



Lanthanum nitrate improves phosphorus-use efficiency and tolerance to phosphorus-deficiency stress in *Vigna angularis* seedlings

Huida Lian¹ · Cheng Qin² · Li Zhang³ · Cong Zhang¹ · Hongbing Li³ · Suiqi Zhang^{1,3}

Received: 19 April 2018 / Accepted: 24 August 2018 / Published online: 30 August 2018
© Springer-Verlag GmbH Austria, part of Springer Nature 2018

Abstract

Here, we examined the effects of La^{3+} on growth, photosynthetic ability, and phosphorus-use efficiency (PUE) in various organs of adzuki bean (*Vigna angularis*) seedlings. La^{3+} substantially alleviated P-deficiency symptoms. Treatment of young seedlings with La^{3+} at 150 mg L^{-1} effectively improved PUE in roots, stems, and leaves via the regulation of root elongation and activation of root physiological responses to P-deficiency, e.g., root activity and acid phosphatase (APase) activity. Root hydraulic conductivity (L_p) was also examined to elucidate the role of La^{3+} in the relationship between water and nutrition transport. We confirmed that La^{3+} increased the level of antioxidant protective enzymes, including superoxide dismutase (SOD) and peroxidase (POD), while it significantly decreased malondialdehyde (MDA) content. The use of La^{3+} to reduce photosynthesis damage under P-deficiency was examined. The negative effects of P-deficiency on net photosynthetic rate (P_n), transpiration rate (T_r), maximum photochemical efficiency (F_v/F_m), and chlorophyll content in leaves were alleviated by La^{3+} treatment. These results clarify the regulatory functions of La^{3+} in stress tolerance and P utilization in adzuki bean seedlings.

Keywords Adzuki bean · Lanthanum nitrate · Phosphorus deficiency stress · Phosphorus use efficiency

Introduction

Adzuki beans (*Vigna angularis*) are one of the most important legume crops in China. Great attention has been paid to the remarkable health benefits and functional components of the species, including starch, fat, protein, and phytochemicals, among other aspects (Shi et al. 2017). Adzuki beans are particularly suited for production in arid and semiarid regions due to their high adaptability to unfavorable environments.

Phosphorus (P) has been reported as one of the most limiting mineral nutrients for the growth of legume crops; further,

almost 30% of the arable soils around the world are P-deficient because of P-conversion to unavailable forms, which cannot be easily utilized by plants (Wissuwa 2003; MacDonald et al. 2011). Low P availability in soils directly reduces photosynthetic ability and finally limits plant growth and development (Yan et al. 2006; Thuynsma et al. 2014; Sulieman and Tran 2015). A lower soluble P content in different tissues of legume crops grown under P-deficiency stress contrasts with the high corresponding content in plant tissues properly fertilized (Sulieman and Tran 2015). However, the use of P chemical fertilizers has been demonstrated unsustainable and a serious hazard to human health and to the environment (Li et al. 2011). Therefore, there is a growing need to develop more P-use-efficient (PUE) crops as well as more precise methods to monitor P-status in plant tissues.

Many studies have reported that plant growth regulators (PGRs) play a central role in controlling the development of the root system in response to P-deficiency or to localized low soil-nutrient patches (Casson and Lindsey 2003). Additionally, it is possible that phosphate-solubilizing microorganisms (PSM) utilize soil phosphate reserves in semiarid regions, thereby contributing to the enhancement of crop yields (Khan et al. 2007; Gupta et al. 2011).

Handling Editor: Bhumi Nath Tripathi

✉ Suiqi Zhang
sqzhang@ms.iswc.ac.cn

¹ State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling 712100, China

² College of Life Sciences, Northwest A&F University, Yangling 712100, Shaanxi, China

³ State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Northwest A&F University, 26 Xinong Road, Yangling 712100, China

Seed priming is a common, effective, low-cost practice for seed quality improvement, aimed to foster biotic/abiotic stress resistance, thus enhancing crop yield (Paparella et al. 2015; Szafrńska et al. 2016). The positive effects of priming are due to specific metabolic changes induced at the physiological level, such as activation of DNA repair and triggering of antioxidant mechanisms (Foyer and Noctor 2005). Previous studies have demonstrated that the application of a biostimulator as a seed primer at pre-sowing improves oxidative stress tolerance in growing seedlings (Szafrńska et al. 2016).

Rare earth elements (REE) are used as micro-fertilizers in Chinese agriculture owing to their ability to improve growth and productivity (Thomas et al. 2014). Indeed, beneficial effects of REE foliar sprays, seed treatments or addition to soil or liquid rooting media have been reported (Tyler 2004; El-Ramady 2010).

Lanthanum (La), a representative REE, reportedly improves plant resistance to environmental stress (Wang et al. 2011a, 2012). The lanthanum cation (La^{3+}) plays an important role in plant growth, particularly by stimulating photosynthesis and increasing chlorophyll content (Goecke et al. 2015; Řezanka et al. 2016). La^{3+} has been used as a plasma membrane calcium channel antagonist to activate abscisic acid (ABA) signaling (Hagenbeek et al. 2000), and for altering reactive oxygen species (ROS) homeostasis (Wang et al. 2005). ROS are vital signaling components in many biological processes (Mittler et al. 2011) and responses to environmental stimuli (Baxter et al. 2014). Photosynthesis often entails the production of ROS (Foyer and Shigeoka 2011) and is inhibited by P-deficiency stress. Antioxidant enzymes function as a protective mechanism to eliminate ROS by-products and are the first line of defense in response to stress (Balabusta et al. 2016). However, very few studies have examined the physiological effects of La^{3+} in plants exposed to environmental stress conditions.

P-deficiency may result in altered root elongation in plants. However, a previous study showed that La^{3+} caused nutrient alterations, disrupted root swelling (Wang et al. 2011b), and seemingly regulated the architecture of the root system (Ruizherreria et al. 2012). Some evidence indicates that root hydraulic conductivity (L_p) influences the supply of certain mineral nutrients, such as nitrogen (N), P, and sulfur (S) (Clarkson et al. 2000). The enhancement of root L_p optimized the delivery of water from the soil to the shoot (Thompson et al. 2007). REEs show a strong affinity for phosphate ions in vitro (Ding et al. 2005). However, current understanding of a role of phosphate in directing La^{3+} to specific plant responses to P-deficiency is poor.

In this study, we evaluated the hypothesis that La^{3+} improves legume performance under conditions of P-deficiency. We also aimed to determine whether La^{3+} decreases ROS accumulation by activating the photosynthetic protection

machinery and by modulating the antioxidant defense system. Further, we wished to clarify the relationship between La^{3+} and specific plant characteristics associated with P-deficiency, such as root acid phosphatase (APase) and root activity.

Materials and methods

Plant material

Vigna angularis c.v. 'Baohong 8824-17' plump seeds were selected and sterilized with 3% H_2O_2 for 25 min and then washed three times with distilled water. These seeds were primed by one of the following treatments: water, 50, 100, 150, or 200 mg L^{-1} $\text{La}(\text{NO}_3)_3$ /water solutions. They were then placed in closed glass bottles for about 7 h. Eight seeds were sown in each pot (10 cm in diameter) filled with sterilized universal sand in an artificial climate incubator set to 28 °C/18 °C and 12 h/12 h (light/dark regime). After incubation, seeds were air-dried to their initial water content. Seedlings were then allowed to grow until the first pair of true leaves was fully expanded.

P-deficiency treatment

Seedlings were transplanted to a plastic box (40 × 30 × 15 cm), and cotyledons were removed to minimize the effect of the seed P-reserve. After 2 days, treatment difference was set by cultivation in 1/2 Hoagland nutrient solution in the absence or presence of P. In addition, the concentration of P [potassium dihydrogen phosphate (KH_2PO_4)] was 10 mg L^{-1} in the control treatment and 0 mg L^{-1} in the P-deficiency treatment; KCl was added to the P-deficiency treatment to ensure sufficient potassium in the absence of KH_2PO_4 . Thereafter, adzuki bean seedlings were treated with CK1 (seeds were primed with water and seedlings grew in normal Hoagland solution); CK2 (seeds were primed with water and seedlings grew in P-deficiency Hoagland solution); L50 (seeds were primed with 50 mg L^{-1} of $\text{La}(\text{NO}_3)_3$ and seedlings grew in P-deficiency Hoagland solution); L100 (seeds were primed with 100 mg L^{-1} of $\text{La}(\text{NO}_3)_3$ and seedlings grew in P-deficiency Hoagland solution); L150 (seeds were primed with 150 mg L^{-1} of $\text{La}(\text{NO}_3)_3$ and seedlings grew in P-deficiency Hoagland solution); and, lastly, L200 (seeds were primed with 200 mg L^{-1} of $\text{La}(\text{NO}_3)_3$ and seedlings grew in P-deficiency Hoagland solution). Seedlings from each of three replications were sampled at 20 days after treatment initiation for each treatment.

Morphological characteristics

Total leaf area was measured using a leaf area meter (model 3000A, Li-Cor Inc., Lincoln, NE, USA). Intact plants were

then separated into roots and shoots after they were thoroughly washed with distilled water. Plant height was measured. Roots and shoots were then oven-dried, and the dry weights were measured. The ratios of root to shoot were calculated on a dry weight basis. Root length was measured using a root analysis instrument (WinRHIZO, Regent Instrument Inc., Quebec, Canada).

Photosynthetic ability

Net photosynthetic rate (P_n) and transpiration rate (T_r) were measured using an automatic photosynthesis measuring apparatus, Li-6400 (LI-COR Inc., USA). Maximum photochemical efficiency (F_v/F_m) was measured using the OS6-FL Modulated Chlorophyll Fluorometer (PAM 2500; Walz, Germany) after dark adaptation for 25 min. Total chlorophyll content was estimated in fresh leaves according to the methods of Lichtenthaler (1987).

Root-related enzyme assay

Fresh roots from each treatment were homogenized with a mortar and pestle in 0.05 M sodium phosphate buffer (pH 7.5). The homogenate was centrifuged at 10,000 rpm for 20 min; the supernatant was used to analyze enzyme activity of superoxide dismutase (SOD), peroxidase (POD), and MDA (malondialdehyde) content. These protocols were performed at 4 °C. SOD and POD enzyme activities were determined according to the method of Li et al. (2000). MDA content was measured after Shu et al. (2012).

The method of Tabatabai and Bremner (1969), as modified by Hedley et al. (1982), was used to determine plant root APase activity. Root samples (approximately 0.3 g) were treated with 4 mL of extraction buffer (0.2 mol L⁻¹ NaOAc-HAc at pH 5.8, and 1.35 mol L⁻¹ p-nitrophenol phosphate (p-NPP) as the substrate). After incubation at 37 °C for 30 min, the reaction was stopped by adding 1.5 mL of 6 mol L⁻¹ NaOH; The absorbance at 405 nm using spectrophotometer (Beckman 640 D, USA).

Root activity measurement was performed according to the triphenyl tetrazolium chloride (TTC) method (Wasaki et al. 2003). Roots were collected from the adzuki beans. Each sample (0.5 g) was immersed in a solution (10 mL) containing 0.4% TTC and 66 mmol L⁻¹ phosphate buffer (pH 7.0); samples were stored at 37 °C for 3 h, and the reaction was terminated by the addition of sulfuric acid (1 mol L⁻¹). Roots were homogenized using a mortar and pestle in 3–5 mL of ethyl acetate and a small amount of quartz sand, the red solutions were transferred to 10-ml volumetric flasks, and the residues were washed 2 or 3 times with ethyl acetate. Samples were placed in volumetric flasks, ethyl acetate was added, and OD at 485 nm was determined spectrophotometrically.

Root hydraulic conductivity (L_p)

Root L_p was determined according to the method of Trubat et al. (2012) with slight modifications. Each shoot was cut off at the base of the root system, leaving 4 cm of mesocotyl. Next, the whole root was placed in a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, USA), ensuring that the incision was above the seal ring. The pressure in the chamber was raised in steps of 0.1 MPa up to 0.5 MPa. Exuded sap was collected with absorbent cotton and weighed. The root surface area was measured using a root analysis instrument (WinRHIZO) and the root L_p was calculated.

P content and PUE

A total of 0.1 g of dried plant sample (root, stem, and leaf) was placed in a digestive tube, supplemented with 5 mL of H₂SO₄, and left overnight. Samples were then placed in the digestion furnace and heated to 360 °C. Three drops of 30% H₂O₂ were added every 30 min during the digestion period until the solution was colorless and clear. Cooled solutions were removed and added to a tube (100 mL) for determination of P content (Bao 2000). Next, 5 mL of diluted test solutions were pipetted into 50-mL volumetric flasks, diluted with water to 30 mL, and supplemented with two drops of a dinitrophenol indicator in 4 mol L⁻¹ NaOH solution, until solutions turned yellow. Then, the solution faded significantly upon addition of 2 mol L⁻¹ H₂SO₄. Molybdenum antimony reagent (5 mL) was added and distilled water was also added to a final volume of 50 mL; sample solutions thus processed were maintained for 30 min with slight shaking. Finally, OD was determined spectrophotometrically at 880 nm.

The determination of PUE was performed according to previously reported methods (Wang et al. 2014), with modifications. PUE was calculated according to the following equation: $PUE = (U_L - U_0)/F$, where U_L is the P absorbed by plants treated with La³⁺, U_0 is P absorbed by plants without P application (P-deficiency treatment), and F is the total amount of P applied.

Membership function value method to assess the ability of La to induce a plant response to P-deficiency

P-deficiency resistance involving multiple traits was evaluated by the Membership function value method (MFV), using the membership functions based on the modified theory of fuzzy mathematics (Zadeh 1965; Liu et al. 2015).

According to the phosphorus-deficiency coefficient (PC), the MFVP was calculated using the following the equations:

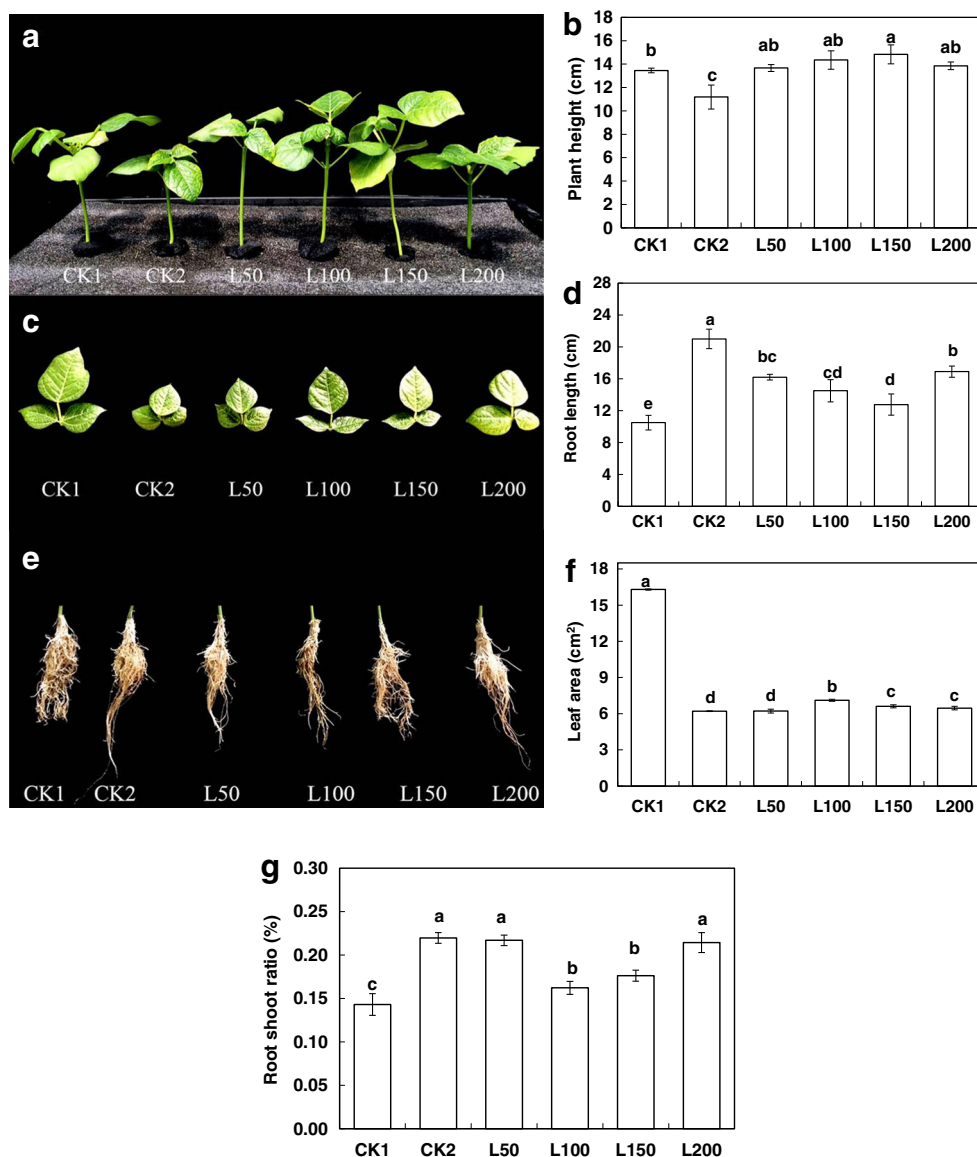
$$U_{ij} = \frac{PC_{ij} - PC_{j\min}}{PC_{j\max} - PC_{j\min}} \quad U_i = \frac{1}{n} \sum_{j=1}^n U_{ij}$$

where U_{ij} is the membership function value of the trait (j) for the treatment (i) for phosphorus-deficiency resistance; $PC_{j\max}$ and $PC_{j\min}$ are the maximum and minimum values for the phosphorus-deficiency resistance coefficient for the trait (j) of all the treatments, respectively; U_i is the average value of the membership function values of all traits measured for the treatment (i).

Experimental design and statistical analysis

The experiment had a completely randomized design. Analysis of variance (Duncan's multiple range test) was conducted using SPSS software (Chicago, IL, USA). Results shown are means \pm standard errors (SEs) of three replicates for each treatment. $p \leq 0.05$ was considered significant.

Fig. 1 Plants after 20 days under P-deficiency stress. **a** Phenotype of above ground. **b** Plant height. **c** Phenotype of roots. **d** Root length. **e** Phenotype of true leaves. **f** Leaf area. **g** Root-shoot ratio. CK1, control; CK2, P-deficiency; L50, P-deficiency plus 50 mg L⁻¹ La³⁺; L100, P-deficiency plus 100 mg L⁻¹ La³⁺; L150, P-deficiency plus 150 mg L⁻¹ La³⁺; L200, P-deficiency plus 200 mg L⁻¹ La³⁺. Data are means \pm SE for three biological replicates. Statistical differences ($p < 0.05$) among La³⁺ concentrations are indicated by different letters



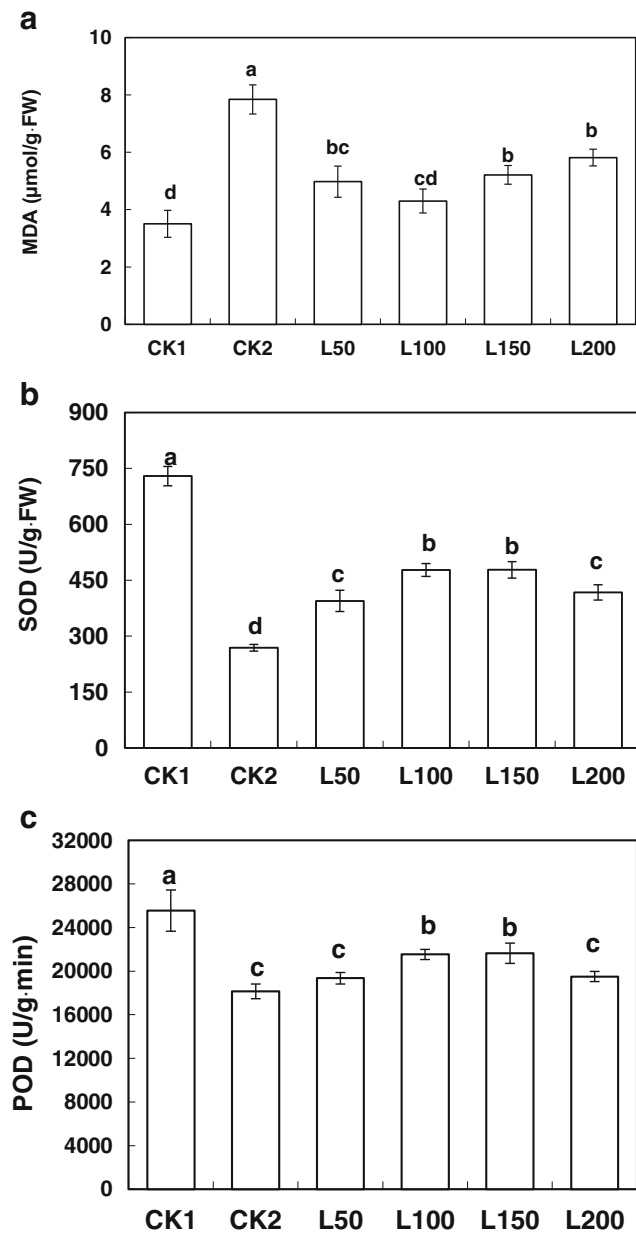
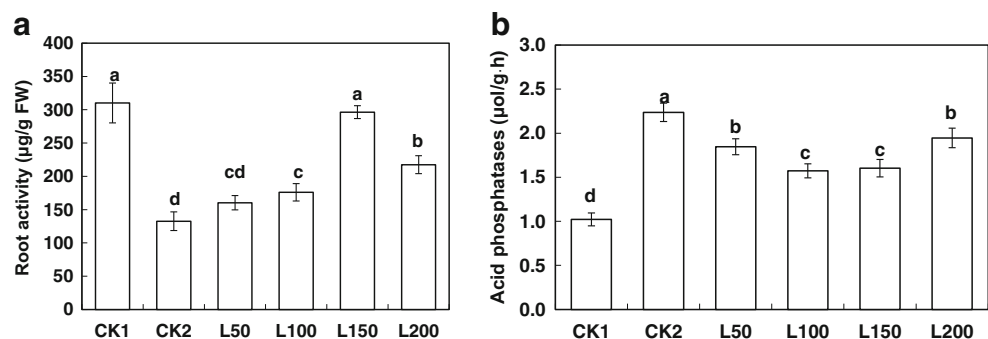


Fig. 2 Malondialdehyde and antioxidant enzyme activities in roots of adzuki bean seedlings grown under P-deficiency. **a** MDA content. **b** SOD activity. **c** POD activity. Data are means \pm SE of three replicates. Different letters above bars indicate significant difference at $p < 0.05$

Fig. 3 Root activity and acid phosphatase activity in adzuki bean seedlings were measured after a 20-day exposure to P-deficiency. **a** TTC activity. **b** Root APase activity. Data are means \pm SE for three biological replicates. Statistical differences ($P < 0.05$) among La^{3+} concentrations are indicated by different letters



La^{3+} treatment reduced P-deficiency-induced ROS accumulation

We examined the effect of P-deficiency on membrane damage and antioxidant enzyme activity under P-deficiency stress (Fig. 2). P-deficiency significantly increased MDA content by 2.24-fold compared with MDA content in controls. However, La^{3+} treatment reduced MDA accumulation, and $100 \text{ mg L}^{-1} \text{La}^{3+}$ was associated to a decrease of 45.19% in MDA content, compared to MDA content in plants subjected to the P-deficiency stress treatment alone. Concomitantly, SOD and POD activities differed similarly depending on exogenous La^{3+} treatment. Namely, SOD and POD activity levels were improved after treatment with La^{3+} by 77.57% and 18.74% in the $100 \text{ mg L}^{-1} \text{La}^{3+}$ treatment, and by 77.72% and 11.77% in the $150 \text{ mg L}^{-1} \text{La}^{3+}$ treatment, respectively, compared with the corresponding activity levels measured in plants grown under P-deficiency alone.

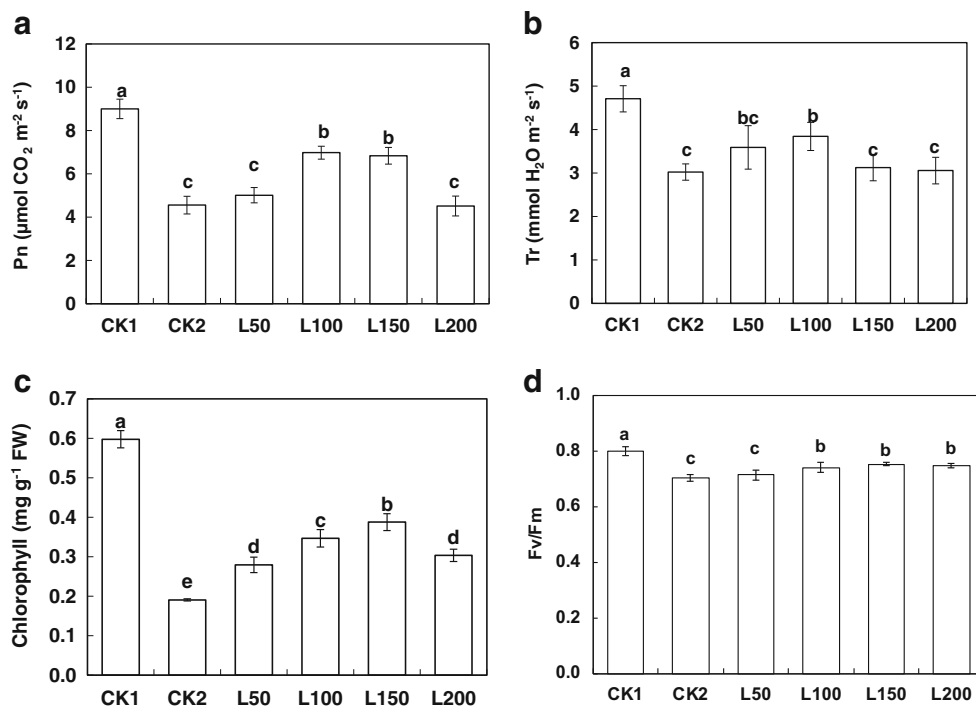
La^{3+} treatment activated root physiological traits associated with P absorption

The effect of seed priming with La^{3+} on APase activity and root activity was estimated (Fig. 3). APase activity improved, while root activity was weakened P-deficient, compared to control seedlings. APase activity in seedlings initially increased with La^{3+} concentration but did not increase any further at La^{3+} concentrations higher than 150 mg L^{-1} . However, the opposite results were recorded for root activity. APase and root activity differed substantially by 72% and 224%, between seeds treated with La^{3+} at 150 mg L^{-1} and seeds subjected to P-deficiency stress respectively.

La^{3+} treatment reduced P-deficiency-induced photosynthetic damage mechanism

P-deficiency stress significantly decreased F_v/F_m , P_n , Tr, and chlorophyll content compared with controls. La^{3+} seed priming alleviated this P-deficiency-induced leaf photosynthesis damage. As shown in Fig. 4, several leaf photosynthesis-

Fig. 4 Net photosynthesis rate (P_n), transpiration rate (Tr), maximum photochemical efficiency (F_v/F_m), and chlorophyll content in adzuki bean seedlings grown under P-deficiency. **a** P_n . **b** Tr . **c** F_v/F_m . **d** Chlorophyll. Data are means \pm SE of three replicates. Different letters above bars indicate significant difference at $P < 0.05$



related symptoms in the third leaves were investigated. P_n decreased after 20 days of P-deficiency, but La^{3+} significantly alleviated this decrease (Fig. 4a). Similarly, F_v/F_m , Tr , and chlorophyll content also decreased after 20 days in the P-deficiency treatment, but La^{3+} treatment significantly alleviated these P-deficiency-induced changes (Fig. 4b–d).

La^{3+} treatment ameliorated P-deficiency effects on root hydraulic conductivity

P-deficiency stress significantly decreased root L_p of seedlings after 20 days, which was 64.16% of the L_p observed in controls (Fig. 5). La^{3+} treatment improved root L_p , but for low concentrations of La^{3+} , there was no significant difference in root L_p from that of seedlings subjected to P-deficiency alone. Root L_p increased as La^{3+} increased to a maximum of

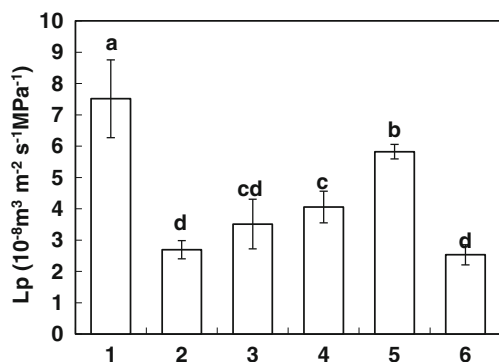


Fig. 5 Root hydraulic conductivity of adzuki bean seedlings grown under P-deficiency. Data are means \pm SE of three replicates. Different letters above bars indicate significant difference at $P < 0.05$

150 mg L^{-1} but decreased rapidly for La^{3+} concentrations above 150 mg L^{-1} . Root L_p at 150 mg L^{-1} La^{3+} significantly increased by 116.27% with respect to the P-deficiency stress treatment.

La^{3+} treatment countered P-deficiency-induced reduction in P content and improved PUE

P content in the root, shoot, and leaf were evaluated (Table 1). P-deficiency decreased P content in all plant parts studied. Under P-deficiency—and in the presence of La^{3+} —there was a significant increase in root P content, but no significant differences in P content in other plant parts were observed. Finally, extensive variation in P content was observed after treatment with 150 mg L^{-1} La^{3+} , i.e., differences of 5.22% (root), 54.09% (stem), and 29.11% (leaf), compared with the corresponding values for the P-deficiency treatment. PUE in

Table 1 Phosphorus content in roots, stems, and leaves of adzuki bean seedlings under P-deficiency

	Root (mg/g)	Shoot (mg/g)	Leaf (mg/g)
Control	1.79 \pm 0.01 a	0.89 \pm 0.07 a	1.82 \pm 0.07 a
P-0	0.61 \pm 0.01 c	0.36 \pm 0.11 c	0.72 \pm 0.026 c
P-50	0.51 \pm 0.02 e	0.48 \pm 0.02 bc	0.74 \pm 0.07 c
P-100	0.74 \pm 0.03 b	0.37 \pm 0.056 c	0.90 \pm 0.08 b
P-150	0.65 \pm 0.03 c	0.56 \pm 0.021 b	0.93 \pm 0.01 b
P-200	0.55 \pm 0.01 d	0.40 \pm 0.10 c	0.74 \pm 0.09 c

Values are means \pm SE of three replicates. Different letters above bars indicate a significant difference at $P < 0.05$

the root, stem, and leaf improved by 1.25%, 1.96%, and 2.09% after treatment with 150 mg L⁻¹ La³⁺, respectively (Fig. 6).

Discussion

Enhanced yield and improved quality of crops and vegetables can be attained by using REE-microfertilizers due to their specific properties (Mcdowell et al. 2015; Ma et al. 2014). La and other REEs have been demonstrated to play a role in alleviating stress-induced oxidative damage in plants caused by a variety of agents, including cadmium (Wang et al. 2012), salt (Liu et al. 2016; Huang and Shan 2018), drought (Zhang et al. 2006), acid rain (Liang et al. 2017), ultraviolet-B radiation (Huang et al. 2013), and others. However, thus far, there had been no reports on La inducing the antioxidant plant response to improve plant resistance to P-deficiency stress, especially in adzuki beans. In addition, here we addressed the relationship between PUE and La-treatment concentration.

Excess ROS accumulation results in lipid peroxidation damage and cell death under various environmental stress conditions (Shi et al. 2013). Hu et al. (2016) suggested that MDA can be used as an indicator of free radical damage to cell membranes. In the present study, root MDA content increased dramatically (Fig. 2) under P-deficiency because of ROS production. However, this adverse effect was reversed by La³⁺ treatment, a finding which is consistent with observations in other studies, such as those that have shown La treatment to decrease MDA content and to increase the activity of the plant antioxidative defense system under cold stress (Wang et al. 2011a). Thus, it was revealed that La was clearly involved in the regulation of antioxidant systems (D'Aquino et al. 2009). Moreover, the antioxidant defense ability is an important mechanism in plant adaptation to P-deficiency (Chen et al. 2015). Similarly, alterations in the antioxidant activities of SOD, POD, and CAT enzymes are induced by oxidative stress

in many plant species upon REE treatment. In this study, both SOD and POD activities decreased under P-deficiency, while La³⁺ treatment induced the activation of antioxidative enzymes to reduce ROS-induced oxidative stress by P-deficiency. These results are consistent with the findings of Pang et al. (2002) indicating the potential ability of La for the enhancement of scavenging of excess oxygen radicals and, eventually, for improving wheat resistance to lead stress.

The fact that photosynthesis can provide immediate and direct evidence of P-starvation effects on plants was systemically demonstrated by Hammond and White (2008). In turn, our own work confirmed the benefit of La³⁺ treatment on two aspects (stomatal and non-stomatal limitations) to maintain photosynthesis activity of adzuki bean under P-deficiency (Fig. 4). In the present study, F_v/F_m , P_n , T_r , and chlorophyll content of adzuki bean leaves were significantly reduced under P-deficiency compared to controls. These results were reversed in the presence of the proper concentration of La. Similar results suggested that a low concentration of La³⁺ could increase the photosynthetic rate by promoting the activation of RuBPCase (Chen and Zhao 2000; Chen et al. 2001). We also found that the highest concentration La³⁺ tested (200 mg L⁻¹) did not have any significant effect.

Various physiological and biochemical traits are involved in crop PUE (Wang et al. 2010). Roots play a critical role in plant tolerance to nutrient deficiency. Altered root architecture and activation of gene expression related to signal transduction and transcriptional regulation in response to P starvation has been reported (Rubio et al. 2001; Wu et al. 2003; Misson et al. 2005). Richardson et al. (2009) showed that enhancement of root elongation is the most pronounced plant adaptation strategy under low-P conditions. Additionally, we found that root length of adzuki bean seedlings increased significantly under P-deficiency stress (Fig. 1d). However, La³⁺ treatment could shorten root length. These findings are consistent with previous results indicating that La³⁺ inhibits primary root growth (Ruizherreria et al. 2012).

Enhanced synthesis and excretion of root APase has been reported in numerous plant species under P-deficiency (Liu et al. 2004; Richardson et al. 2009). Root activity could reflect root absorption, synthesis, oxidation, and reduction ability

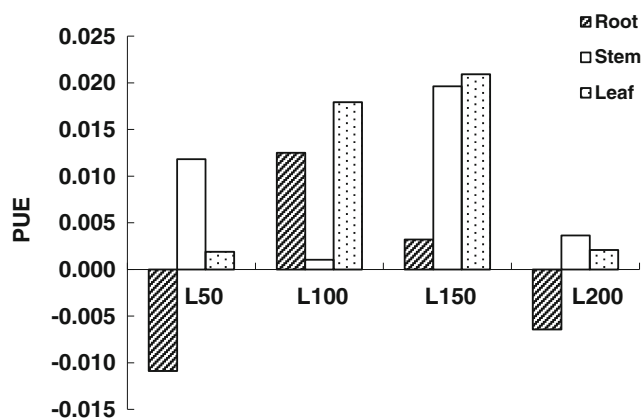


Fig. 6 Phosphorus use efficiency in roots, stems, and leaves of adzuki bean seedlings grown under P-deficiency

Table 2 MFVP method to access the difference of different concentration of La under P-deficiency

La(NO ₃) ₃	
Concentration (mg/L)	MFVP
CK1	0.74
CK2	0.24
L50	0.30
L100	0.34
L150	0.43
L200	0.32

(Sechenbater 2001; Dresbøll and Kristian 2012). In this study, we found that root activity decreased and APase activity increased under P-deficiency (Fig. 3a, b). However, these effects were alleviated in seedlings treated with $150 \text{ mg L}^{-1} \text{ La}^{3+}$.

Modification of root water-uptake capacity is important for avoiding stress-induced growth reduction (Matsuo et al. 2009; Aroca et al. 2012). Moreover, water absorption is required for nutrient absorption by roots; therefore, root hydraulic conductivity (L_p) may be involved in plant adaptation to diverse environments, such as drought, salinity, low temperature, and nutrient availability (Steudle 2000). In this study, L_p decreased significantly under P-deficiency (Fig. 5), in agreement with the reduction in root L_p by N, P, and S deficiencies observed by Clarkson et al. (2000); however, La^{3+} treatment restored root L_p , thereby increasing P content and PUE in roots, stems, and leaves (Table 1 and Fig. 6). Xu and Wang (2007) showed that P uptake could be regulated by the application of REE-containing fertilizers. Similar results were obtained for the influence of La on nutrient uptake and distribution (Xie et al. 2002).

In summary, when adzuki bean seedlings are pretreated with La^{3+} they showed greater tolerance and adaptability to subsequent P-deficiency stress. La^{3+} reduced MDA content by inducing high levels of antioxidant enzyme activities under P-deficiency. Further, our results prove that La^{3+} improves P absorption in various plant tissues, possibly as a consequence of changes to root system architecture and to the activation of physiological traits associated with responses to P-deficiency, such as APase activity. Root L_p results indicate that La^{3+} played an important role in regulating root water uptake and in facilitating nutrient transport to the leaves. La^{3+} treatment effectively alleviated the photosynthetic damage induced by P-deficiency stress. Therefore, clearly our results have widened our understanding of the mechanisms by which La^{3+} mediates plant tolerance to P-deficiency.

Conclusions

REEs are widely used chemical soil fertilizers throughout China. Our results showed that La^{3+} as a novel seed priming agent associated with the physiological mechanisms that protect the plant against P-deficiency stress. La^{3+} -primed adzuki bean seeds exhibited significant amelioration of the adverse effects of P-deficiency stress. La^{3+} priming treatment of the seeds effectively activated SOD and POD protective activities which resulted in prevention of membrane lipid peroxidation, as demonstrated by the low levels of MDA observed. Acting as a protective molecule, La^{3+} triggered the P-deficiency stress response which induced the photosynthesis protective mechanisms and maintained pigment content. La^{3+} also improved PUE in roots, stems, and leaves of adzuki bean seedlings by promoting root elongation and modulating activity associated with root responses to P-deficiency. In addition, root hydraulic

conductivity was involved in the process. Our results also indicate that the usefulness of the MFV method to compare the relative ability of different concentrations of La^{3+} to alleviate P-deficiency effects depends on the agronomic trait in question, the photosynthetic parameters, and on the root physiological characters for different treatments (Table 2). The fact that a high dose of La^{3+} (i.e., 150 mg L^{-2}) actually improved the tolerance and PUE of adzuki bean seedlings under P-deficiency stress.

Funding information This study received financial support from the National Science and Technology Supporting Programs (2015BAD22B01) and the Special Funds for Scientific Research Programs of the State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau (A314021403-CS).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Aquino LD, Pinto MC, Nardi L, Morgana M, Tommasi F (2009) Effect of some light rare earth elements on seed germination, seedling growth and antioxidant metabolism in *Triticum durum*. *Chemosphere* 75: 900–905. <https://doi.org/10.1016/j.chemosphere.2009.01.026>
- Aroca R, Porcel R, Ruizlozano JM (2012) Regulation of root water uptake under abiotic stress conditions. *J Exp Bot* 63:43–57. <https://doi.org/10.1093/jxb/err266>
- Balabusta M, Szafrńska K, Posmyk MM (2016) Exogenous melatonin improves antioxidant defense in cucumber seeds (*Cucumis sativus* L.) germinated under chilling stress. *Front Plant Sci* 7(575). <https://doi.org/10.3389/fpls.2016.00575>
- Bao SD (2000) Soil agro-chemistry analysis. China Agr Press, Beijing, pp 268–270
- Baxter A, Mittler R, Suzuki N (2014) ROS as key players in plant stress signalling. *J Exp Bot* 65:1229–1240. <https://doi.org/10.1093/jxb/ert375>
- Casson SA, Lindsey K (2003) Genes and signalling in root development. *New Phytol* 158:11–38. <https://doi.org/10.1046/j.1469-8137.2003.00705.x>
- Chen WJ, Zhao GW (2000) Effects of rare earth ions on activity of Rubpase in tobacco. *Plant Sci* 152:78–84
- Chen WJ, Tao Y, Gu YH, Zhao GW (2001) Effect of lanthanide chloride on photosynthesis and dry matter accumulation in tobacco seedlings. *Biol Trace Elem Res* 79:169–176. <https://doi.org/10.1385/BTER:79:2:169>
- Chen S, Zhao H, Ding G, Xu F (2015) Genotypic differences in antioxidant response to phosphorus deficiency in *brassica napus*. *Plant Soil* 391:19–32. <https://doi.org/10.1007/s11104-015-2395-7>
- Clarkson DT, Carvajal M, Henzler T, Waterhouse RN, Smyth AJ, Cooke DT, Steudle E (2000) Root hydraulic conductance: diurnal aquaporin expression and the effects of nutrient stress. *J Exp Bot* 51:61–70. <https://doi.org/10.1093/jexbot/51.342.61>
- Ding SM, Liang T, Zhang CS, Yan JC, Zhang ZL (2005) Accumulation and fractionation of rare earth elements (REEs) in wheat: controlled by phosphate precipitation, cell wall absorption and solution complexation. *J Exp Bot* 56:2765–2775. <https://doi.org/10.1093/jxb/eri270>

- Dresbøll DB, Kristian KT (2012) Spatial variation in root system activity of tomato (*Solanum lycopersicum*, L.) in response to short and long-term waterlogging as determined by ^{15}N uptake. *Plant Soil* 357: 161–172. <https://doi.org/10.1007/s11104-012-1135-5>
- El-Ramady H (2010) Ecotoxicol of REEs. Vdm Verlag Dr Müller, Saarbrücken
- Foyer CH, Noctor G (2005) Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell* 17:1866–1875. <https://doi.org/10.1105/tpc.105.033589>
- Foyer CH, Shigeoka S (2011) Understanding oxidative stress and antioxidant functions to enhance photosynthesis. *Plant Physiol* 155:93–100. <https://doi.org/10.1104/pp.110.166181>
- Goecke F, Jerez CG, Zachleder V, Figueroa FL, Bišová K, Řezanka T, Vítová M (2015) Use of lanthanides to alleviate the effects of metal ion-deficiency in *Desmodium quadricauda* (Sphaeropleales, Chlorophyta). *Front Microbiol* 6(2). <https://doi.org/10.3389/fmicb.2015.00002>
- Gupta M, Bishit S, Singh B, Gulati A, Tewari R (2011) Enhanced biomass and steviol glycosides in stevia rebaudiana treated with phosphate-solubilizing bacteria and rock phosphate. *Plant Growth Regul* 65: 449–457. <https://doi.org/10.1007/s10725-011-9615-9>
- Hagenbeek D, Quatrano RS, Rock CD (2000) Trivalent ions activate abscisic acid-inducible promoters through an *ABI1*-dependent pathway in rice protoplasts. *Plant Physiol* 123:1553–1560
- Hammond JP, White PJ (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. *J Exp Bot* 59:93–109. <https://doi.org/10.1093/jxb/erm221>
- Hedley MJ, White RE, Nye PH (1982) Plant-induced changes in the rhizosphere of rape (*Brassica Napus* var. emerald) seedlings. *New Phytol* 91:45–56. <https://doi.org/10.1111/j.1469-8137.1982.tb03291.x>
- Hu LX, Zhang ZF, Xiang ZX, Yang ZJ (2016) Exogenous application of citric acid ameliorates the adverse effect of heat stress in Tall Fescue (*Lolium arundinaceum*). *Front Plant Sci* 7:179. <https://doi.org/10.3389/fpls.2016.00179>
- Huang G, Shan C (2018) Lanthanum improves the antioxidant capacity in chloroplast of tomato seedlings through ascorbate-glutathione cycle under salt stress. *Sci Hortic* 232:264–268. <https://doi.org/10.1016/j.scienta.2018.01.025>
- Huang G, Wang L, Zhou Q (2013) Lanthanum (iii) regulates the nitrogen assimilation in soybean seedlings under ultraviolet-B radiation. *Biol Trace Elem Res* 151:105–112. <https://doi.org/10.1007/s12011-012-9528-0>
- Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agron Sustain Dev* 27:29–43. <https://doi.org/10.1051/agro:2006011>
- Li H, Sun Q, Zhao S, Zhang W (2000) Principles and techniques of plant physiological biochemical experiment. Higher Edu, Beijing, pp 195–197
- Li H, Huang G, Meng Q, Ma L, Yuan L, Wang F (2011) Integrated soil and plant phosphorus management for crop and environment in China. A Review. *Plant Soil* 349:157–167. <https://doi.org/10.1007/s11104-013-1799-5>
- Liang C, Li L, Su L (2017) Effect of lanthanum on plasma membrane H⁺-ATPase in rice (*Oryza sativa*) under acid rain stress. *J Plant Growth Regul* 3:1–11. <https://doi.org/10.1007/s00344-017-9740-4>
- Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol* 148:350–382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
- Liu Y, Mi GH, Chen FJ, Zhang JH, Zhang FS (2004) Rhizosphere effect and root growth of two maize (*Zea mays* L.) genotypes with contrasting P efficiency at low P availability. *Plant Sci* 167:217–223. <https://doi.org/10.1016/j.plantsci.2004.02.026>
- Liu C, Yang Z, Hu YG (2015) Drought resistance of wheat alien chromosome addition lines evaluated by membership function value based on multiple traits and drought resistance index of grain yield. *Field Crop Res* 179:103–112. <https://doi.org/10.1016/j.fcr.2015.04.016>
- Liu RQ, Xu XJ, Wang S, Shan CJ (2016) Lanthanum improves salt tolerance of maize seedlings. *Photosynthetica* 54:148–151. <https://doi.org/10.1007/s11099-015-0157-7>
- Ma JJ, Ren YJ, Yan LY (2014) Effects of spray application of lanthanum and cerium on yield and quality of Chinese cabbage (*Brassica chinensis*, L.) based on different seasons. *Biol Trace Elem Res* 160: 427–432. <https://doi.org/10.1007/s12011-014-0062-0>
- Macdonald GK, Bennett EM, Potter PA, Ramankutty N (2011) Agronomic phosphorus imbalances across the world's croplands. *PNAS* 108:3086–3091. <https://doi.org/10.1073/pnas.1010808108>
- Matsuo N, Ozawa K, Mochizuki T (2009) Genotypic differences in root hydraulic conductance of rice (*Oryza sativa* L.) in response to water regimes. *Plant Soil* 316:25–34. <https://doi.org/10.1007/s11104-008-9755-5>
- McDowell RW, Catto W, Orchiston T (2015) Can the application of rare earth elements improve yield and decrease the uptake of cadmium in ryegrass-dominated pastures? *Soil Res* 53:826–834. <https://doi.org/10.1071/SR15073>
- Misson J, Raghothama KG, Jain A, Jouhet J, Block MA, Bagny R, Ortel P, Creff A, Somerville S, Rolland N, Doumas P, Nacry P, Herrera-Estrella L, Nussaume L, Thibaud MC (2005) A genome-wide transcriptional analysis using *Arabidopsis thaliana* Affymetrix gene chips determined plant responses to phosphate deprivation. *PNAS* 102:33. <https://doi.org/10.1073/pnas.0505266102>
- Mittler R, Vanderauwera M, Suzuki N, Miller G, Tognetti VB, Vandepoele K, Gollery M, Shulaev V, Breusegem FV (2011) ROS signaling: the new wave? *Trends Plant Sci* 16:300–309. <https://doi.org/10.1016/j.tplants.2011.03.007>
- Pang X, Wang DH, Xing XY, Peng A, Zhang FS, Li CJ (2002) Effect of La^{3+} on the activities of antioxidant enzymes in wheat seedlings under lead stress in solution culture. *Chemosphere* 47(10):1033–1039
- Paparella S, Araújo SS, Rossi G, Wijayasinghe M, Carbonera D, Balestrazzi A (2015) Seed priming: state of the art and new perspectives. *Plant Cell Rep* 34:1281–1293. <https://doi.org/10.1007/s00299-015-1784-y>
- Řezanka T, Kaineder K, Mezricky D, Řezanka M, Bišová K, Zachleder V, Vítová M (2016) The effect of lanthanides on photosynthesis, growth, and chlorophyll profile of the green alga *Desmodium quadricauda*. *Photosynth Res* 130:335–346. <https://doi.org/10.1007/s11120-016-0263-9>
- Richardson AE, Hocking PJ, Simpson RJ, George TS (2009) Plant mechanisms to optimise access to soil phosphorus. *Crop Pasture Sci* 60: 124–143. <https://doi.org/10.1071/CP07125>
- Rubio V, Linhares F, Solano R, Martín AC, Iglesias J, Leyva A, Paz-Ares J (2001) A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. *Genes Dev* 15:2122–2133. <https://doi.org/10.1101/gad.204401>
- Ruizherreria LF, Sánchez-Calderón L, Herrera-Estrella L, López-Bucio J (2012) Rare earth elements lanthanum and gadolinium induce phosphate-deficiency responses in *Arabidopsis thaliana* seedlings. *Plant Soil* 353:231–247. <https://doi.org/10.1007/s11104-011-1026-1>
- Sechenbater, Wu HY (2001) Effect of different stress on roots activity and nitrate reductase activity in *Zea mays* L. *Agric Res Arid Areas* 19: 67–70.
- Shi H, Ye T, Chen F, Cheng ZM, Wang YP, Yang PF, Zhang YS, Chan ZL (2013) Manipulation of arginase expression modulates abiotic stress tolerance in *Arabidopsis*: effect on arginine metabolism and ROS accumulation. *J Exp Bot* 64:1367–1379. <https://doi.org/10.1093/jxb/ers400>
- Shi ZX, Yao Y, Zhu YY, Ren GX (2017) Nutritional composition and biological activities of 17 Chinese adzuki bean (*Vigna angularis*)

- varieties. *Food Agric Immunol* 28:78–89. <https://doi.org/10.1080/09540105.2016.1208152>
- Shu X, Yin LY, Zhang QF, Wang WB (2012) Effect of Pb toxicity on leaf growth, antioxidant enzyme activities, and photosynthesis in cuttings and seedlings of *Jatropha curcas* L. *Environ Sci Pollut Res* 19:893–902. <https://doi.org/10.1007/s11356-011-0625-y>
- Steudle E (2000) Water uptake by plant roots: an integration of views. *Plant Soil* 226:45–56. <https://doi.org/10.1023/A:1026439226716>
- Suliaman S, Tran LS (2015) Phosphorus homeostasis in legume nodules as an adaptive strategy to phosphorus deficiency. *Plant Sci* 239:36–43. <https://doi.org/10.1016/j.plantsci.2015.06.018>
- Szafrańska K, Reiter RJ, Posmyk MM (2016) Melatonin application to *pisum sativum* L. seeds positively influences the function of the photosynthetic apparatus in growing seedlings during paraquat-induced oxidative stress. *Front Plant Sci* 7(1663). <https://doi.org/10.3389/fpls.2016.01663>
- Tabatabai MA, Bremner JM (1969) Use of *p*-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol Biochem* 1:301:307–301:307. [https://doi.org/10.1016/0038-0717\(69\)90012-1](https://doi.org/10.1016/0038-0717(69)90012-1)
- Thomas PJ, Carpenter D, Boutin C, Allison JE (2014) Rare earth elements (REEs): effects on germination and growth of selected crop and native plant species. *Chemosphere* 96:57–66. <https://doi.org/10.1016/j.chemosphere.2013.07.020>
- Thompson AJ, Andrews J, Mulholland BJ, Mckee JM, Hilton HW, Horridge JS, Farquhar GD, Smeeton RC, Smillie IR (2007) Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion. *Plant Physiol* 143:1905–1917. <https://doi.org/10.1104/pp.106.093559>
- Thuysma R, Valentine A, Kleinert A (2014) Phosphorus deficiency affects the allocation of below-ground resources to combined cluster roots and nodules in *lupinus albus*. *J Plant Physiol* 171:285–291. <https://doi.org/10.1016/j.jplph.2013.09.001>
- Trubat R, Cortina J, Vilagrosa A (2012) Root architecture and hydraulic conductance in nutrient deprived *pistacia lentiscus* L. seedlings. *Oecologia* 170:899–908. <https://doi.org/10.1007/s00442-012-2380-2>
- Tyler G (2004) Rare earth elements in soil and plant systems—a review. *Plant Soil* 267:191–206
- Wang X, Shi GX, Xu QS, Wang CT (2005) Toxic effects of lanthanum, cerium, chromium and zinc on *Potamogeton malaianus*. *J Rare Earths* 23:367–371
- Wang XR, Shen JB, Liao H (2010) Acquisition or utilization, which is more critical for enhancing phosphorus efficiency in modern crops? *Plant Sci* 179:302–306. <https://doi.org/10.1016/j.plantsci.2010.06.007>
- Wang CR, Lu XW, Tian Y, Cheng T, Hu LL, Chen FF, Jinag CJ, Wang XR (2011a) Lanthanum resulted in unbalance of nutrient elements and disturbance of cell proliferation cycles in *V. faba* L. seedlings. *Biol Trace Elem Res* 143:1174–1181. <https://doi.org/10.1007/s12011-010-8939-z>
- Wang Y, Zhou M, Gong X, Liu C, Hong MM, Wang L, Hong F (2011b) Influence of lanthanides on the antioxidative defense system in maize seedlings under cold stress. *Biol Trace Elem Res* 142:819–830. <https://doi.org/10.1007/s12011-010-8814-y>
- Wang C, Luo X, Tian Y, Xie Y, Wang SC, Li YY, Tian L, Wang X (2012) Biphasic effects of lanthanum on *vicia faba* L. seedlings under cadmium stress, implicating finite antioxidation and potential ecological risk. *Chemosphere* 86:530–537. <https://doi.org/10.1016/j.chemosphere.2011.10.030>
- Wang E, Bell M, Luo ZK, Moody P, Probert ME (2014) Modelling crop response to phosphorus inputs and phosphorus use efficiency in a crop rotation. *Field Crop Res* 155:120–132. <https://doi.org/10.1016/j.fcr.2013.09.015>
- Wasaki J, Yamamura T, Shinano T, Osaki M (2003) Secreted acid phosphatase is expressed in cluster roots of lupin in response to phosphorus deficiency. *Plant Soil* 248:129–136
- Wissuwa M (2003) How do plants achieve tolerance to phosphorus deficiency? small causes with big effects. *Plant Physiol* 133:1947–1958. <https://doi.org/10.1104/pp.103.029306>
- Wu P, Ma LG, Hou XL, Wang MY, Wu YR, Liu FY, Deng XW (2003) Phosphate starvation triggers distinct alterations of genome expression in *Arabidopsis* roots and leaves. *Plant Physiol* 132:1260–1271. <https://doi.org/10.1104/pp.103.021022>
- Xie ZB, Zhu JG, Chu HY, Zhang YL, Zeng Q, Ma HL (2002) Effect of lanthanum on rice production, nutrient uptake, and distribution. *J Plant Nutr* 25:2315–2331. <https://doi.org/10.1081/PLN-120014078>
- Xu XK, Wang ZJ (2007) Phosphorus uptake and translocation in field-grown maize after application of rare earth-containing fertilizer. *J Plant Nutr* 30:557–568. <https://doi.org/10.1080/01904160701209287>
- Yan X, Wu P, Ling H, Xu G, Xu F, Zhang Q (2006) Plant nutriomics in China: an overview. *Ann Bot* 98:473–482. <https://doi.org/10.1093/aob/mcl116>
- Zadeh L (1965) Fuzzy sets. *Inf Control* 8:338–353. [https://doi.org/10.1016/S0019-9958\(65\)90241-X](https://doi.org/10.1016/S0019-9958(65)90241-X)
- Zhang L, Yang T, Gao Y, Liu Y, Zhang T, Xu S (2006) Effect of Lanthanum Ions (La³⁺) on Ferritin-Regulated Antioxidant Process Under PEG Stress. *Biol Trace Elem Res* 113:193–208. <https://doi.org/10.1385/BTER:113:2:193>