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RESEARCH ARTICLE

Foliar spray of La_2O_3 nanoparticles regulates the growth, antioxidant parameters, and nitrogen metabolism of fragrant rice seedlings in wet and dry nurseries

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Abstract

Nanoparticles (NPs) have been widely used in agriculture, and lanthanum oxide nanoparticles (La_2O_3) NPs can regulate plant growth. La_2O_3 NPs treatment was hypothesized to affect the accumulation and distribution of substances in rice seedlings under wet and dry nursery conditions. The objective of the present study was to ascertain the effects of La_2O_3 NPs foliar spray on the morphology and physiology of fragrant rice seedlings under wet and dry nursery conditions. Seedlings of two fragrant rice cultivars, namely 'Xiangyaxiangzhan' and 'Yuxiangyouzhan,' were grown under wet and dry nursery conditions with La_2O_3 NPs treatments at three concentrations (CK, La_2O_3 NPs 0 mg L^{-1}; T1, La_2O_3 NPs 20 mg L^{-1}; and T2, La_2O_3 NPs 40 mg L^{-1}). The results showed that the seedling-raising method was significantly associated with La_2O_3 NPs application ($P < 0.05$), affecting the leaf area of both cultivars. Changes in plant morphological parameters, such as dry weight and root–shoot ratio, were the reasons for the differences in cultivars in response to La_2O_3 NPs application. Changes were also observed in the plant morphological and physiological parameters of leaf area, specific leaf area, chlorophyll contents, antioxidant properties, and activities of nitrogen metabolism enzymes. The relationship between morphological and physiological processes in fragrant rice was investigated to test the hypothesis. In both wet and dry nursery methods, the T2 concentration of La_2O_3 NPs was beneficial for rice seedlings and significantly increased their leaf area due to changes in morphological and physiological parameters. Therefore, the results of this study provide a theoretical basis for expanding the research on La_2O_3 NPs application in rice, as well as relevant references for strengthening rice seedlings in the nursery, which has a positive effect on the grain yield improvement in fragrant rice.

Keywords Fragrant rice · Leaf area · Physiological parameters · Photosynthetic pigment · Rare earth oxide nanoparticles

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Abbreviations

WN	Wet nursery
DN	Dry nursery
CK	La_2O_3 NPs concentration of 0 mg L^{-1}
T1	La_2O_3 NPs concentration of 20 mg L^{-1}
T2	La_2O_3 NPs concentration of 40 mg L^{-1}

Introduction

Rice, a globally important nutritious crop, heavily relies on proper seedling development for its production (Qing et al. 2022). China, where fragrant rice is prized for its delectable flavor, produces approximately 30% of rice in the world (Deng et al. 2022). Mechanical transplanting is popular in rice production because it is a simplified cultivation technique with low labor costs (Dou et al. 2021). In mechanical transplanting, different seedling-raising methods greatly influence seedling growth and rice yield (Cheng et al. 2018).

Nano-fertilizer application is a brand-new approach to crop management. Nanoparticles (NPs) are frequently used in agriculture to increase crop growth and productivity (Li et al. 2021). Valojai et al. (2021) demonstrated that nano-fertilizer application improved the yield of milled rice grains. Ahanger et al. (2021) also showed that using nano-vermicompost as organic fertilizer promoted plant growth. Therefore, NPs application during seedling raising can be used to improve rice growth and production.

Technical requirements for rice seedling establishment are crucial when mechanically transplanting rice seedlings (Dou et al. 2021). The establishment of strong and uniform rice seedlings is a prerequisite and key for the consistent development of field transplants to achieve a high rice yield (Li et al. 2020). The water level is undeniably essential to rice seedling quality (Lampayan et al. 2015). High-quality rice mechanical transplanting in paddy fields depends on the high quality of rice seedlings raised using an optimized seedling-raising method (Zhang and Gong 2014). The dry nursery (DN) method had higher seedling establishment rates than the wet nursery (WN) method (Cheng et al. 2018). As both are common rice-raising methods, comparative studies on fragrant rice seedlings grown using these nursery methods may draw more attention to the mechanized production of fragrant rice.

NPs show promise in agriculture, especially in the optimal use of chemical fertilizers and pesticides. Typically, at least one of their dimensions is smaller than 100 nm (Chen et al. 2021). The use of NPs aids in our understanding of plant mechanisms against abiotic stress (Mohapatra et al. 2023). Rare earth oxide (REO) NPs are emerging materials with unique properties (Xu and Qu 2014), which have led to their increasing use in optics, agronomic screening, thermoelectric materials, and so forth (Kumar and Seth 2021). Lanthanum oxide (La_2O_3) NPs are REO NPs famed for their strong interaction, significant specific surface area, and quick absorption (Yue et al. 2017). Because of extensive use, La_2O_3 NPs are inevitably released into agricultural soils (Garner et al. 2017). This may result in environmental damage and a serious threat to the health of living organisms (Liu et al. 2020a). Researchers in the scientific community are divided on whether NPs are beneficial or detrimental to crops. For example, Liu et al. (2020a) demonstrated a decrease in the biomass of maize and soybean exposed to 50 mg L^{-1} La_2O_3 NPs. In contrast, Zhang et al. (2022) showed that Mo_3 NPs promoted plant growth and N_3^- utilization in rice. Xiao et al. (2022) found that La_2O_3 NPs exposure significantly increased leaf sucrose content and plant biomass in radish. A recent study confirmed that La_2O_3 NPs affected plant growth and physiological metabolism (Liu et al. 2020a). La_2O_3 NPs may change plant growth and maturation. However, the effects of La_2O_3 NPs application on fragrant rice seedlings cultivated using WN and DN methods remain unclear.

Different nursery methods are critical to the quality of rice seedlings, and the application of La_2O_3 NPs used as nano-fertilizer promotes the growth of fragrant rice seedlings. However, the effects of both seedling raising methods and La_2O_3 NPs concentration applied to rice seedlings are not known, so the aim of this study was to assess the effects of La_2O_3 NPs foliar spray on the morphological and physiological characteristics of fragrant rice seedlings using WN and DN methods. La_2O_3 NPs treatment was hypothesized to affect the accumulation and distribution of organic substances in rice seedlings in both nursery methods. As there could be plant physiological changes, this study also evaluated the biomass, growth, antioxidant properties, and nitrogen metabolism of fragrant rice seedlings under La_2O_3 NPs application using the two seedling-raising methods. Furthermore, the relationship between morphological and physiological parameters of fragrant rice was investigated to test the hypothesis.

Materials and methods

Experimental description

The experiments were performed in September 2021 at the South China Agricultural University (SCAU), Guangzhou, Guangdong Province, China. Fragrant rice cultivars 'Xiangyaxiangzhan' and 'Yuxiangyouzhan,' both of which are popular in South China, were used in this study. 'Xiangyaxiangzhan' is a conventional rice variety, whereas 'Yuxiangyouzhan' is a super rice variety with great potential for high productivity and wide adaptability (Liu et al. 2020b). Seeds of both cultivars were obtained from the College of Agriculture, SCAU. La_2O_3 NPs that were 99.99% pure and of 50 nm particle size were obtained from Macklin Biochemical Co., Ltd., Shanghai, China.

Experimental treatments

The experimental design was completely randomized. Approximately 500 g seeds of each cultivar was used. Under room temperature, the seeds were disinfected with 75% alcohol for 10 min, followed by three washes with sterile water, and then soaked in distilled water for 24 h. Two approaches were used to sow and raise seeds, i.e., WN and DN (Cheng et al. 2018). Until the three-leaf stage, rice seedlings were treated with three La_2O_3 NPs concentrations of 0, 20, and 40 mg L^{-1}. La_2O_3 NPs solutions were agitated for 5 min before being subjected to ultrasonic treatment (HZS-H, China) (25 °C, 800 W, 40 kHz) for 30 min to facilitate their dispersion (Li et al. 2021). Each treatment solution was prepared just before use. There were 12 treatments, with each treatment repeated four times. During the experiment, the average temperature was 24.9 °C, the average humidity was 71.8%, the average sunshine hours were 173.9 h, and the average light intensity was 263.7 μmolm-^{2} s-1.

Measurement indicators and methods

Determination of biomass and morphological traits

Three days after treatment, 40 randomly selected rice seedlings were collected from each treatment, i.e., four replicates for each treatment and ten rice seedlings for each replicate. Leaf sheath length, plant height, and leaf area of these seedlings were determined with a digimatic caliper. An electronic balance was used to measure the fresh and dry biomass. Dry biomass was obtained by drying the fresh biomass in an oven at 60 °C for 3 days until constant weight. Formulae based on Awan et al. (2014) were used to determine the shoot dry weight to plant height ratio, root–shoot ratio, specific leaf area, and dry weight ratio of distinct plant tissues.

Determination of chlorophyll contents

Fresh leaf samples (0.1 g) were extracted with 7.5 mL of 95% ethanol and placed in a dark room to determine their chlorophyll levels. After 24 h, samples were centrifuged at 5000 rpm for 5 min. Supernatant chlorophyll and carotenoid contents were evaluated by sequentially measuring the absorbance at 665, 649, and 470 nm (Li et al. 2021).

Determination of physiological characteristics

The plant samples were collected 3 days after treatment, divided into stems, leaves, and roots, flash-frozen in liquid nitrogen for 5 min, and stored at – 80 °C for biochemical analysis.

Determination of antioxidant enzyme activities and malondialdehyde (MDA) content Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities, as well as MDA content, were measured as previously reported (Li et al. 2021). Spectrophotometer UV-2550 (Shimadzu Corporation, Kyoto, Japan) was used to measure the absorbance. Fresh samples were homogenized (0.15 g) with 1.5 mL of 0.05 mol L^{-1} phosphate-buffered saline (PBS) solution in an ice bath and then centrifuged at 14,000 rpm for 15 min at 4 °C, and the supernatant was used to determine the parameters. Each treatment was repeated three times for physiological measurements.

SOD activity was measured as follows. Exactly 5 μL of the crude enzyme solution was added to a reaction system, which consisted of 150 μL of 50 mM sodium phosphate buffer, 30 μL of 130 mM methionine, 30 μL of 750 μM nitrotetrazolium blue chloride (NBT), and 30 μL of 100 μM ethylenediaminetetraacetic acid disodium salt. After mixing them, 30 μL of 20 μM riboflavin was added, the light was set to 4000 lx, and the mixture was allowed to react for 20 min. 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 measured in units of U gFW^{-1}.

POD activity was measured as follows. Exactly 100 μL of PBS (pH 7.0) and 95 μL of 0.2% guaiacol were mixed, 5 μL of crude enzyme solution and 100 μL of 0.3% hydrogen peroxide solution were added. To detect absorbance at 470 nm, the data were recorded every 30 s for 2.5 min. The absorbance increase of one unit per minute (U gFW^{-1}) was chosen as the standard unit of POD activity.

CAT activity was measured as follows. First, 1.95 mL of ultrapure water and 1 mL of 0.3% hydrogen peroxide solution were added to the reaction system. Then, 0.05 mL of the crude enzyme solution was added, and the absorbance of the reaction system was measured at 240 nm for 2 min every 30 s. The walls of the quartz cuvette prevent light absorption in the ultraviolet region. CAT activity was calculated as U gFW^{-1}, and one CAT activity unit (U) was the quantity of enzyme that catalyzed the reaction to cause a decrease in 0.01 absorbance per minute.

MDA content was determined by reacting 0.4 mL of 0.5% thiobarbituric acid solution and 0.2 mL of crude enzyme solution in a boiling water bath for 30 min and measuring the absorbance of the supernatant at 532, 600, and 450 nm. It was expressed as μmol gFW^{-1}.

Determination of nitrogen metabolism enzyme activities The method developed by Liao et al. (2022) was used to measure nitrate reductase (NR) activity in rice seedlings. Fresh samples (0.1 g) were homogenized in 2 mL of ice-cold PBS before being centrifuged at 4000 rpm and 4 °C for 15 min. The original enzyme extract was collected in the supernatant. Absorbance reduction was used to define one NR activity unit (U), which was then quantified as U gFW^{-1}.

Glutamine synthetase (GS) and glutamate synthase (GOGAT) activities were calculated using the methods of Qing et al. (2022). Extraction buffer was used to homogenize fresh samples (0.2 g), and the samples were then centrifuged at 13,000 rpm for 25 min at 4 °C. GS and GOGAT activities were measured using the supernatants. The absorbance for GS was measured at 540 nm using the spectrophotometer. GS activity was calculated as U gFW^{-1}, and one GS activity unit (U) was the amount of the enzyme required to catalyze the formation of 1 μmol of γ-glutamyl isohydroxamic acid in 15 min of reaction time. Total activity was the number of μmol of γ-glutamyl isohydroxamic acid catalyzed per gram of fresh crude enzyme in 15 min of reaction time. To determine GOGAT activity, the absorbance at 340 nm was measured every 30 s for 11 consecutive times. U gFW^{-1} is the unit of measure for the absorbance reduction in one micromole of NADH per min.

Statistical analyses

Statistix v8 (Analytical Software, Tallahassee, FL, USA) was used for data analysis, analysis of variance (ANOVA), and correlation analysis, and the least significant difference (LSD) test was used to compare sample means at the 0.05 level of significance.

Correlations between variables were analyzed using the Pearson correlation coefficient. Correlations with an absolute value of less than 0.3 were considered weak, those between 0.3 and 0.7 were considered moderate, and those greater than 0.7 were considered strong. Significance was set at $P < 0.05$.

Results

Biomass

Method (*M*) and $M \times \mathrm{La_2O_3}$ NPs (*La*) significantly ($P < 0.05$) impacted dry weight. Statistically significant differences ($P < 0.05$) were observed in the dry weight of stem, leaf, shoot, and total plant after $\mathrm{La_2O_3}$ NPs application. Under T1 treatment, DN increased the root dry weight of ‘Xiangyaxiangzhan’ and ‘Yuxiangyouzhan’ by 18.65% and 31.88%, respectively, compared with WN. Under T2 treatment, WN increased the stem dry weight of ‘Xiangyaxiangzhan’ and ‘Yuxiangyouzhan’ by 24.58% and 20.77%, respectively, compared with DN. Moreover, T1 and T2 treatments decreased the shoot dry weight of ‘Yuxiangyouzhan’ by 11.49% and 7.99%, respectively, compared with CK (Table 1). Statistically significant differences ($P < 0.05$) were observed in the fresh weight of stem, leaf, shoot, and total plant for *M* and *La*. WN increased the root fresh weight of ‘Xiangyaxiangzhan’ by 20.47% under T2 treatment, compared with DN. It also increased the stem fresh weight of ‘Yuxiangyouzhan’ by 34.88%, 64.28%, and 55.15% under CK, T1, and T2 treatments, respectively, compared with DN. Nonetheless, on average, compared with CK, T1 treatment decreased the leaf fresh weight of ‘Yuxiangyouzhan’ by 7.96% (Table 2).

Table 1 Effects of foliar spray of $\mathrm{La_2O_3}$ nanoparticles on the dry weight of fragrant rice seedlings in wet and dry nurseries

Treatment		Root dry weight (mg)	Stem dry weight (mg)	Leaf dry weight (mg)	Shoot dry weight (mg)	Total dry weight (mg)
‘Xiangyaxiangzhan’						
CK	WN	18.95±1.13abcde	43.63±3.15bcd	62.50±3.19abc	106.13±5.74ab	125.08±6.87abcd
	DN	19.68±1.55abcd	40.05±1.77de	54.85±4.48 cd	94.90±6.14c	114.58±6.10de
	Mean	19.31A	41.84A	58.68AB	100.51AB	119.83A
T1	WN	18.10±1.15cde	38.60±3.15de	52.28±3.87d	90.88±6.79c	108.98±6.57e
	DN	21.48±0.48ab	39.23±0.86de	53.55±1.28d	92.78±1.63c	114.25±1.88de
	Mean	19.79A	38.91A	52.91B	91.83B	111.61A
T2	WN	21.93±0.46a	47.65±2.08ab	66.98±2.59ab	114.63±3.90a	125.90±6.96abcd
	DN	20.78±1.22abcd	38.25±2.73e	52.93±3.94d	91.18±5.43c	111.95±5.59e
	Mean	21.35A	42.95A	59.95AB	102.90A	118.93A
‘Yuxiangyouzhan’						
CK	WN	18.28±1.06bcde	48.28±1.25ab	68.93±1.79a	117.20±2.28a	135.48±2.68ab
	DN	18.30±0.90abcde	51.65±1.68a	60.88±2.38bcd	112.53±3.88a	131.50±3.08abc
	Mean	18.29A	49.96A	64.90A	114.86A	133.49A
T1	WN	16.08±0.77e	45.45±0.63bcd	62.63±2.07abc	108.08±2.15a	124.15±1.79bcde
	DN	21.20±1.77abc	41.50±0.82cde	56.48±2.48 cd	97.98±3.17bc	119.18±4.93cde
	Mean	18.64A	43.48B	59.55B	103.03B	121.66B
T2	WN	20.35±0.33abcd	48.70±1.79ab	68.43±1.39ab	117.13±2.48a	137.48±2.36a
	DN	18.00±1.34de	40.33±1.30de	55.29±0.34 cd	95.61±1.41bc	113.61±1.24de
	Mean	19.18A	44.51B	61.86AB	106.37B	125.54B
ANOVA	Cultivar (*C*)	ns	ns	ns	ns	ns
	Method (*M*)	*	*	**	**	**
	$\mathrm{La_2O_3}$ NPs (*La*)	ns	**	**	**	**
	$C \times M$	ns	ns	ns	ns	ns
	$C \times La$	ns	*	ns	*	ns
	$M \times La$	*	*	*	*	*

Values followed by different lowercase/uppercase letters in a column represent significant differences among the treatments in cultivars at $P < 0.05$. * and ** represent a significant difference at $P < 0.05$ and $P < 0.01$, respectively; ns represents a non-significant difference

Distribution of growth parameters

Under T1 treatment, DN increased the root weight ratio of ‘Yuxiangyouzhan’ by 36.50%, compared with WN. Under T2 treatment, WN increased the leaf weight ratio of ‘Xiangyaxiangzhan’ by 13.25%, compared with DN (Table 3). Cultivar (C) and $M \times La$ significantly affected the shoot dry weight to plant height ratio. Compared with ‘Xiangyaxiangzhan,’ the shoot dry weight to plant height ratio of ‘Yuxiangyouzhan’ under CK-WN, CK-DN, T1-WN, and T2-WN treatments increased by 26.09%, 18.82%, 24.54%, and 19.43%, respectively. Under T1 treatment, DN increased the root–shoot ratio of ‘Yuxiangyouzhan’ by 44.35%, compared with WN (Table 4).

Morphological traits

M significantly affected plant height. WN increased the plant height of ‘Xiangyaxiangzhan’ by 25.14%, 29.15%, and 22.85% under CK, T1, and T2 treatments, respectively, compared with DN. Compared with ‘Yuxiangyouzhan,’ the leaf sheath length of ‘Xiangyaxiangzhan’ under CK-WN treatment increased by 27.35%. C, M, La, and $C \times M$ significantly affected the leaf area. T2 treatment increased the mean value of the leaf area of ‘Xiangyaxiangzhan’ in the two seedling nursery methods by 16.66%, compared with CK. In contrast, the T1 treatment reduced the mean value of the leaf area of ‘Yuxiangyouzhan’ in WN and DN by 15.44%, compared with CK. Moreover, WN increased the leaf area of ‘Yuxiangyouzhan’ by 52.69%, 27.90%, and 49.80% under CK, T1, and T2 treatments, respectively, compared with DN. C, $C \times M$, and $M \times La$ significantly affected the specific leaf area. DN increased the specific leaf area of ‘Xiangyaxiangzhan’ by 24.14% and 42.87% under CK and T2 treatments, compared with WN. While DN increased the specific leaf area of ‘Yuxiangyouzhan’ by 4.52% under T2 treatment, compared with WN (Table 5).

Table 2 Effects of foliar spray of La_2O_3 nanoparticles on the fresh weight of fragrant rice seedlings in wet and dry nurseries

Treatment		Root fresh weight (mg)	Stem fresh weight (mg)	Leaf fresh weight (mg)	Shoot fresh weight (mg)	Total fresh weight (mg)
‘Xiangyaxiangzhan’						
CK	WN	230.25 ± 11.93b	402.50 ± 22.08a	298.25 ± 11.97ab	700.75 ± 26.67a	931.00 ± 37.50ab
	DN	226.00 ± 18.92b	299.50 ± 10.84b	222.50 ± 18.40d	522.00 ± 27.86c	748.00 ± 36.61de
	Mean	228.125A	351.00A	260.38A	611.38A	839.50A
T1	WN	255.25 ± 15.28ab	376.75 ± 19.80a	260.25 ± 8.10c	637.00 ± 27.82b	892.25 ± 16.62b
	DN	240.75 ± 9.36b	282.25 ± 19.58bc	212.75 ± 11.32d	495.00 ± 25.42cde	735.75 ± 29.80de
	Mean	248A	329.50A	236.50A	566.00A	814.00A
T2	WN	279.50 ± 8.53a	389.75 ± 16.16a	303.00 ± 16.25ab	692.75 ± 29.22a	972.25 ± 31.98a
	DN	232.00 ± 14.75b	296.00 ± 10.66b	213.00 ± 5.12d	509.00 ± 15.56 cd	741.00 ± 28.16de
	Mean	255.75A	342.88A	258.00A	600.88A	856.63A
‘Yuxiangyouzhan’						
CK	WN	187.25 ± 9.21c	384.75 ± 13.52a	300.00 ± 6.77ab	684.75 ± 18.42ab	872.00 ± 18.93bc
	DN	157.25 ± 13.94 cd	285.25 ± 7.78b	225.75 ± 4.09d	511.00 ± 11.71c	668.25 ± 21.53ef
	Mean	172.25A	335.00A	262.88A	597.88A	770.13A
T1	WN	146.25 ± 10.43d	384.00 ± 16.31a	281.00 ± 4.30bc	665.00 ± 16.74ab	811.25 ± 18.02 cd
	DN	159.50 ± 14.86 cd	233.75 ± 8.73c	206.00 ± 6.26d	439.75 ± 13.95e	599.25 ± 26.33f
	Mean	152.88A	308.88A	243.50B	552.38B	705.25B
T2	WN	168.50 ± 5.81 cd	376.25 ± 11.77a	312.25 ± 6.45a	688.50 ± 12.73a	857.00 ± 14.61bc
	DN	167.50 ± 8.96 cd	242.50 ± 13.60c	199.25 ± 8.91d	441.75 ± 22.46de	609.25 ± 23.68f
	Mean	168.00A	309.38A	255.75AB	565.13AB	733.13AB
ANOVA	Cultivar (C)	**	ns	ns	ns	**
	Method (M)	*	**	**	**	**
	La_2O_3 NPs (La)	ns	*	**	**	*
	$C \times M$	ns	*	ns	*	ns
	$C \times La$	ns	ns	ns	ns	ns
	$M \times La$	ns	ns	*	ns	ns

Values followed by different lowercase/uppercase letters in a column represent significant differences among the treatments in cultivars at $P < 0.05$. * and ** represent a significant difference at $P < 0.05$ and $P < 0.01$, respectively; ns represents a non-significant difference

Table 3 Effects of foliar spray of La_2O_3 nanoparticles on the dry weight ratio of different plant tissues of fragrant rice seedling in wet and dry nurseries

Treatment		Root weight ratio (%)	Stem weight ratio (%)	Leaf weight ratio (%)
‘Xiangyaxiangzhan’				
CK	WN	15.14±0.12bcdef	34.82±1.05c	50.04±1.16abc
	DN	17.30±1.57abcd	35.02±0.50bc	47.68±1.81bcd
	Mean	16.22A	34.92A	48.86A
T1	WN	16.81±1.54abcd	35.29±0.84bc	47.90±1.47bcd
	DN	18.80±0.34a	34.34±0.63c	46.86±0.63 cd
	Mean	17.80A	34.81A	47.38A
T2	WN	17.55±0.92abc	38.25±2.94ab	53.43±1.94a
	DN	18.67±1.35a	34.15±1.54c	47.18±2.26 cd
	Mean	18.11A	36.20A	50.30A
‘Yuxiangyouzhan’				
CK	WN	13.48±0.67ef	35.63±0.61bc	50.89±1.06ab
	DN	14.49±0.96def	39.26±0.56a	46.25±0.78d
	Mean	13.99A	37.45A	48.57A
T1	WN	12.96±0.70f	36.62±0.51abc	50.41±1.14abc
	DN	17.70±0.76ab	34.92±0.83bc	47.38±0.41bcd
	Mean	15.33A	35.77AB	48.90A
T2	WN	14.82±0.40cdef	35.40±0.79bc	49.78±0.82abcd
	DN	15.83±1.13bcde	35.49±1.08bc	48.67±0.31bcd
	Mean	15.33A	35.45B	49.23A
ANOVA	Cultivar (*C*)	**	ns	ns
	Method (*M*)	**	ns	*
	La_2O_3 NPs (*La*)	*	ns	ns
	C×*M*	ns	*	ns
	C×*La*	ns	ns	ns
	M×*La*	ns	ns	ns

Values followed by different lowercase/uppercase letters in a column represent significant differences among the treatments in cultivars at $P<0.05$. * and ** represent a significant difference at $P<0.05$ and $P<0.01$, respectively; ns represents a non-significant difference

Chlorophyll contents

La_2O_3 NPs treatment significantly affected leaf chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid, and total chlorophyll (Chl) contents, and the chlorophyll a/b (Chl a/b) ratio, *C*×*La*, and *M*×*La*. T2 treatment increased the mean value of Chl a, Chl b, carotenoid, and total Chl contents of ‘Xiangyaxiangzhan’ in the two seedling nursery methods by 20.61%, 11.39%, 46.12%, and 17.47%, respectively, compared with CK (Fig. 1). On average, T1 treatment increased the Chl a, Chl b, carotenoid, and total chlorophyll contents of ‘Yuxiangyouzhan’ by 26.39%, 17.42%, 39.57%, and 23.25%, respectively, compared with CK (Fig. 1). Compared with ‘Xiangyaxiangzhan,’ the carotenoid contents of ‘Yuxiangyouzhan’ under CK-WN, T1-WN, and T1-DN treatments increased by 40.15%, 88.49%, and 18.89%, respectively (Fig. 1d). For ‘Xiangyaxiangzhan,’ compared with CK-WN treatment, the Chl a, Chl b, carotenoid, and total Chl contents, and the Chl a/b ratio showed no significant change under CK-DN treatment but increased under T1- and T2-DN treatments (Fig. 1).

Antioxidant enzyme activities and MDA content

C, *M*, and *La* all had a considerable impact on SOD activity. *C*×*M*, *C*×*La*, and *M*×*La* had an impact as well. Compared with CK, the mean value of SOD activity in leaves of ‘Xiangyaxiangzhan’ substantially improved by 15.28% under T2 treatment (Fig. 2a). Much improvement was observed in the mean value of SOD activity in leaves of ‘Yuxiangyouzhan,’ by 39.93% and 36.69% under T1 and T2 treatments, respectively (Fig. 2a). T2 treatment marginally increased the mean value of SOD activity in roots of ‘Yuxiangyouzhan’ by 50.52% in the two seedling nursery methods (Fig. 2b). Compared with CK, T2 treatment in DN and WN led to a dramatic increase in POD activity in roots of ‘Xiangyaxiangzhan’ by 78.11% and 26.73%, respectively, and a wild surge in POD activity in roots of ‘Yuxiangyouzhan’ by 45.50% and 22.04%, respectively (Fig. 2d). CAT activity was significantly affected by *M*, *La*, *C*×*M*, and *M*×*La*. Compared with CK, T2 treatment led to a significant increase in the mean value of CAT activity in leaves of ‘Xiangyaxiangzhan’ by 15.53% in the two seedling nursery

Table 4 Effects of foliar spray of La_2O_3 nanoparticles on the total dry weight to plant height ratio and root–shoot ratio of fragrant rice seedling in wet and dry nurseries

Treatment		Shoot dry weight to plant height ratio(mg cm^{-1})	Root–shoot ratio
'Xiangyaxiangzhan'			
CK	WN	0.48 ± 0.03de	0.18 ± 0.00bcdef
	DN	0.54 ± 0.03bcd	0.21 ± 0.02abc
	Mean	0.51A	0.19A
T1	WN	0.42 ± 0.03e	0.20 ± 0.02abcd
	DN	0.55 ± 0.01abcd	0.23 ± 0.01a
	Mean	0.48A	0.22A
T2	WN	0.51 ± 0.02 cd	0.19 ± 0.01bcde
	DN	0.50 ± 0.04de	0.23 ± 0.02a
	Mean	0.51A	0.21A
'Yuxiangyouzhan'			
CK	WN	0.61 ± 0.01abc	0.16 ± 0.01ef
	DN	0.64 ± 0.03a	0.17 ± 0.01def
	Mean	0.62A	0.16A
T1	WN	0.52 ± 0.01bcd	0.15 ± 0.01f
	DN	0.55 ± 0.08abcd	0.22 ± 0.01ab
	Mean	0.53B	0.18A
T2	WN	0.61 ± 0.02ab	0.17 ± 0.01cdef
	DN	0.54 ± 0.02bcd	0.19 ± 0.02bcde
	Mean	0.58AB	0.18A
ANOVA	Cultivar (*C*)	*	**
	Method (*M*)	ns	**
	La_2O_3 NPs (*La*)	ns	ns
	$C \times M$	ns	ns
	$C \times La$	ns	ns
	$M \times La$	**	ns

Values followed by different lowercase/uppercase letters in a column represent significant differences among the treatments in cultivars at $P < 0.05$. * and ** represent a significant difference at $P < 0.05$ and $P < 0.01$, respectively; ns represents a non-significant difference

methods (Fig. 2e). T1 and T2 treatments significantly enhanced the mean value of CAT activity in leaves of 'Yuxiangyouzhan' by 47.43% and 75.10%, respectively (Fig. 2e). However, CAT activity in roots of 'Yuxiangyouzhan' was decreased by 28.42% in T1 treatment, compared with CK (Fig. 2f). On average, T1 and T2 treatments led to a significant decrease in MDA content in leaves of 'Xiangyaxiangzhan by 46.51% and 49.56%, respectively, compared with CK (Fig. 2g). MDA content was adversely impacted by *M*, *La*, $C \times M$, $C \times La$, and $M \times La$. MDA content in leaves of 'Yuxiangyouzhan' in DN was reduced by 35.77%, 49.66%, and 36.98% under CK, T1, and T2 treatments, respectively, compared with WN (Fig. 2h). Moreover, MDA content in roots of 'Yuxiangyouzhan' in DN was reduced by 64.19% and 66.67% under CK and T1 treatments, respectively, compared with WN (Fig. 2h).

Nitrogen metabolism enzyme activities

On average, T1 and T2 treatments increased GS activity in leaves of 'Xiangyaxiangzhan' by 8.03% and 11.68%, respectively, and 'Yuxiangyouzhan' by 5.00% and 8.57%, respectively, compared with CK (Fig. 3a). T2 treatment marginally improved the mean value of GS activity in roots in the two seedling nursery methods for 'Yuxiangyouzhan,' whereas no discernible changes were found for 'Xiangyaxiangzhan' after *La* application (Fig. 3b). T1 treatment decreased the mean value of NR activity in leaves of 'Xiangyaxiangzhan' and 'Yuxiangyouzhan' by 19.14% and 15.16%, respectively, compared with CK (Fig. 3c). T2 treatment decreased the mean value of NR activity in leaves of 'Xiangyaxiangzhan' and 'Yuxiangyouzhan' by 10.54% and 19.16%, respectively, compared with CK (Fig. 3c). T1 and T2 treatments increased the mean value of GOGAT activity in leaves of 'Xiangyaxiangzhan' by 20.03% and 18.35%, respectively, compared with CK (Fig. 3e). T1 and T2 treatments increased the mean value of GOGAT activity in leaves of 'Yuxiangyouzhan' by 24.36% and 36.79%, respectively, compared with CK (Fig. 3e).

Correlation analysis

The root–shoot ratio was significantly negatively correlated with the fresh weight of leaf and shoot; the dry weight of stem, leaf, shoot, and total plant; and GOGAT activity in roots. Moreover, the root–shoot ratio revealed negative and obvious correlations with root dry weight, root dry weight ratio, and CAT activity in leaves. Leaf fresh weight was significantly positively correlated with leaf area, stem fresh weight, shoot fresh weight, total fresh weight, stem dry weight, leaf dry weight, shoot dry weight, total dry weight, plant height, leaf weight ratio, POD activity in roots, and GOGAT activity in roots. POD activity in roots was significantly positively correlated with the fresh weight of root, leaf, shoot, and total plant; the dry weight of leaf, shoot, and total plant; the leaf weight ratio; and the GOGAT activity in leaves (Fig. 4).

Discussion

WN has been widely promoted since the 1960s, while DN, a water-saving seedling-raising method, has been recently adopted in many places. These seedling-raising methods with their own characteristics are the two main ways in China (Lampayan et al. 2015). Water is a key factor among various seedling-raising techniques, which strongly impacts the morphological traits of both rice cultivars. Coleoptile elongation and seedling emergence were brought about by

Table 5 Effects of foliar spray of La_2O_3 nanoparticles on the morphological traits of fragrant rice seedling in wet and dry nurseries

Treatment		Plant height (cm)	Leaf sheath length (cm)	Leaf area (cm^2)	Specific leaf area (cm^2 g^{-1})
'Xiangyaxiangzhan'					
CK	WN	22.12 ± 0.25a	6.14 ± 0.18ab	3.67 ± 0.07bcd	59.16 ± 3.48 cd
	DN	17.68 ± 0.47bd	5.48 ± 0.21bc	3.48 ± 0.17 cd	73.44 ± 7.64 ab
	Mean	19.90A	5.81A	3.57B	61.84A
T1	WN	21.79 ± 0.33a	5.66 ± 0.08bc	3.75 ± 0.17abcd	63.08±1.02bcd
	DN	16.87 ± 0.19c	5.86 ± 0.19bc	2.95 ± 0.26ef	64.52±5.84bc
	Mean	19.33A	5.76A	3.35B	64.28A
T2	WN	22.40 ± 0.57a	6.99 ± 1.16c	4.22 ± 0.10a	55.12±4.50cdef
	DN	18.23 ± 0.63bc	5.68 ± 0.10bc	4.12 ± 0.13f	78.75±4.60a
	Mean	20.31A	6.33A	4.16A	61.84A
'Yuxiangyouzhan'					
CK	WN	19.38 ± 0.36abc	4.82 ± 0.08c	4.07 ± 0.19ab	59.17±3.01def
	DN	17.68 ± 0.47bc	5.48 ± 0.21bc	2.67 ± 0.14f	52.27±3.49cde
	Mean	18.53A	5.15B	3.37A	51.59A
T1	WN	20.77 ± 0.36ab	5.34 ± 0.18bc	3.27 ± 0.25de	57.34±2.84f
	DN	19.45 ± 3.56abc	5.86 ± 0.19bc	2.56 ± 0.22f	44.02±3.05f
	Mean	20.11A	5.60A	2.92B	48.72A
T2	WN	19.18 ± 0.64abc	4.96 ± 0.08c	3.91 ± 0.13abc	45.18±2.64ef
	DN	17.75 ± 0.45bc	5.68 ± 0.10bc	2.61 ± 0.14f	47.22±2.32cd
	Mean	18.46A	5.32AB	3.26AB	52.28A
ANOVA	Cultivar (*C*)	ns	*	**	**
	Method (*M*)	**	ns	**	ns
	La_2O_3 NPs (*La*)	ns	ns	**	ns
	$C \times M$	ns	*	**	*
	$C \times La$	ns	ns	ns	ns
	$M \times La$	ns	ns	ns	*

Values followed by different lowercase/uppercase letters in a column represent significant differences among the treatments in cultivars at $P < 0.05$. * and ** represent a significant difference at $P < 0.05$ and $P < 0.01$, respectively; ns represents a non-significant difference

hypoxic conditions in flooded soil (Cheng et al. 2018). An earlier study also indicated that dry soil led to a reduced number of tillers (Tao et al. 2014). The results were consistent with previous research. Plant height was significantly higher and root dry weight was significantly lower in WN-raised rice seedlings than in DN-raised rice seedlings (Tables 1 and 5). Overall, WN-raised rice seedlings were morphologically better than DN-raised rice seedlings. WN-raised rice seedlings had improved fresh weight, dry weight, plant height, leaf sheath length, and leaf area (Tables 1, 2, and 5).

Lanthanum (La) has been used as a plant growth regulator in agricultural production for nearly 50 years (Dai et al. 2017). La application at certain concentrations can reduce growth inhibition and promote growth in wheat and rice plants (Yang et al. 2021; Luo et al. 2021). NPs application plays an important role in food security and green agricultural development (Kumar and Seth 2021). However, nanoagriculture in China is still in the nascent stage (Li et al. 2021). NPs concentrations as low as 50 mg L^{-1} can stunt plant growth (Yue et al. 2017), whereas concentrations of more than 50 mg L^{-1} have a more pronounced effect on physiological and biochemical processes in plants (Li et al. 2018; Nhan et al. 2015). Other studies indicated that plant growth may benefit from exposure to NPs at levels as low as 0–50 mg L^{-1} (Rastogi et al. 2017). Thus, exposure dosages of 0, 20, and 40 mg L^{-1} were chosen. Studies on La_2O_3 NPs application have been increasing, and a recent study found that La_2O_3 NPs treatment affected root development, aerial part growth, and pigment concentration in *Pfaffia glomerata* plantlets (Neves et al. 2022). Thus, we assessed La_2O_3 NPs application in regulating growth by using different rice seedling-raising methods. Xiao et al. (2021) showed that the storage root biomass of radish decreased by 38% and 60% on exposure to 100 and 300 mg L^{-1} La_2O_3 NPs, respectively. However, the present study found that La_2O_3 NPs treatment significantly reduced shoot fresh weight but increased root dry weight of WN- and DN-raised rice seedlings (Tables 1 and 2). The difference may be because the La_2O_3 NPs concentrations used in our study were lower than those used in

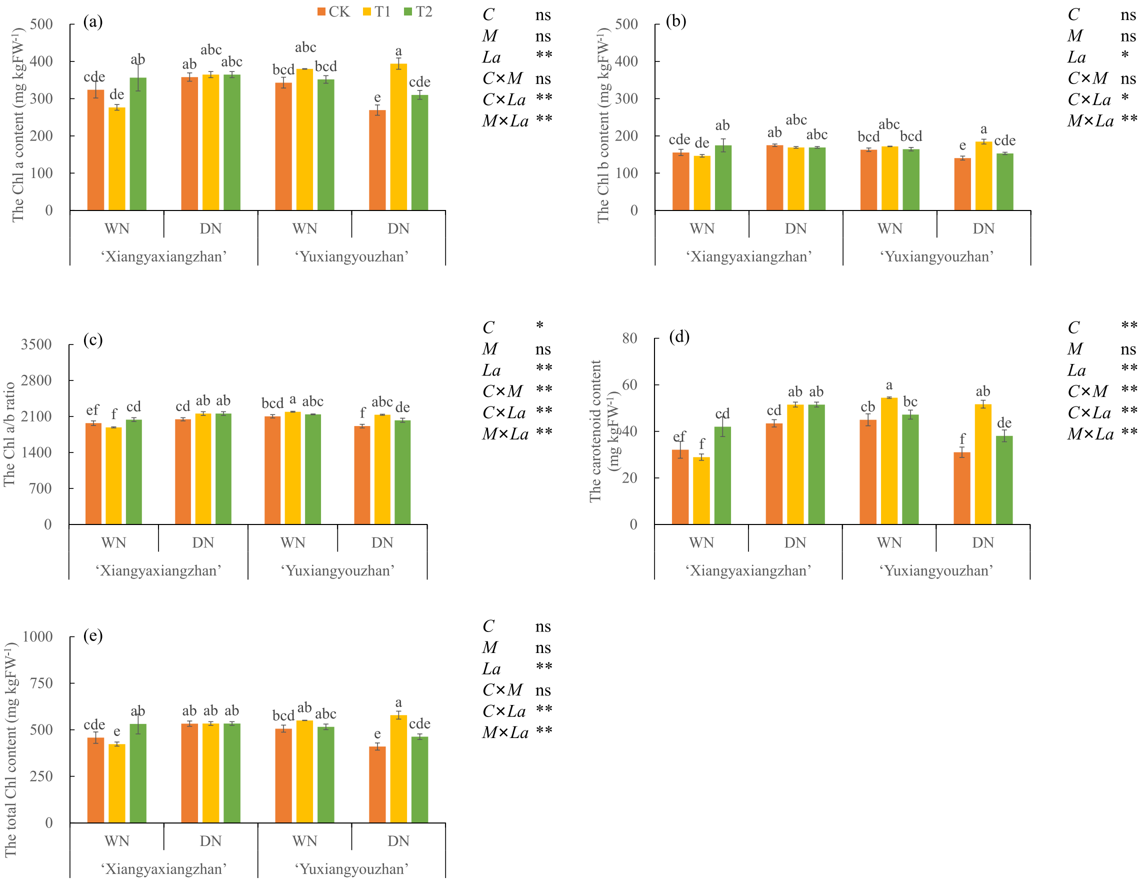


Fig. 1 The chlorophyll a content (**a**), the chlorophyll b content (**b**), the chlorophyll a/b ratio (**c**), the carotenoid content (**d**), and the total chlorophyll content (**e**) of the two fragrant rice cultivars in wet and dry nurseries with foliar spray of La_2O_3 nanoparticles. *C*, cultivar; *M*, method; *La*, La_2O_3 NPs. Lowercase letters represent significant differences between treatments (LSD test, $P < 0.05$)

previous studies; thus, it was demonstrated that low concentrations of La_2O_3 NPs treatment can promote the root growth of plants.

The effects of drought pressure markedly reduced leaf area (Timmusk et al. 2014). Karimi et al. (2018) also established that the loss of leaves or the development of smaller leaves is one of the ecological responses to drought. Similarly, our study illustrated that the leaf area was smaller in DN-raised rice seedlings than in WN-raised rice seedlings (Table 5). Moreover, La_2O_3 NPs application under T2 treatment improved the leaf area and specific leaf area compared with plants under T1 and CK treatments for 'Xiangyaxiangzhan' (Table 5). Specifically, the increase in the leaf area led to an increase in the photosynthetic area, thereby La_2O_3 NPs under DN treatment significantly improved plant growth parameters. Positive effects of other NPs on leaf area have been reported in plants under drought stress. In feverfew plants, increased biomass accumulation after treatment with nano-silicon complexes has been linked to enhanced nutrient uptake as a result of improved root growth, and may also be associated with enhanced photosynthetic efficiency under adverse conditions (Esmaili et al. 2022). Therefore, the T2 treatment was able to mitigate the reduction in leaf area under DN.

Chlorophyll (Chl) plays an active role in photosynthesis, a process by which plants assimilate, distribute, and transform solar energy (Agnihotri and Seth 2016). The physiological characteristics of rice seedlings are influenced by the rice cultivation method. Enhanced photosynthesis from more Chl contents in leaves resulted in healthier, stronger seedlings (Kumar et al. 2023). Under drought conditions, the decrease in photochemical efficiency of photosystem II may be associated with the decrease in photosynthetic pigment contents of leaves (Song et al. 2014). Yadav et al. (2022) also showed that under cadmium stress, the Chl a, Chl b, total Chl, and carotenoid contents significantly decreased. Carotenoids are important antioxidants, which can dissipate the excess excitation energy in the photosystem II light-harvesting antenna, especially under stress (Li et al. 2021). Azim et al. (2022) also showed that ZnO NPs enhanced the Chl and carotenoid contents in tomato seedlings. Similarly, in this study, La_2O_3 NPs application

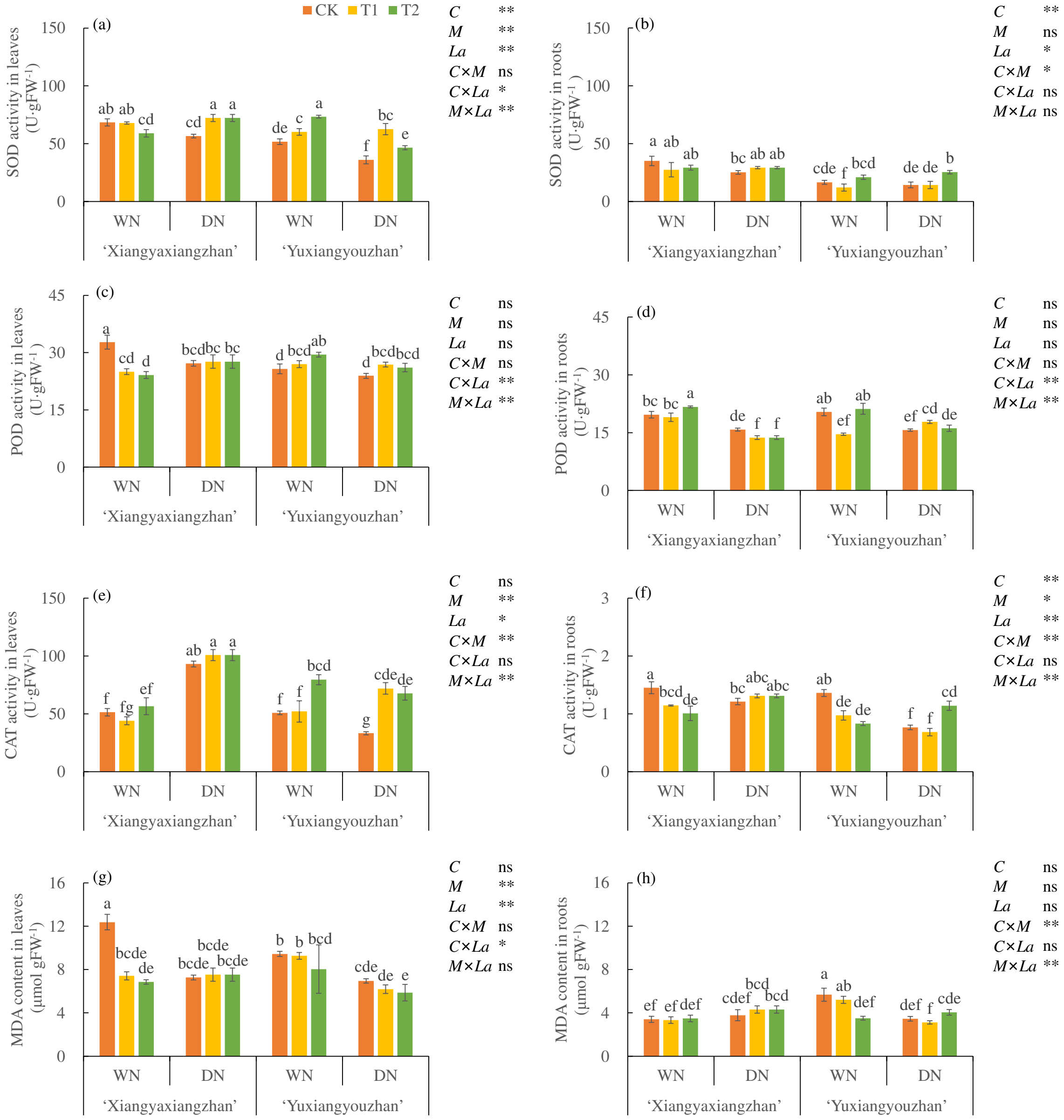


Fig. 2 Superoxide dismutase (SOD) activity in leaves (**a**) and roots (**b**), peroxidase (POD) activity in leaves (**c**) and roots (**d**), catalase (CAT) activity in leaves (**e**) and roots (**f**), malondialdehyde (MDA) content in leaves (**g**) and roots (**h**) of the two fragrant rice cultivars in wet and dry nurseries with foliar spray of La_2O_3 nanoparticles. *C*, cultivar; *M*, method; *La*, La_2O_3 NPs. Lowercase letters represent significant differences between treatments (LSD test, $P < 0.05$)

significantly alleviated the reduction in Chl a, Chl b, and carotenoid contents of DN seedlings (Fig. 1). This improves phytochrome content and ensures photosynthesis in rice seedling leaves. Thus, better photosynthesis led to the rapid growth of fragrant rice seedlings. Considering that La_2O_3 NPs can significantly regulate light utilization and electron transport in photosynthesis (Liu et al. 2020a), we deduced that fragrant rice seedlings uptake La_2O_3 NPs and consequently enhance photosynthesis.

In addition, increased activities of SOD, POD, and CAT and decreased MDA contents were observed in the fragrant rice seedlings growing in the presence of La_2O_3 NPs under T1 treatment (Fig. 2). Sun et al. (2020) suggested that stress causes an increase in reactive oxygen species (ROS), resulting in lipid peroxidation in the cell membrane system and MDA formation. MDA is an essential measure of oxidative stress. Gupta and Seth (2020) illustrated that the activation of the cellular antioxidant system is an essential method for safeguarding against diverse environmental stresses by scavenging ROS generated therein. SOD, POD, and CAT play significant roles in the antioxidative defense mechanisms of plants to

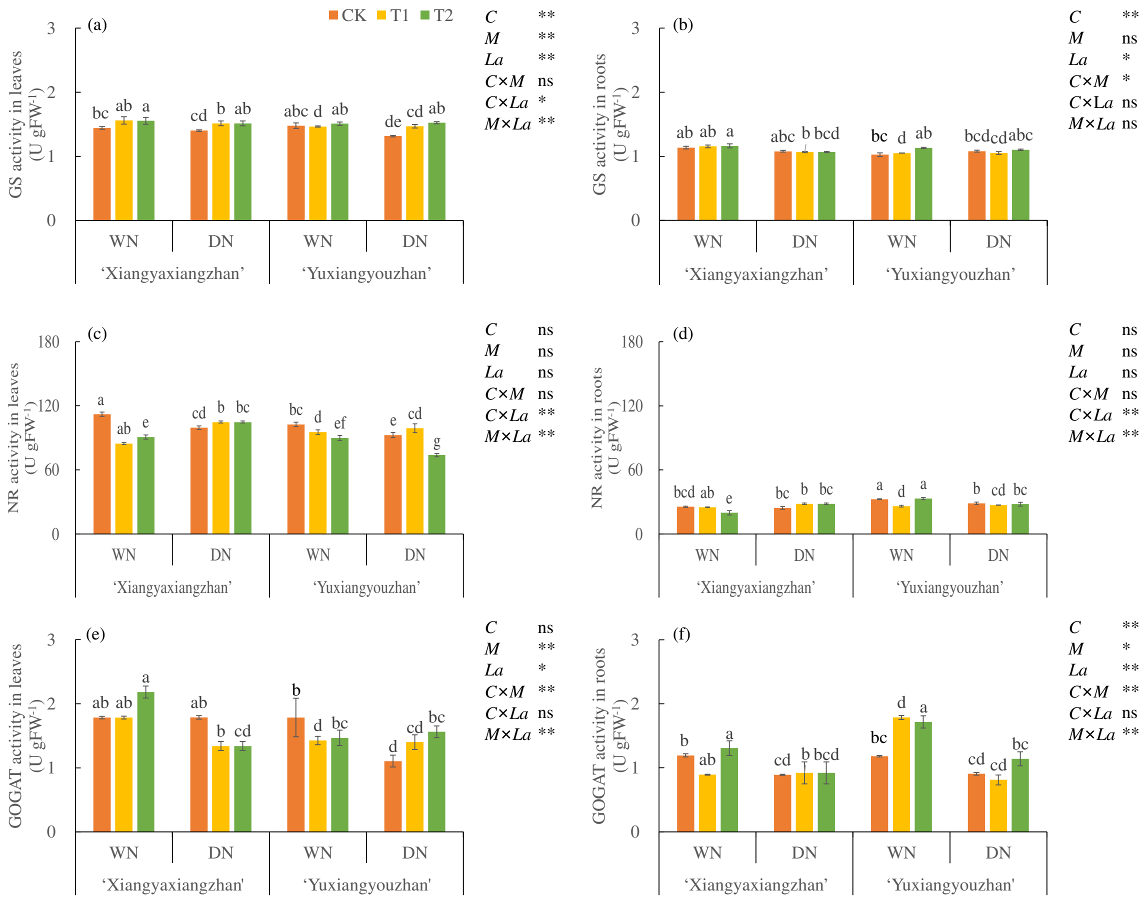


Fig. 3 Glutamine synthetase (GS) activity in leaves (**a**) and roots (**b**), nitrate reductase (NR) activity in leaves (**c**) and roots (**d**), and glutamate synthase (GOGAT) activity in leaves (**e**) and roots (**f**) of the two fragrant rice cultivars in wet and dry nurseries with foliar spray of La_2O_3 nanoparticles. *C*, cultivar; *M*, method; *La*, La_2O_3 NPs. Lowercase letters represent significant differences between treatments (LSD test, $P < 0.05$). $^*P \leq 0.05$

suppress ROS (Ashraf et al. 2018). Some substances can help plants to resist adversity. For example, 24-Epibrassinolide could modulate the antioxidant defense mechanism of *Brassica juncea* L., inhibit oxidative damage to cell membranes by salt stress, and reduce the accumulation of oxides, improving plant growth (Gupta and Seth 2022). Rice under stress experienced less oxidative damage because of ZnO NPs application (Li et al. 2021). This might be because these NPs helped to neutralize ROS by triggering antioxidant enzyme systems (Khan et al. 2017). Similarly, in this study, to reduce ROS accumulation under DN conditions, La_2O_3 NPs treatment greatly increased SOD, POD, and CAT activities (Fig. 2). ROS and MDA levels in rice leaves decreased due to higher antioxidant enzyme activities in plants after La_2O_3 NPs application, which also decreased the induction of lipid peroxidation. Thus, when applied in proper concentrations, La_2O_3 NPs could enhance seedling resistance and increase the contents of carbon and nitrogen compounds, thus laying a good foundation for field transplanting.

Nitrogen absorbed by crop roots must be converted by nitrogen metabolism-related enzymes, including GS, NR, and GOGAT, into organic substances that can be used by plants. These three enzymes are necessary and play significant roles in nitrogen metabolism (Hu et al. 2021). However, Gupta et al. (2017) showed that salt stress led to a reduction in NR, GS, and GOGAT activities, and total nitrogen content, thus impeding nitrogen metabolism. GS has multiple functions and is associated with various nitrogen metabolic pathways (Sanz-Luque et al. 2015). Enhanced GS and GOGAT activities facilitate the generation of organic nitrogen (Hu et al. 2021). Increasing irrigation can increase both GOGAT and GS activities (Zhang et al. 2022). Wang et al. (2018) also claimed that endogenous stress-resistant compounds that can withstand stress increase because of the increase in GOGAT activity. Similarly, in this study, GS activity in leaves in both cultivars was found to increase with the increase in La_2O_3 NPs concentration (Fig. 3a). GOGAT activity in leaves of 'Xiangyaxiangzhan' under La_2O_3 NPs treatment was dramatically increased compared with CK

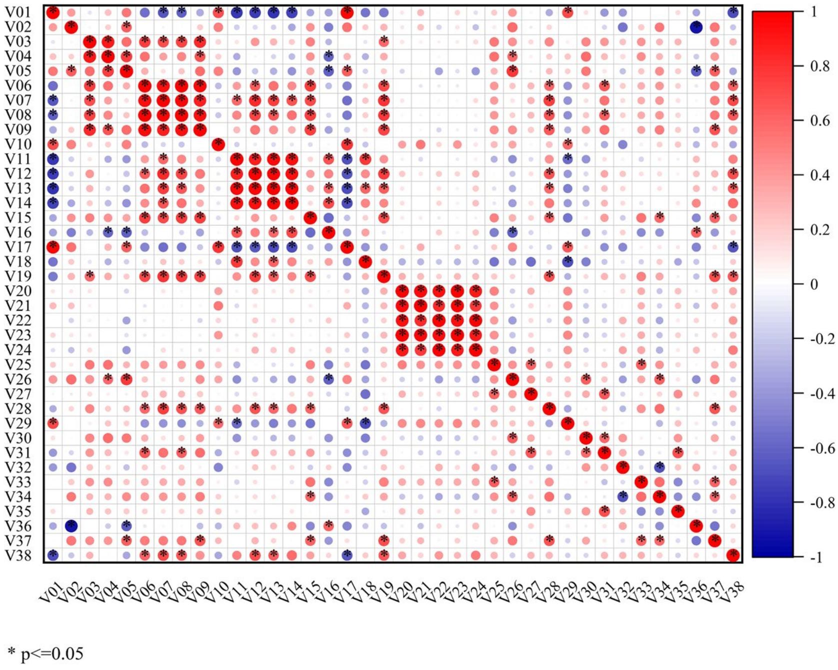
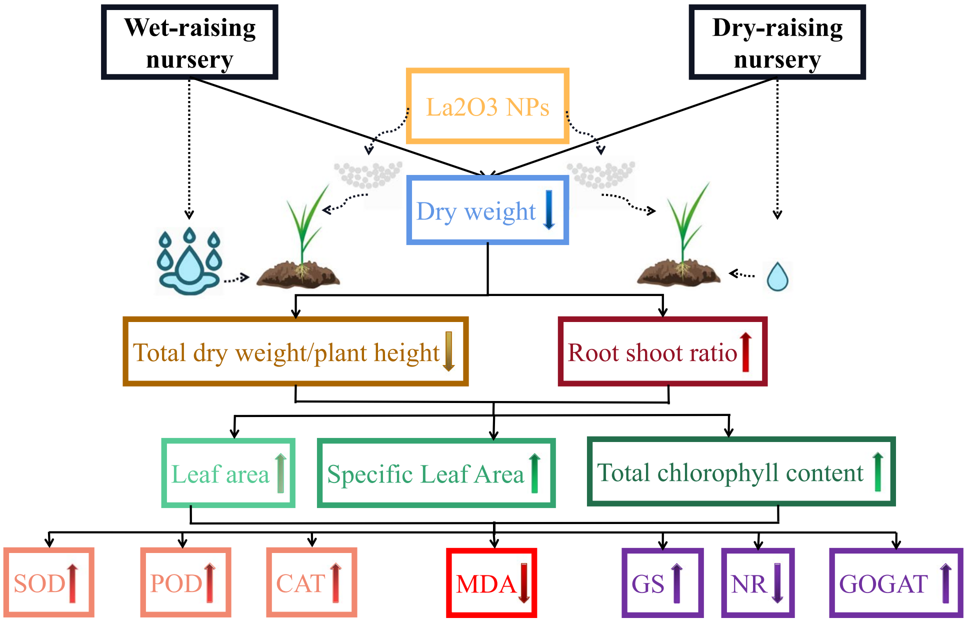


Fig. 4 Correlation analysis between the investigated parameters. V01, root–shoot ratio; V02, leaf sheath length; V03, leaf area; V04, specific leaf area; V05, root fresh weight; V06, stem fresh weight; V07, leaf fresh weight; V08, shoot fresh weight; V09, total fresh weight; V10, root dry weight; V11, stem dry weight; V12, leaf dry weight; V13, shoot dry weight; V14, total dry weight; V15, plant height; V16, shoot dry weight/plant height; V17, root weight ratio; V18, stem weight ratio; V19, leaf weight ratio; V20, the Chl a content; V21, the Chl b content; V22, carotenoid content; V23, total Chl content; V24, the Chl a/b ratio; V25, SOD activity in leaves; V26, SOD activity in roots; V27, POD activity in leaves; V28, POD activity in roots; V29, CAT activity in leaves; V30, CAT activity in roots; V31, MDA content in leaves; V32, MDA content in roots; V33, GS activity in leaves; V34, GS activity in roots; V35, NR activity in leaves; V36, NR activity in roots; V37, GOGAT activity in leaves; V38, GOGAT activity in roots

Fig. 5 Seedling raising methods and their interaction with La_2O_3 NPs regulated the dry weight of fragrant rice. Changes in plant morphological parameters (e.g., shoot dry weight/plant height and root–shoot ratio) were the reason for the differences in fragrant rice cultivars in response to La_2O_3 NPs application. These changes resulted from the changes in the leaf area, specific leaf area, the chlorophyll contents, antioxidant properties, and activities of nitrogen metabolism enzymes



(Fig. 3e). NR is the main nitrogen source entering plants and is considered the primary signaling enzyme in plants (Chamizo-Ampudia et al. 2017). This study revealed that NR activities in leaves and roots were lower under La_2O_3 NPs treatment than under CK treatment and significantly varied with La_2O_3 NPs concentration and rice cultivars (Fig. 3 a and b). These findings agree with those of Ahanger et al. (2021), which demonstrated that applying nano-compost dramatically reduced NR activity due to drought treatment. Gupta and Seth (2020) also suggested that the reduction in NR activity during salt stress was because of the oxidative modification of the NR protein caused by an excessive amount of ROS generated by stress. Therefore, this study found that La_2O_3 NPs application increased the activity of GS and GOGAT in rice seedling leaves and decreased the activity of NR in leaves and roots compared to CK.

Overall, La_2O_3 NPs treatment at different concentrations could affect chlorophyll contents, antioxidant enzyme activities, MDA content, and nitrogen metabolism in the studied rice cultivars under different raising nurseries. The potential cause of the observed results was the slight difference in the morphological index under La_2O_3 NPs treatment but a significant change in the physiological indexes (Fig. 5).

Conclusion

Our study indicated that the La_2O_3 NPs concentration of 40 mg L^{-1} was beneficial, as it significantly increased the leaf area of seedlings grown by both WN and DN methods due to changes in morphological and physiological parameters, thereby providing a theoretical basis for La_2O_3 NPs application in agriculture. However, this study lacks molecular biology analysis of rice seedlings after La_2O_3 NPs application using different seedling-raising methods and electron microscopy studies after La_2O_3 NPs enter rice seedlings. The results of this study provide a theoretical basis for expanding the research on La_2O_3 NPs application in rice, as well as relevant references for strengthening rice seedlings in the nursery, which has a positive outcome of grain yield increase.

Author contribution Zhaowen Mo designed the experiments; Weifen Chen, Gaoxin Liao, and Feiyang Sun examined the characteristics; Zhaowen Mo, Weifen Chen, Zhilong Chen, Feiyang Sun, and Haoming Chen did the data analysis and paper writing; Weifen Chen, Yixian Ma, Gaoxin Liao, Zhilong Chen, Haoming Chen, Xiangru Tang, and Zhaowen Mo revised and edited the manuscript. The final manuscript was read and approved by all authors.

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Data availability All data generated or analyzed during this study are included in this article.

Declarations

Ethical approval Not relevant.

Consent to participate Not relevant.

Consent for publication Not relevant.

Competing interests The authors declare no competing interests.

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