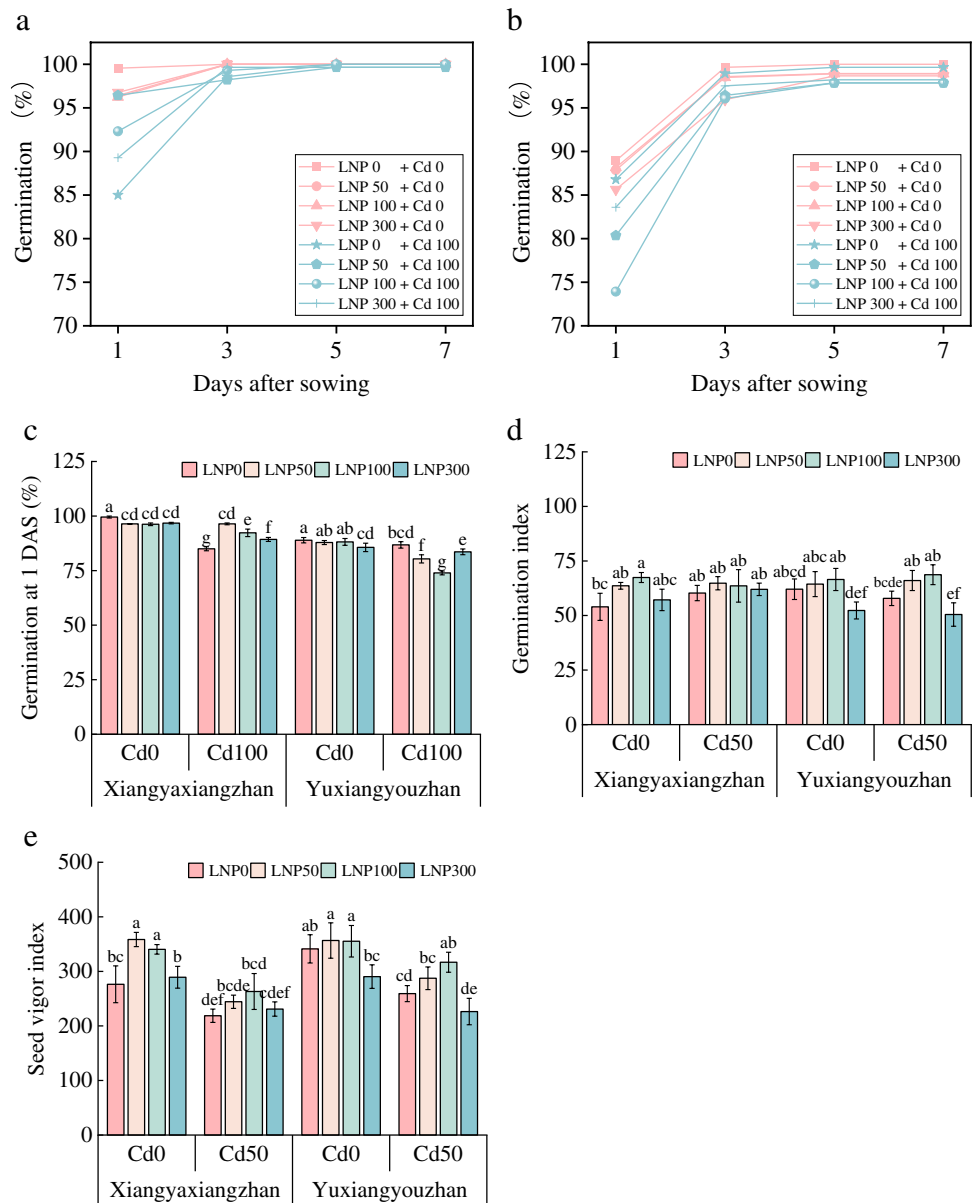
\*, significant at  $P < 0.05$ ; \*\*, significant at  $P < 0.01$ ; ns, nonsignificant at  $P > 0.05$  level (LSD). V, variety; Cd, cadmium; DAS, days after sowing; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde; GSH, glutathione; MT, metallothionein

with LNP0. In Yuxiangyouzhan, the LNP100 significantly improved the shoot length under Cd stress (Fig. 4a). Under the Cd50, the LNP100 increased the root length by 33.41% compared with LNP0, whereas LNP300 decreased the root length 22.27%. In Yuxiangyouzhan, the treatment of LNP and Cd had a co-inhibitory effect on root length (Fig. 4b). The coleoptile length was increased by 4.58% under Cd50, compared with the Cd0 in non-primed seeds. Compared to LNP0, the LNP100 increased the coleoptile length of Xiangyaxiangzhan by 10.47% under Cd50 (Fig. 4c). In addition, Cd treatment reduced prophyll leaf length by 22.99% compared to the Cd0. For Xiangyaxiangzhan, under Cd treatment, the LNP100 significantly increased the length of prophyll

leaf by 8.39% compared with the LNP0 (Fig. 4d). Furthermore, the leaf sheath length was reduced by 22.98–31.06% under both Cd stress, compared with Cd0. On the other hand, the leaf sheath length was increased by 16.02% and 9.17%, respectively, at LNP100 compared with the LNP0 under Cd50 for both varieties (Fig. 4e, Additional file: Table S3).

For Xiangyaxiangzhan, total dry weight of seedlings was increased by 34.03%, 43.35% and 109.54% at the LNP50, LNP100 and LNP300, respectively, compared with LNP0 under Cd0. For Yuxiangyouzhan, the total dry weight was decreased by 20.84% at LNP300 under Cd0, compared with LNP0. (Fig. 5a). For Xiangyaxiangzhan, the shoot dry weight was significantly improved by

**Fig. 2** Effect of  $\text{La}_2\text{O}_3$  NPs on the dynamic of seed germination. Xiangyaxiangzhan (a) and Yuxiangyouzhan (b), germination at 1 DAS (c), germination index (d), seed vigor index (e). DAS: days after sowing; LNP 50, LNP 100 and LNP 300: 0, 50, 100 and 300  $\text{mg L}^{-1}$  of  $\text{La}_2\text{O}_3$  NPs. Cd 0 and Cd 100: 0 and 100  $\text{mg L}^{-1}$  of  $\text{CdCl}_2$ . Values were represented as mean  $\pm$  SD ( $n=4$ ). Lower-case letters represent significant differences between treatments (LSD test,  $p < 0.05$ )

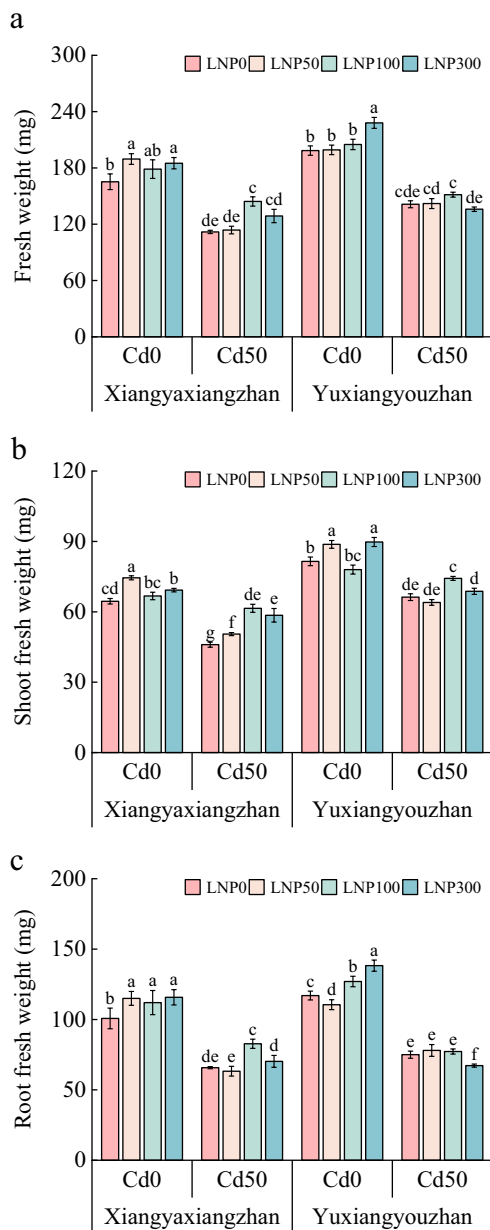


13.19% and 13.56% at LNP50 and LNP100, respectively, compared with LNP0, under Cd0. However, for Yuxiangyouzhan, nano-priming with  $\text{La}_2\text{O}_3$  NPs decreased the dry weight of shoot by 12.10%-19.01%, compared with non-priming (Fig. 5b). For Xiangyaxiangzhan, the root dry weight was significantly enhanced by 84.96% and 256.39% at the LNP100 and the LNP300, respectively, compared with the LNP0 under Cd0. For Yuxiangyouzhan, the root dry weight was significantly improved by 25.92% at LNP100 under Cd50. (Fig. 5c). Furthermore, for Xiangyaxiangzhan, the shoot / root ratio was significantly increased by 247.95% at LNP300 under Cd0 whereas LNP100 significantly improved the shoot / root ratio by 94.86% under Cd50, compared with LNP0 for Yuxiangyouzhan (Fig. 5d, Additional file: Table S4).

### 3.3 Physio-Biochemical Attributes

#### 3.3.1 Antioxidant Enzymes Activity

V, Cd, Cd  $\times$  V,  $\text{La}_2\text{O}_3$  NPs  $\times$  V, Cd  $\times$   $\text{La}_2\text{O}_3$  NPs and V  $\times$  Cd  $\times$   $\text{La}_2\text{O}_3$  NPs significantly affected the activity of SOD, POD, and CAT in shoots of rice seedlings (Table 1). The Cd100 treatment significantly reduced the SOD activity by 41.07% in Xiangyaxiangzhan compared with Cd0, whilst the SOD activity in shoots was significantly increased by 247.32% in Yuxiangyouzhan, compared with Cd0 (Additional file: Table). The  $\text{La}_2\text{O}_3$  NPs treatments significantly improved the SOD activity of the shoots for Xiangyaxiangzhan, but had no significant effect for Yuxiangyouzhan under Cd0. At Cd50, the SOD activity was significantly



**Fig. 3** Fresh weight of rice seedlings. Total fresh weight (a), shoot fresh weight (b), root fresh weight (c) of rice seedlings. LNP 50, LNP 50, LNP 100, and LNP 300: 0, 50, 100, and 300 mg L<sup>-1</sup> of La<sub>2</sub>O<sub>3</sub> NPs. Cd 0 and Cd 50: 0 and 50 mg L<sup>-1</sup> of CdCl<sub>2</sub>. were represented as mean ± SD (n=4). Lowercase letters represent significant differences between treatments (LSD test,  $p < 0.05$ )

increased by 48.75% and 21.31% at LNP50 and LNP100, respectively, compared to LNP0 (Fig. 6a). The POD activity was significantly enhanced by 78.86% under Cd50, compared with Cd0. On the other hand, the La<sub>2</sub>O<sub>3</sub> NPs significantly reduced the POD activity, and the effect was more pronounced in Xiangyaxiangzhan. On an average, the La<sub>2</sub>O<sub>3</sub> NPs significantly reduced the POD activity in Xiangyaxiangzhan by 16.95% under Cd50 (Fig. 6b). Under

Cd50, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs significantly reduced the CAT activity in Xiangyaxiangzhan and Yuxiangyouzhan in shoots by 33.28% and 13.03%, respectively, compared with non-primed seeds (Fig. 6c). However, under Cd100, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs significantly enhanced the CAT activity by 19.52% compared with non-primed seeds (Additional file: Table S5).

### 3.3.2 The MDA, Proline, and Soluble Protein Contents

Individual factors and their interaction i.e., Cd, Cd × V, La<sub>2</sub>O<sub>3</sub> NPs, La<sub>2</sub>O<sub>3</sub> NPs × V, Cd and V × La<sub>2</sub>O<sub>3</sub> NPs × Cd significantly affected the MDA content in shoots (Table 1). The Cd50 significantly enhanced the shoot MDA content by 16.49% for Xiangyaxiangzhan, but the MDA content was significantly decreased by 16.72% under the across Cd treatments for Yuxiangyouzhan, compared with Cd0 in non-primed seeds. For Xiangyaxiangzhan, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs significantly enhanced the MDA content. For example, the MDA content was significantly increased by 18.90% and 19.98% in LNP50 and LNP100, respectively, as compared with LNP0 under Cd0. In contrast, the MDA content was substantially reduced in Yuxiangyouzhan under La<sub>2</sub>O<sub>3</sub> NPs nano-priming. Overall, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs reduced the MDA content by 20.63% and 18.67%, for Xiangyaxiangzhan and Yuxiangyouzhan, respectively, compared with non-priming under Cd50 (Fig. 7a).

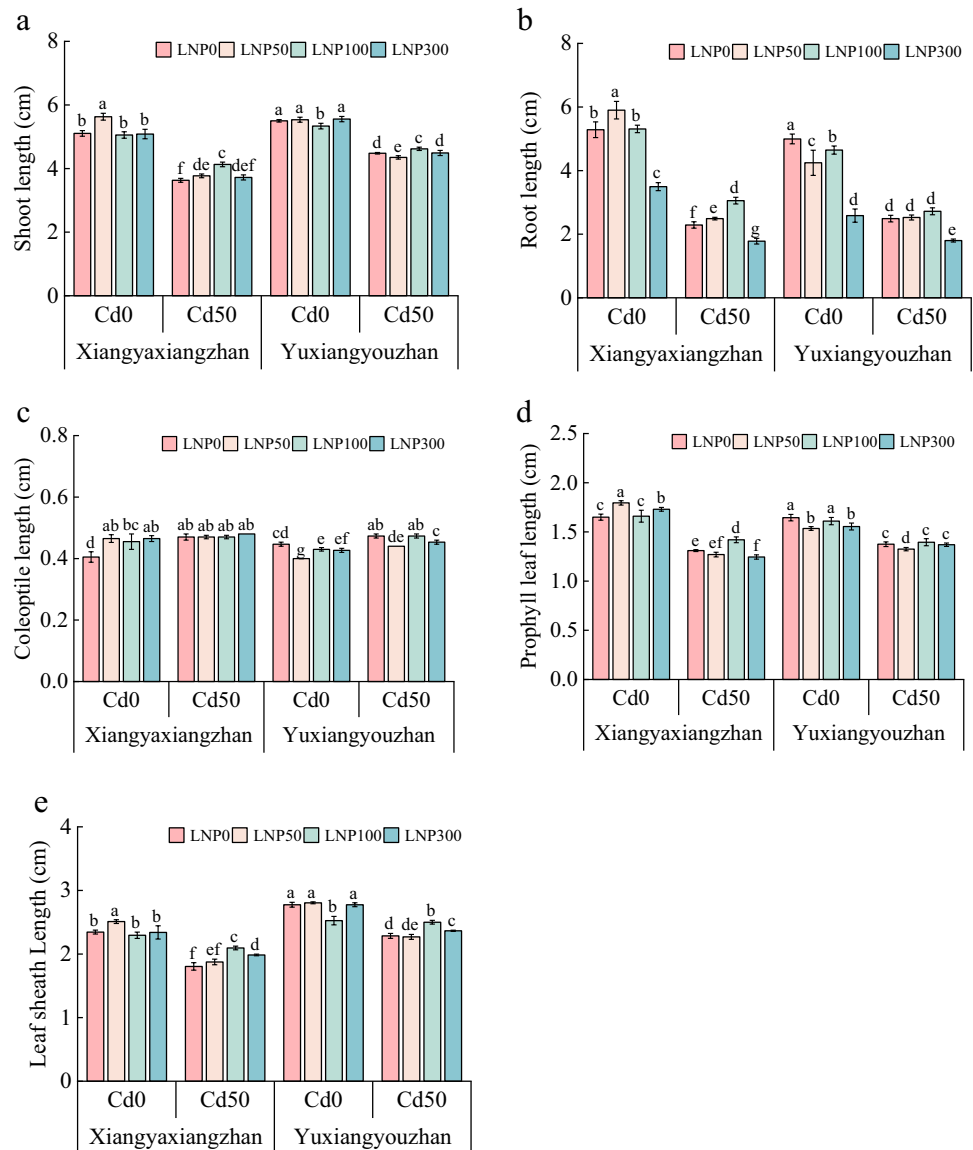
Cd, V, La<sub>2</sub>O<sub>3</sub> NPs, and their interaction affected the proline and protein content in shoots (Table 1). For Xiangyaxiangzhan, the proline content was significantly reduced by 16.40%, 41.68% and 11.19% at the LNP50, LNP100, and LNP300 treatment respectively, under Cd50. Under Cd100, the proline content was significantly reduced by 17.52%, 34.21% and 30.80% at the LNP50, the LNP100 and LNP300 respectively, compared with LNP0. For Yuxiangyouzhan the LNP300 significantly increased the proline content by 51.74% compared with LNP0 (Fig. 7b). Moreover, under the Cd50, the soluble protein content was significantly increased by 28.34% and 19.94% for Xiangyaxiangzhan and Yuxiangyouzhan, respectively, compared with Cd0. On the other hand, seed priming with La<sub>2</sub>O<sub>3</sub> NPs reduced the soluble protein content up to 28.85% and 19.74%, respectively under Cd50 (Fig. 7c) whilst the soluble protein content was enhanced by nano-priming under Cd100 for Yuxiangyouzhan (Additional file: Table S6).

### 3.3.3 The GSH and MT Content

Nano-priming with La<sub>2</sub>O<sub>3</sub> NPs and its interaction with Cd and V substantially affected the GSH and MT content in shoots (Table 1). The GSH content was increased by 62.23% and 142.09% at Cd 50 and Cd100 treatment, respectively, for Xiangyaxiangzhan and increased by 44.44% and 103.01%,



**Fig. 4** The length of each part of rice seedling. Shoot length (a), root length (b), coleoptile length (c), prophyll leaf length (d), leaf sheath length (e). LNP 0, LNP 50, LNP 100, and LNP 300: 0, 50, 100, and 300 mg L<sup>-1</sup> of La<sub>2</sub>O<sub>3</sub> NPs. Cd 0 and Cd 50: 0 and 50 mg L<sup>-1</sup> of CdCl<sub>2</sub>. were represented as mean ± SD (*n* = 4). Lowercase letters represent significant differences between treatments (LSD test,  $\rho < 0.05$ )

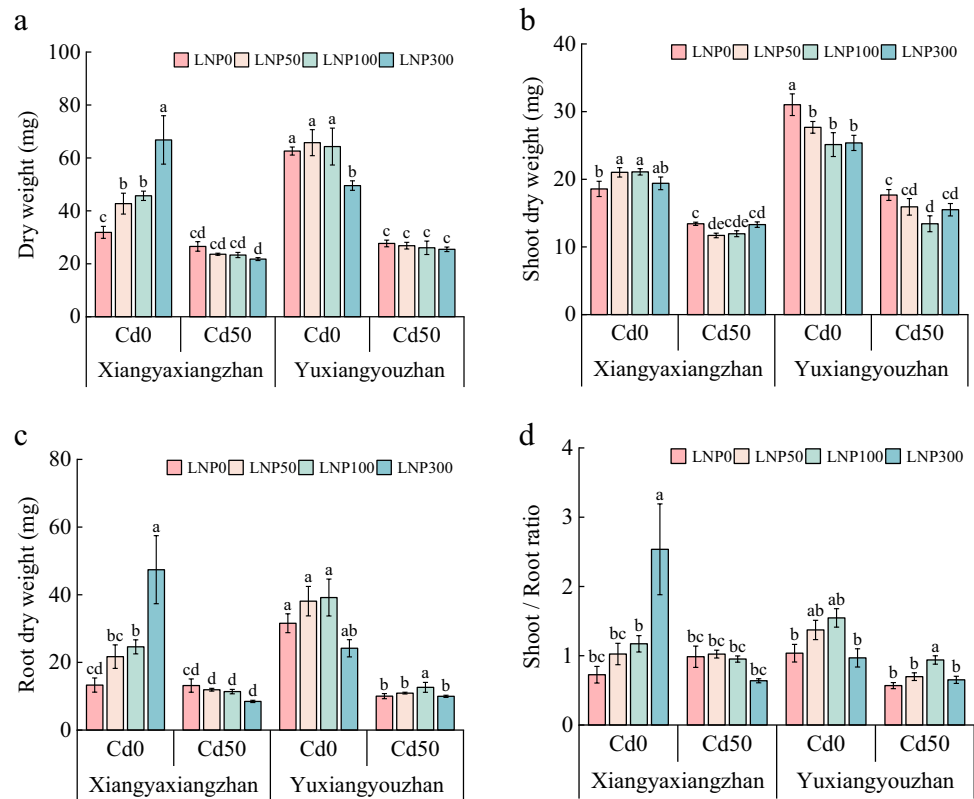


respectively, at the Cd50 and Cd100 treatment for Yuxiangyouzhan (Fig. 8a, Additional file: Table S7). Nano-priming with La<sub>2</sub>O<sub>3</sub> NPs significantly improved the GSH content under normal conditions whereas significantly decreased under Cd stress. For example, seed priming with LNP50 significantly reduced the GSH content by 25.04% and 26.94% compared with LNP0, for Xiangyaxiangzhan and Yuxiangyouzhan respectively, under Cd100. Furthermore, the Cd100 increased the MT content by 85.45% for Xiangyaxiangzhan whereas Cd treatments had no significant effect on Yuxiangyouzhan (Additional file: Table S7). Under Cd50, nano-priming with LNP50 reduced the MT content by 35.28% and 42.85% compared to non-priming treatment for Xiangyaxiangzhan and Yuxiangyouzhan, respectively (Fig. 8b).

### 3.3.4 Activity of $\alpha$ -Amylase Enzymes

The activity of  $\alpha$ -amylase of shoots was significantly affected by Cd, Cd  $\times$  V, La<sub>2</sub>O<sub>3</sub> NPs, La<sub>2</sub>O<sub>3</sub> NPs  $\times$  V, and V  $\times$  La<sub>2</sub>O<sub>3</sub> NPs  $\times$  Cd (Table 1). Cd treatment significantly enhanced the activity of  $\alpha$ -amylase. For instance, the Cd100 significantly increased the  $\alpha$ -amylase activity by 5.52% for Xiangyaxiangzhan and 7.28% for Yuxiangyouzhan compared with Cd0. Moreover, the La<sub>2</sub>O<sub>3</sub> NPs treatments enhanced  $\alpha$ -amylase activity at low concentrations of Cd, but at high concentrations of Cd, the nano-priming with La<sub>2</sub>O<sub>3</sub> NPs treatments showed an inhibitory effect on  $\alpha$ -amylase activity (Fig. 8c, Additional file: Table S7).

**Fig. 5** Dry weight of rice seedlings. Total dry weight (a), shoot dry weight (b), root dry weight (c), shoot to root ratio (d) of rice seedlings. LNP 50, LNP 50, LNP 100, and LNP 300: 0, 50, 100, and 300 mg L<sup>-1</sup> of La<sub>2</sub>O<sub>3</sub> NPs. Cd 0 and Cd 50: 0 and 50 mg L<sup>-1</sup> of CdCl<sub>2</sub>. Values were represented as mean ± SD (n = 4). Lowercase letters represent significant differences between treatments (LSD test,  $p < 0.05$ )



### 3.3.5 The Chlorophyll and Carotenoids Contents

The contents of chlorophyll and carotenoids was significantly affected by Cd, La<sub>2</sub>O<sub>3</sub> NPs, La<sub>2</sub>O<sub>3</sub> NPs × V, and V × La<sub>2</sub>O<sub>3</sub> NPs × Cd (Table 1). Under Cd stress, the chlorophyll a, chlorophyll b and total chlorophyll content was significantly decreased for both rice cultivars and the effects were more pronounced as the Cd concentration increased. However, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs significantly improved the chlorophyll a, chlorophyll b and total chlorophyll content. In contrast, for Yuxiangyouzhan under Cd stress, the nano-priming with La<sub>2</sub>O<sub>3</sub> NPs showed a significant inhibitory effect on the total chlorophyll a, chlorophyll b and chlorophyll content (Fig. 9a-c). Furthermore, the carotenoids content was significantly decreased for all La<sub>2</sub>O<sub>3</sub> NPs treatments with and without Cd stress for Yuxiangyouzhan. On contrary, for Xiangyaxiangzhan, the carotenoids content was increased for nano-priming with La<sub>2</sub>O<sub>3</sub> NPs under Cd stress (Fig. 9e, Additional file: Table S8).

### 3.4 Correlation Analysis

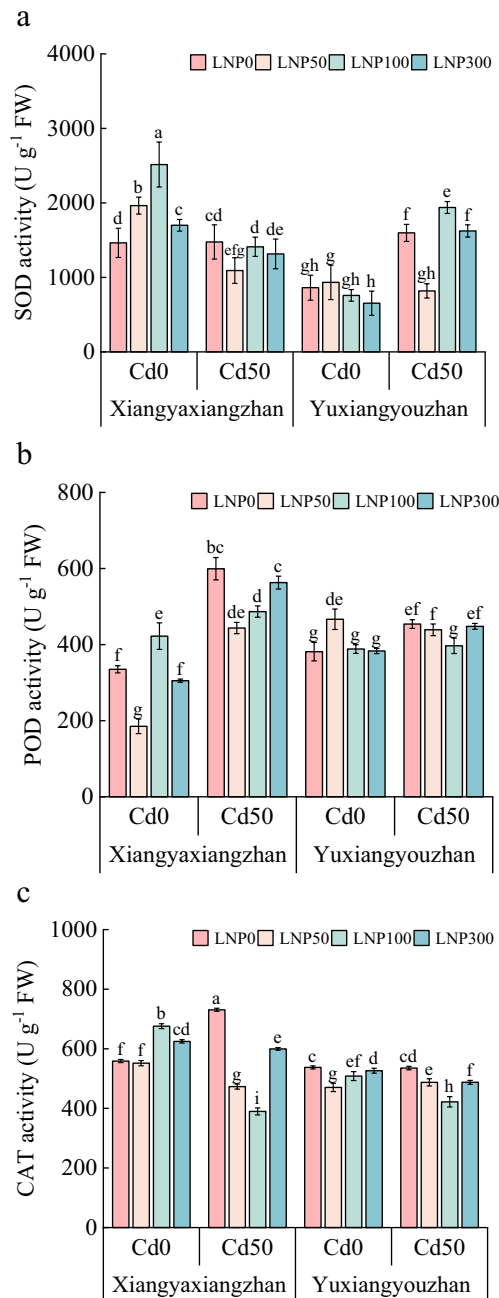
The germination rate had a significant positive correlation with the chlorophyll and MDA content. Moreover, the germination rate showed a significant and negative correlation with the SOD activity, α-amylase activity, as well as the soluble protein content. Additionally, the morphological growth

attributes, i.e., the shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, total fresh weight, the total dry weight, length of shoot and root, prophyll leaf length, and leaf sheath length were significantly and positively associated with the chlorophyll content but negatively associated with POD activity, proline content, and GSH content (Fig. 9).

## 4 Discussion

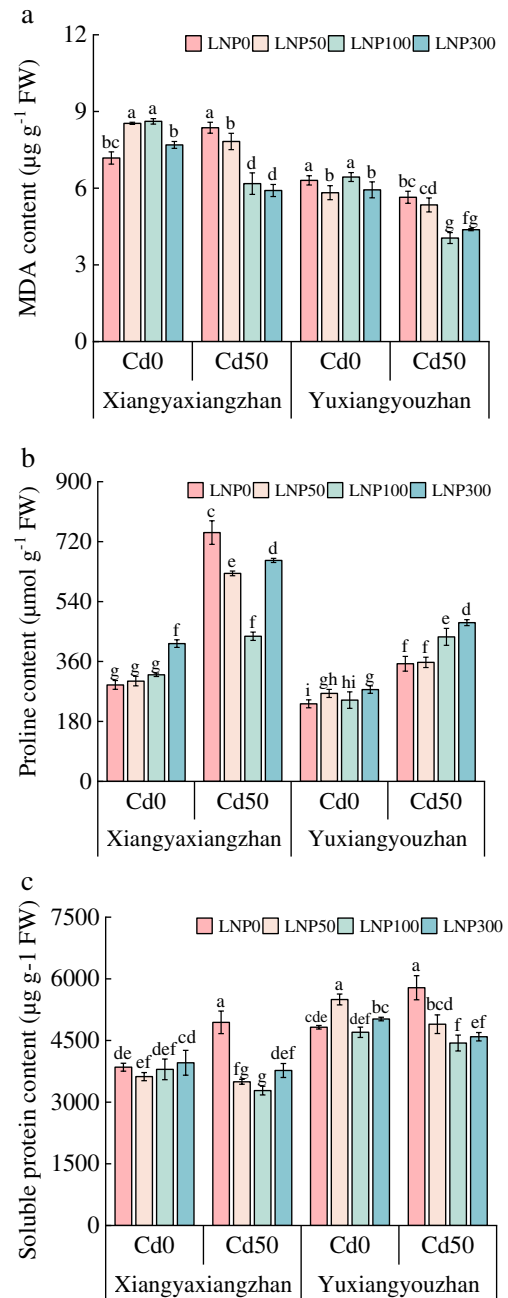
Previously, several reports have shown that the Cd stress could decrease the seed germination in plants of various species (Arezoo et al. 2015; Nabaei and Amooaghaie 2019; Kanu et al. 2019). In this study, Cd treatment showed a severe inhibitory effect on the seed germination rate and seed vigor index of two rice varieties (Fig. 2a-e). In accordance with the present results, previous studies have demonstrated that Cd toxicity significantly reduced the seed germination attributes in rice plants because the Cd stress severely impacts the seeds metabolic pathways such as alanine, aspartate, glutamate, phenylpropanoid, taurine and hypotaurine metabolism (Li et al. 2021b).

In the present study, the shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, total fresh weight, the total dry weight, length of shoot and root, prophyll leaf length, and leaf sheath length of rice seedlings were inhibited by Cd stress, and the inhibitory effects were concentration-dependent (Fig. 3,



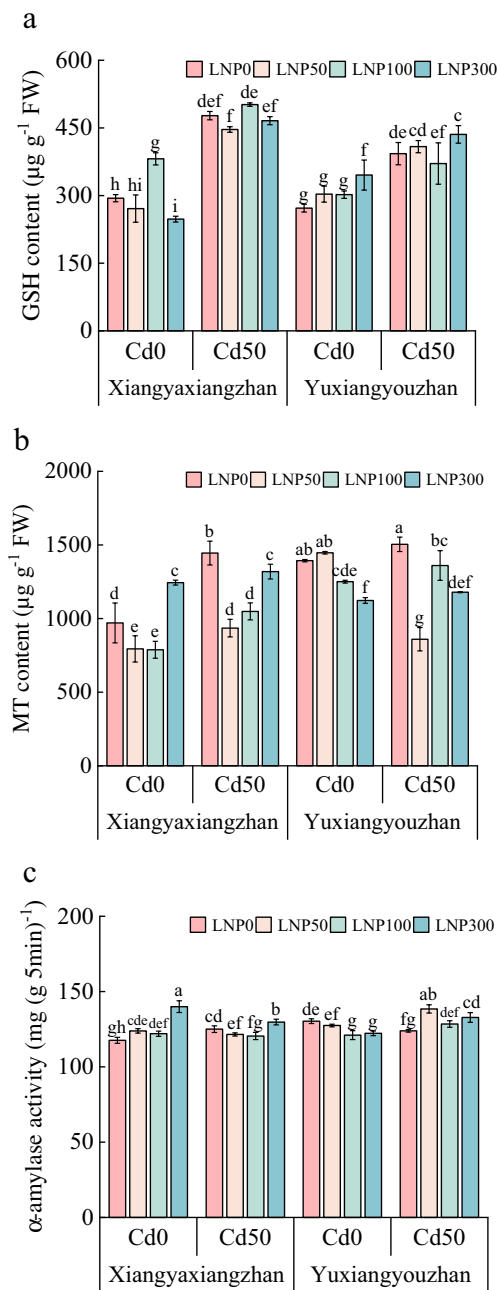
**Fig. 6** The antioxidant enzyme activities in shoots of rice seedlings. SOD activity (a), POD activity (b), CAT activity (c). LNP 50, LNP 100, and LNP 300: 0, 50, 100, and 300 mg L<sup>-1</sup> of La<sub>2</sub>O<sub>3</sub> NPs. Cd 0 and Cd 50: 0 and 50 mg L<sup>-1</sup> of CdCl<sub>2</sub>. Values were represented as mean ± SD (*n* = 4). Lowercase letters represent significant differences between treatments (LSD test, *p* < 0.05)

4 and 5). Our findings were consistent with the previous study. Cd toxicity can influence the morphological growth attributes, physiological responses and protein contents of rice (Ahsan et al. 2007). In this study, the roots were significantly more inhibited than shoots, probably due to the high accumulation of Cd ions in roots as a result of direct exposure to Cd (Fig. 4a, b). Results



**Fig. 7** The MDA content, proline content, and soluble protein content in shoots of rice seedlings. MDA content (a), proline content (b), soluble protein content (c) of rice seedlings. LNP 50, LNP 100, and LNP 300: 0, 50, 100, and 300 mg L<sup>-1</sup> of La<sub>2</sub>O<sub>3</sub> NPs. Cd 0 and Cd 50: 0 and 50 mg L<sup>-1</sup> of CdCl<sub>2</sub>. Values were represented as mean ± SD (*n* = 4). Lowercase letters represent significant differences between treatments (LSD test, *p* < 0.05)

also revealed that the Cd50 treatment significantly increased the coleoptile length (Fig. 4c). This may be a self-regulatory mechanism in rice under Cd stress, as the growth of the coleoptile helps to protect the germination and growth of the embryo and



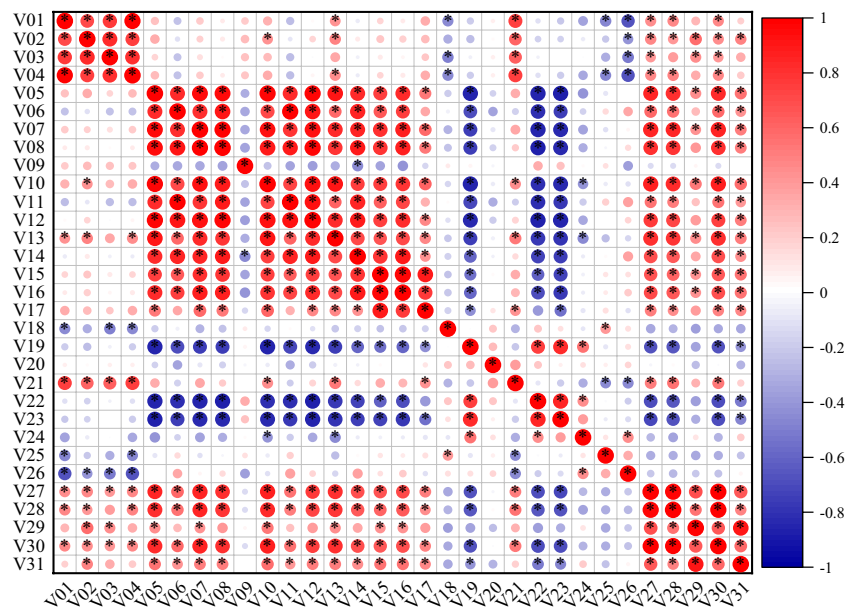
**Fig. 8** The GSH and MT content, and  $\alpha$ -amylase activity of rice seedling. GSH content (a), MT content (b),  $\alpha$ -amylase activity (c) of rice seedlings. LNP 50, LNP 50, LNP 100, and LNP 300: 0, 50, 100 and 300  $\text{mg L}^{-1}$  of  $\text{La}_2\text{O}_3$  NPs. Cd 0 and Cd 50: 0 and 50  $\text{mg L}^{-1}$  of  $\text{CdCl}_2$ . Values were represented as mean  $\pm$  SD ( $n=4$ ). Lowercase letters represent significant differences between treatments (LSD test,  $p < 0.05$ )

improve the plant's resistance to stress (Morgan 1988). At the same time, the plant roots secrete large amounts of cytokinin and transport to the above-ground parts of the plant, promoting the elongation of the coleoptile and inhibiting the growth of the roots, thus enhancing the plant's ability to transport nutrients

(Munzuroglu and Zengin 2006). This may also be one of the mechanisms by which the rice responds to Cd stress.

Previous research has reported the inhibition of shoot and root growth, and biomass of rice seedlings caused by Cd (Ahsan et al. 2007; He et al. 2008), whereas nano-priming with  $\text{La}_2\text{O}_3$  NPs on rice growth remains controversial.  $\text{La}_2\text{O}_3$  NPs can be adsorbed to the plant surface and enter the plant as lanthanum ions through natural nano-channels or micro-scale channels (Ma et al. 2011). Lanthanum can alleviate cadmium stress in plants such as wheat and cucumber (Yang et al. 2019). Here, nano-priming with  $\text{La}_2\text{O}_3$  NPs (at lower concentrations) alleviated the Cd stress on rice early growth by enhancing the morphological growth attributes, i.e., shoot and root length, prophyll leaf length, leaf sheath length, notably in the fresh weight (Fig. 3). Moreover, the LNP50 and LNP100 treatments significantly improved the growth of rice seedling. Our findings were consistent with previous study which reported that low concentration of  $\text{La}_2\text{O}_3$  NPs could enhance the root biomass of radish (Xiao et al. 2021). This may be due to lanthanum can mitigate cadmium-induced oxidative damage by regulating the metabolism of ascorbic acid and glutathione, thereby improving cadmium tolerance in plants (Dai et al. 2016). The positive effect of lanthanum with low concentrations were noticed, however, treatment with high concentrations had a detrimental impact on the development of the early stages of rice (Si et al. 2018). Previous research has also demonstrated the phytotoxicity of  $\text{La}_2\text{O}_3$  NPs (Yue et al. 2017; Ma et al. 2011). In this study, the LNP300 treatment showed a negative impact on the root growth (Fig. 4b).  $\text{La}_2\text{O}_3$  NPs can regulate genes related to cell wall and lignin synthesis, affecting the development of the cell wall and causing embolization and lignification of plant root cells and forming apoplastic barriers of root (Yue et al. 2019). This may explain the inhibition of root length under LNP300 treatment in this study. However, the formation of root apoplastic barriers may also improve the resistance to stress and reduce the uptake of harmful ions through the roots in plants. Therefore, application of  $\text{La}_2\text{O}_3$  NPs at appropriate concentrations would be capable of improving the early growth of rice, while higher concentrations could result in inhibition. In summary, LNP100 treatment has a better growth promotion effect on Xiangyaxiangzhan under Cd stress.

Machinery for ROS scavenging in plants is important and necessary (Noman and Aqeel 2017). Cd impedes plant antioxidative systems as a result of enhanced ROS and MDA accumulation (Mostofa et al. 2019; Hayat et al. 2021). Typically, SOD consumes  $\text{O}_2^-$  through reduction reactions to produce  $\text{H}_2\text{O}_2$  and  $\text{O}_2$ , while the  $\text{H}_2\text{O}_2$  produced by the photorespiration and the  $\beta$ -oxidation of fatty acids are consumed by CAT (Shah et al. 2001; Lin and Kao 2000). POD is found in the extracellular space, cell walls, cell membranes and vesicles and utilizes guaiacol as an electron donor, while using  $\text{H}_2\text{O}_2$  to oxidize a variety of inorganic and organic substrates in ROS scavenging system (Li et al. 2019). In this study, Cd toxicity enhanced the SOD,



**Fig. 9** Correlation analysis between the investigated parameters. V01, Germination rate at 1 DAS; V02, Germination rate at 3 DAS; V03, Germination rate at 5 DAS; V04, Germination index; V05, Seed vigor index; V06, Shoot fresh weight; V07, Root fresh weight; V08, Total fresh weight; V09, Coleoptile length; V10, First leaf length; V11, Leaf sheath length; V12, Shoot length; V13, Root length; V14, Shoot dry weight; V15, Root dry weight; V16, Total dry weight; V17,

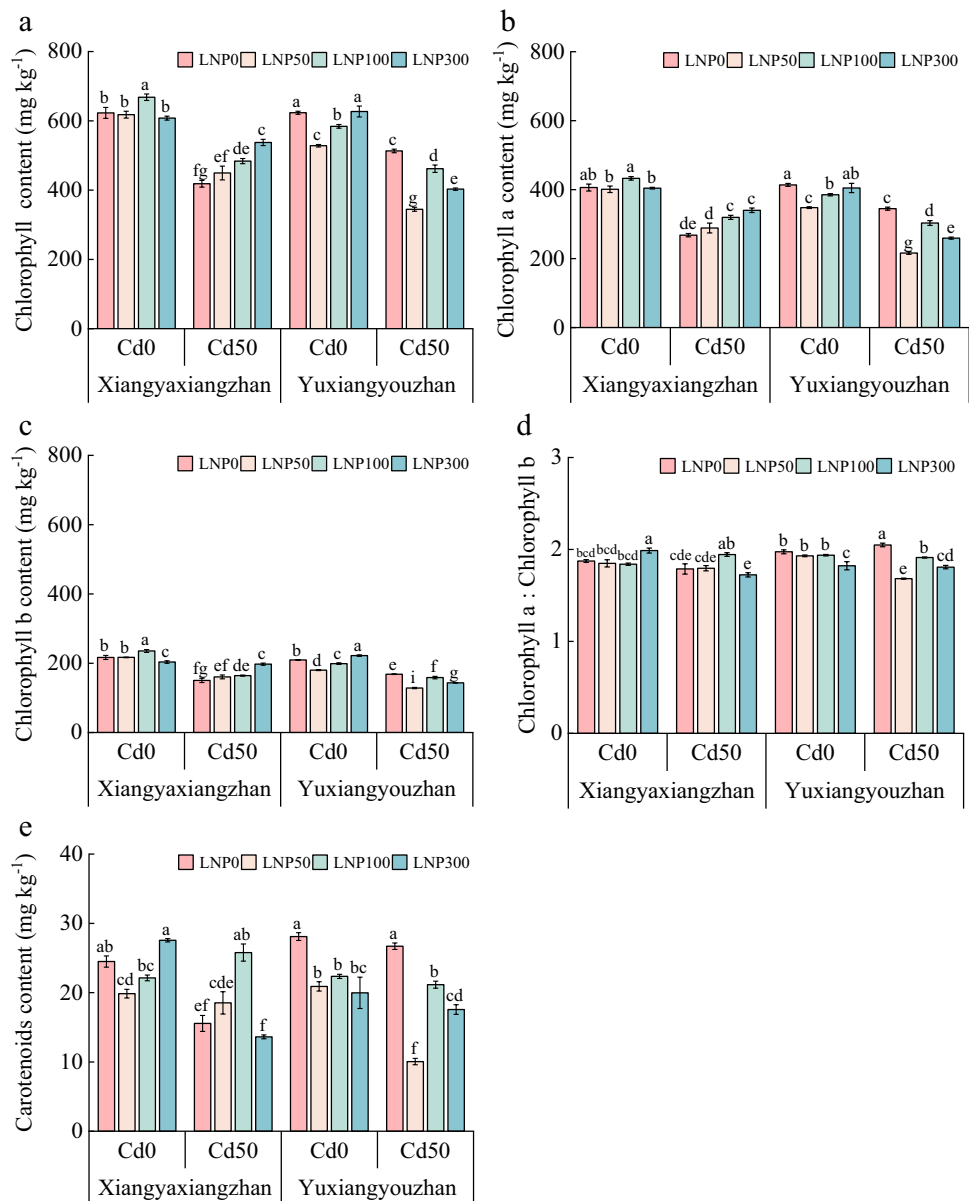
Root/shoot ratio; V18, SOD activity; V19, POD activity; V20, CAT activity; V21, MDA content; V22, Proline content; V23, GSH content; V24, MT content; V25,  $\alpha$ -amylase activity; V26, Soluble protein content; V27, Chlorophyll a content; V28, Chlorophyll b content; V29, Carotenoids content; V30, Chlorophyll content; V31, Chlorophyll a:chlorophyll b

POD and CAT activity in rice seedlings, indicating that Cd can cause oxidative stress and induce membrane lipid peroxidation which is in agreement with previous studies (Qing et al. 2022). Here, nano-priming with  $\text{La}_2\text{O}_3$  NPs reduced the SOD, POD, and CAT activity under Cd stress (Fig. 6a-c). Correlation analysis also revealed that activity of POD showed a negative correlation significantly with the morphological growth attributes e.g., fresh weight, length, dry weight, confirming that  $\text{La}_2\text{O}_3$  NPs alleviated oxidative stress and improved the seedling growth under Cd toxicity. These results revealed that the promotion effect of  $\text{La}_2\text{O}_3$  NPs on the development of rice seedlings under Cd toxicity was related to the alleviation of Cd-induced oxidative stress, which can reduce the ROS production and thus reduce the activities of antioxidant enzymes. A possible explanation for this might be La and Cd probably share the same binding sites in calmodulin. By interacting with calmodulin, La can down-regulate the expression of *TaNramp5* and affect Cd uptake in plant (Yang et al. 2021). It is also reported that  $\text{La}^{3+}$  has a higher charge than  $\text{Cd}^{2+}$ , the greater electropositivity allows it to compete with Cd easily for adsorption in plant tissues (Yang et al. 2019). Therefore, it seems possible that the alleviation of Cd-induced oxidative stress in this study are due to the  $\text{La}_2\text{O}_3$  NPs application negatively affected the Cd accumulation in rice seedlings.

In addition to antioxidants, the content of MDA is a fundamental indicator of oxidative stress, which confers properties

on inter- and intracellular membranes and therefore leads to increased ionic permeability of the cell membrane (Kong et al. 2017). GSH relieves heavy metals in plants and it can function as antioxidants in plant cells (Zhang et al. 2013). The MT is the most effective bioactive substances to scavenge ROS (Cai et al. 2019) whereas the proline and soluble proteins are osmoregulatory substances in plants that prevent disrupting the cell structure and function under heavy metal stress conditions (Ashraf et al. 2017; Ashraf and Tang 2017). In present study, Cd toxicity significantly enhanced the content of MDA, GSH, MT, proline, as well as soluble proteins in the rice seedlings (Fig. 7, 8), indicating that Cd can cause oxidative stress, quite in line with the previous studies of Cd stress in rice (Ahsan et al. 2007; Li et al. 2021b; Qing et al. 2022). Meanwhile, substantial reductions in MDA, GSH, MT, proline, and soluble proteins content were noticed in  $\text{La}_2\text{O}_3$  NPs treatments under Cd stress, indicating that nano-priming with  $\text{La}_2\text{O}_3$  NPs could alleviate the heavy metal stress in rice. A significant negative correlation was found between rice morphological indices and proline contents (Fig. 9) which confirms that  $\text{La}_2\text{O}_3$  NPs can alleviate oxidative and osmotic stress caused by Cd stress and promote rice morphological growth. This also accords with earlier study, which showed that lanthanum decreased the MDA and proline so as to maintain normal plasmolemma permeability (Yan et al. 2007, 2007, 2007). On the contrary, high concentrations of  $\text{La}_2\text{O}_3$  NPs significantly increased the content of MDA, proline, GSH,

**Fig. 10** The chlorophyll and carotenoids content in rice seedling. Total chlorophyll content (a), chlorophyll a content (b), chlorophyll b content (c), chlorophyll a: chlorophyll b content (d), carotenoids content (e). LNP 50, LNP 100, and LNP 300: 0, 50, 100, and 300 mg L<sup>-1</sup> of La<sub>2</sub>O<sub>3</sub> NPs. Cd 0 and Cd 50: 0 and 50 mg L<sup>-1</sup> of CdCl<sub>2</sub>. Values were represented as mean ± SD (*n* = 4). Lowercase letters represent significant differences between treatments (LSD test, *P* < 0.05)



and MT, which indicating that the La<sub>2</sub>O<sub>3</sub> NPs may also cause phytotoxicity, especially at higher concentrations (Fig. 7a, b, Fig. 8a, b). Therefore, optimization of the La<sub>2</sub>O<sub>3</sub> NPs is prerequisite before its practice on large scale.

Starch is an essential energy-storing carbohydrate that participates in the growth of rice seedlings. Previous research has established that by mobilizing resources in the endosperm,  $\alpha$ -amylases stimulates seed germination and seedling growth, while starch is immobilized under Cd stress, leading to growth suppression. (Seneviratne et al. 2019). In this study, nano-priming with La<sub>2</sub>O<sub>3</sub> NPs substantially improved the  $\alpha$ -amylase activity (Fig. 8c) with and without Cd stress, which could be beneficial to the metabolism of stored substances hence improving the early growth of rice.

Chlorophyll is the main pigment for photosynthesis in plants, converting light energy into chemical energy and regulating a variety of biochemical processes in plants (Sardar et al. 2022). Cd toxicity could inactivate the chlorophyll-generation-related enzymes. It has previously been observed that Cd toxicity resulted in substantial reduction in chlorophyll content and stomatal conductance in plants (Chaudhary and Sharma 2009; Kilic et al. 2017). In this study, it was found that Cd could lead to an inhibitory effect of chlorophyll and carotenoids contents (Fig. 10). Previously, application of NPs such as ZnO NPs was found to affect the chlorophyll content and carotenoids content of rice (Li et al. 2021a). Here, it was noticed that application of La<sub>2</sub>O<sub>3</sub> NPs could significantly increase the chlorophyll content for Xiangyaxiangzhan (Fig. 10a). These results corroborate the

findings that La can lead to the chlorophyll protein complexes increasing which results in the improvement of chlorophyll content and the absorption of photoelectron as well as energy transport in the process of photosynthesis (Zeng et al. 2000). It is also reported that La<sub>2</sub>O<sub>3</sub> NPs can significantly regulate the light utilization and electron transport in photosynthesis (Liu et al. 2020). In summary, we deduced that the application of La<sub>2</sub>O<sub>3</sub> NPs can enhance the photosynthesis of rice seedling with and without Cd toxicity.

## 5 Conclusion

In summary, early growth of rice seedlings was inhibited under Cd toxicity. Seed priming with La<sub>2</sub>O<sub>3</sub> NPs substantially enhanced the early growth of rice seedlings by improving morphological attributes and modulating the physiological and biochemical responses under Cd toxicity. Comparatively, the LNP100 treatment is the most effective in mitigating Cd toxicity in rice. No doubt, nano-priming of rice seeds with La<sub>2</sub>O<sub>3</sub> NPs improved the early growth of rice under Cd toxic conditions, nevertheless, the precise mechanisms of La<sub>2</sub>O<sub>3</sub> NPs in regulating and improving early growth of rice under Cd stress remain to be elucidated. The insights gained from this study may be of assistance to understand the effects of La<sub>2</sub>O<sub>3</sub> NPs on early growth of rice, and its potential applications in enhancing the Cd tolerance of crops.

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**Authors' contributions** Feiyang Sun, Weifen Chen, and Yong Ren: Investigation, Data Curation, Formal analysis, Visualization, and Writing Original Draft;

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**Data Availability** The data sets supporting the results of this article are included within the article.

**Code Availability** No code.

## Declarations

**Ethics Approval** Not applicable.

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