



Studies on the Effects of OsRGA1 Involved in Responses to Low Phosphorus Stress in Rice

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Abstract

Phosphorus (P) is an essential macronutrient that plays a critical role in plant growth, energy transfer, and metabolic regulation, yet its low availability in soils severely limits crop productivity. The heterotrimeric G protein α -subunit gene OsRGA1 has been implicated in nutrient signaling pathways, but its role in low phosphorus (LP) stress adaptation in rice remains insufficiently characterized. In this study, we investigated the functional contribution of OsRGA1 to LP stress responses using wild-type Nipponbare (NIP), OsRGA1 knockout mutants (R-5, R-8), and overexpression lines (ROE1, ROE2) grown hydroponically under normal phosphorus (NP, 300 μ M KH_2PO_4) and LP (5 μ M KH_2PO_4) conditions. Phenotypic analysis revealed that LP stress markedly promoted primary root elongation in all genotypes, with the most pronounced increase observed in ROE2 (56.14%) and the least in mutants R-5 and R-8 (23.73% and 21.14%, respectively). LP treatment significantly enhanced antioxidant enzyme activities (SOD, POD, CAT), acid phosphatase activity, and the expression of phosphorus transporter genes (OsPT2, OsPT6, OsPT8), while reducing root oxidation capacity. Notably, these LP-induced responses were amplified in overexpression lines, particularly ROE2, and attenuated in knockout mutants relative to NIP. Strigolactone (5DS) content also increased substantially under LP stress, with the magnitude of change correlating with OsRGA1 expression levels, suggesting hormonal cross-talk in root adaptive responses. Gene expression profiling demonstrated that OsRGA1 transcript abundance peaked at 5 μ M P, confirming its sensitivity to LP conditions. The data collectively indicate that OsRGA1 acts as a positive regulator in LP stress adaptation by promoting primary root elongation, enhancing antioxidant defenses, stimulating acid phosphatase activity, and upregulating P transporter genes. These coordinated physiological and molecular adjustments improve phosphorus acquisition efficiency under nutrient-limited conditions. Our findings provide new insights into the

regulatory network linking G protein signaling, hormonal pathways, and nutrient stress responses in rice. Targeted manipulation of OsRGA1 may represent a promising strategy for breeding rice cultivars with improved phosphorus-use efficiency, contributing to sustainable agricultural productivity in P-deficient soils.

Subject Areas

Science

Keywords

OsRGA1, Low Phosphorus Stress, Root Development, Antioxidant Enzymes, Acid Phosphatase, Phosphorus Transporter, Strigolactone, Rice

1. Introduction

Phosphorus (P) is a vital plant macronutrient, second only to nitrogen in its importance for growth and development. It is a structural component of nucleic acids, phospholipids, and energy molecules such as ATP. It plays a central role in numerous physiological and biochemical processes, including energy transfer, signal transduction, and metabolic regulation. Despite its importance, P availability in soils is often limited because most soil phosphorus exists in insoluble forms bound to calcium, iron, or aluminum, rendering it inaccessible for direct plant uptake. Consequently, phosphorus deficiency is a major constraint to crop productivity worldwide.

Traditional approaches to mitigate P deficiency rely heavily on applying phosphate fertilizers. However, excessive use of such inputs can lead to environmental problems, including eutrophication and resource depletion. Therefore, enhancing plant phosphorus-use efficiency through genetic and physiological improvement is an important goal for sustainable agriculture. Plants have evolved diverse adaptive strategies to cope with low phosphorus (LP) availability, including morphological modifications, physiological adjustments, and changes in gene expression to improve phosphorus acquisition and utilization.

The root system is the primary interface for phosphorus uptake, and both external nutrient status and internal hormonal regulation strongly influence its architecture and function. Among plant hormones, strigolactones (SLs) have emerged as key regulators of root development and architecture, especially under P-limiting conditions. SLs interact with other phytohormones such as auxins and cytokinins to modulate root elongation, lateral root formation, and root hair growth, enhancing the plant's ability to explore the soil for nutrients. Previous studies have shown that SL production increases under phosphorus deficiency and contributes to improved activity of protective enzymes in roots [1].

The heterotrimeric G protein α -subunit gene OsRGA1 in rice (*Oryza sativa* L.) is known to participate in various signaling pathways, including those related to

nutrient responses. However, its role in mediating adaptation to LP stress remains poorly understood. Given that G protein signaling can influence root morphology, hormone signaling, and stress responses, understanding the function of OsRGA1 under P deficiency is essential for elucidating the molecular networks that regulate phosphorus-use efficiency.

This study investigates the role of OsRGA1 in the physiological, morphological, and molecular responses of rice seedlings to LP stress. Using wild-type Nipponbare, OsRGA1 knockout mutants, and overexpression lines, we evaluated root system architecture, antioxidant enzyme activities, acid phosphatase activity, strigolactone levels, and phosphorus transporter gene expression under NP and LP conditions. Our findings provide new insights into the regulatory mechanisms of OsRGA1 in phosphorus acquisition and offer potential strategies for breeding rice cultivars with enhanced adaptation to phosphorus-deficient environments.

2. Literature Review

2.1. Phosphorus Role and Deficiency in Rice

Phosphorus (P) is an essential macronutrient required for rice growth, serving as a key structural component of nucleic acids, phospholipids, and energy molecules such as ATP. It plays a central role in energy transfer, signal transduction, and metabolic regulation, influencing processes from seed germination to grain filling. Adequate phosphorus availability supports strong root development, tillering, and early maturation, directly contributing to yield and grain quality.

In most soils, however, phosphorus exists predominantly in insoluble forms bound to calcium, iron, or aluminum, making it inaccessible for plant uptake. The concentration of soluble inorganic phosphate in the soil solution is typically too low to meet crop demands, leading to phosphorus deficiency, a major limitation in rice production worldwide. Symptoms include stunted growth, reduced tiller numbers, dark green foliage, and delayed maturity [2].

Farmers often address P deficiency through phosphate fertilizer application, but overuse can cause environmental degradation and deplete finite phosphate rock reserves. As a sustainable alternative, improving phosphorus-use efficiency (PUE) through genetic and physiological approaches is critical. Understanding the mechanisms that regulate P acquisition and utilization, particularly those involving root system architecture and nutrient signaling pathways, is vital for breeding rice cultivars adapted to low-phosphorus environments.

2.2. OsRGA1 and G-Protein Signaling

Heterotrimeric G proteins are key molecular switches that mediate signal transduction between extracellular stimuli and intracellular responses in plants. They consist of three subunits— α , β , and γ —with the α -subunit playing a central role in binding and hydrolyzing GTP to regulate downstream [3] signaling pathways. In rice, OsRGA1 encodes the G protein α -subunit and is implicated in controlling various physiological processes, including growth, development, and stress

responses.

G-protein signaling influences a wide range of plant traits such as hormone perception, root architecture, and nutrient acquisition. Evidence suggests that OsRGA1 interacts with phytohormonal pathways, including auxins, cytokinins, and strigolactones, which are critical regulators of root system architecture under nutrient stress conditions. Through these interactions, OsRGA1 can modulate root elongation, lateral root formation, and adaptive responses to environmental cues.

Under low phosphorus (LP) conditions, root system modifications are essential for improving phosphorus [4] uptake. OsRGA1 is hypothesized to function as a positive regulator in this adaptation by enhancing root elongation, antioxidant defense, and nutrient transporter expression. Understanding OsRGA1's role within the broader G-protein signaling network provides valuable insight into the molecular mechanisms underlying phosphorus-use efficiency in rice and offers potential targets for crop improvement.

2.3. Current Knowledge Gaps

Although the role of phosphorus in rice growth and the general mechanisms of low phosphorus (LP) adaptation are well-documented, the specific molecular pathways linking G-protein signaling to phosphorus acquisition remain poorly understood. While OsRGA1, encoding the G protein α -subunit, has been associated with growth regulation, hormone signaling, and stress responses, its direct contribution to LP stress adaptation has not been fully clarified.

Previous studies have shown that phosphorus deficiency alters root system architecture, increases acid phosphatase activity, and induces phosphorus transporter genes, often mediated by hormonal signals such as strigolactones. However, it is unclear how OsRGA1 integrates these hormonal pathways with physiological and morphological changes under LP conditions. Furthermore, the extent to which OsRGA1 modulates antioxidant defense systems, root oxidative capacity, and strigolactone biosynthesis during phosphorus stress remains largely unexplored.

Another gap lies in comparative functional analysis among wild-type, knockout, and overexpression lines under controlled phosphorus conditions, which can reveal the regulatory strength and direction of OsRGA1-mediated responses. Addressing these gaps is essential to determine whether OsRGA1 acts as a central regulatory hub in LP adaptation and to assess its potential as a target for breeding rice varieties with improved phosphorus-use efficiency.

2.4. Study Objectives

This study aims to elucidate the role of OsRGA1, a heterotrimeric G protein α -subunit gene, in regulating rice responses to low phosphorus (LP) stress. Specifically, we sought to:

- 1) Assess OsRGA1 expression patterns under varying phosphorus concentrations to determine its sensitivity and peak induction point during LP stress.

2) Compare phenotypic responses, including primary root elongation and overall seedling morphology, among wild-type Nipponbare, OsRGA1 knockout mutants, and overexpression lines under normal phosphorus (NP) and LP conditions.

3) Evaluate physiological changes such as antioxidant enzyme activities, acid phosphatase activity, root oxidation capacity, and strigolactone content in relation to OsRGA1 function.

4) Examine molecular responses by quantifying the expression of phosphorus transporter genes (OsPT2, OsPT6, OsPT8) in different genotypes during LP stress [5].

By integrating morphological, physiological, and molecular analyses, this research seeks to clarify whether OsRGA1 acts as a positive regulator in LP stress adaptation. The findings will contribute to a deeper [6] understanding of the signaling networks linking G-protein activity to nutrient acquisition, offering potential strategies for developing rice varieties with enhanced phosphorus-use efficiency for sustainable agriculture.

3. Materials and Methods

3.1. Plant Materials and Growth Conditions

Five rice genotypes were used in this study: wild-type *Oryza sativa* L. japonica cv. Nipponbare (NIP), two OsRGA1 knockout mutants (R-5, R-8) generated via CRISPR/Cas9 in the NIP background, and two overexpression lines (ROE1, ROE2) produced through transgenic transformation.

Seeds were surface-sterilized, germinated, and transferred to a hydroponic culture system in a controlled [7] growth chamber. Plants were maintained under a 28°C/25°C day/night temperature regime, 12-hour photoperiod, and 70% relative humidity. Nutrient solutions were prepared according to standard rice hydroponic formulations and refreshed every three days to ensure consistent nutrient availability.

Two phosphorus treatments were applied: normal phosphorus (NP) at 300 µM KH₂PO₄ [8] and low phosphorus (LP) at 5 µM KH₂PO₄. Seedlings were grown under NP conditions for initial establishment before being subjected to their respective phosphorus treatments.

Plants were arranged in a randomized design with three biological replicates per genotype and treatment. Growth was monitored regularly, and morphological, physiological, and molecular analysis samples were collected after defined treatment durations to evaluate OsRGA1-mediated responses to LP stress.

3.2. Low Phosphorus Treatment and Measurements

After seed germination and initial establishment under normal phosphorus (NP) conditions, seedlings of all five genotypes, NIP, R-5, R-8, ROE1, and ROE2, were transferred to hydroponic solutions containing either NP (300 µM KH₂PO₄) or low phosphorus (LP, 5 µM KH₂PO₄). Treatments were maintained in a controlled

environment with solutions replaced every three days to ensure stable nutrient levels [9].

Morphological measurements included seedling height, primary root length, root number, and dry biomass of shoots and roots. Root traits were assessed using a root scanner and image analysis software, while biomass was determined after drying samples to a constant weight.

Physiological indexes measured included root oxidation capacity via the α -naphthylamine method, antioxidant enzyme activities (SOD, POD, CAT) using spectrophotometric assays, and acid phosphatase activity using p-nitrophenyl phosphate as a substrate. Strigolactone (5DS) [10] content was quantified by LC-MS after solvent extraction.

For molecular analysis, total RNA was extracted from leaf tissues, reverse transcribed, and used for qRT-PCR to measure expression levels of OsRGA1 and phosphorus transporter genes (OsPT2, OsPT6, OsPT8). Each treatment was performed with three biological replicates, and data were analyzed statistically to assess genotype-specific responses to LP stress.

3.3. Biochemical and Molecular Analyses

Biochemical assays were performed to evaluate physiological responses to low phosphorus (LP) stress. Antioxidant enzyme activities, superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were measured from fresh leaf and root extracts using spectrophotometric [11] methods. Root oxidation capacity was determined via the α -naphthylamine method. Acid phosphatase activity was assayed using p-nitrophenyl phosphate (p-NPP) as a substrate, with absorbance recorded at 410 nm to estimate enzymatic conversion of organic phosphorus to inorganic forms.

Acid phosphatase activity

1) Take 50 mg of rice leaves and roots, respectively, add 1 mL of pre-cooled acetic acid-sodium acetate solution, and grind the liquid nitrogen to powder. 4°C, 8000 rpm centrifuges for 10 min, Supernatant is the enzyme solution.

2) Configure the reaction fluid in the 2 mL EP tube according to **Table 1**.

Table 1. Preparation of acid phosphatase assay solution.

Number	Blank	Control
Enzyme extract (μ L)	0	2.5
Disodium p-nitrophenyl phosphate (μ L)	500	500
Acetic acid-sodium acetate solution (μ L) 37°C, 1 h	50	47.5
2 M NaOH (μ L)	500	500

3) Draw a standard curve and configure p-Nitrophenol (p-NP) standard liquid with different concentration gradients in the 2 mL EP tube according to **Table 2**.

Table 2. Production of standard curves of p-NP.

Number	1	2	3	4	5	6
P NP content (μL)	0	6.25	18.75	31.25	43.75	56.75
Acetic acid sodium acetate solution (μL)	550	543.75	531.25	518.75	506.25	493.75
p NP content (μg)	0	6.25	18.75	31.25	43.75	56.25
NaOH (μL)	550	500	500	500	500	500

4) The reaction liquid and p-NP standard liquid are centrifuged at 8000 rpm for 5 minutes in the centrifuge.

5) Take 200 μL and add it to the 96-hole enzyme plate to measure the light absorption value of the sample at 410 nm.

Strigolactone (5DS) [12] content, a key hormone regulating root architecture under nutrient stress, was quantified from leaf samples using liquid chromatography-mass spectrometry (LC-MS) following solvent extraction.

For molecular analysis, total RNA was extracted from leaves of each genotype using an RNAPure Plant Kit, followed by DNase I treatment to eliminate genomic DNA. Reverse transcription was performed with HiFiScript gDNA Removal RT MasterMix, and quantitative real-time PCR (qRT-PCR) was conducted using gene-specific primers. Target genes included OsRGA1 and phosphorus transporter genes OsPT2, OsPT6, and OsPT8. Relative expression levels were determined using internal reference genes and the $2^{-\Delta\Delta Ct}$ method [13].

All biochemical and molecular measurements were carried out in three biological replicates per treatment, ensuring reliable evaluation of genotype-specific responses to LP stress.

3.4. Statistical Analysis

All experimental data were analyzed using SPSS 25.0 statistical software. Values are expressed as mean \pm standard deviation (SD) from three independent biological replicates. Differences between treatments or genotypes were evaluated with one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significance levels [14]. For pairwise comparisons, independent-sample t-tests were used where appropriate.

A significance threshold of $P < 0.05$ was used to indicate statistically significant differences, while $P < 0.01$ was considered highly important. Data visualization, including bar graphs and line charts, was performed using GraphPad Prism 9.0, with error bars representing the standard deviation across replicates.

Before statistical testing, data were examined for normality and homogeneity of variance to ensure the validity [15] of parametric analyses. In cases where assumptions were not met, data transformations were applied to normalize the distribution. All reported statistical results are based on untransformed data for ease of interpretation.

This approach ensured rigorous evaluation of morphological, physiological, biochemical, and molecular parameters, allowing for accurate assessment of genotype-specific responses to low phosphorus (LP) stress and the functional role of OsRGA1 in rice phosphorus-use efficiency.

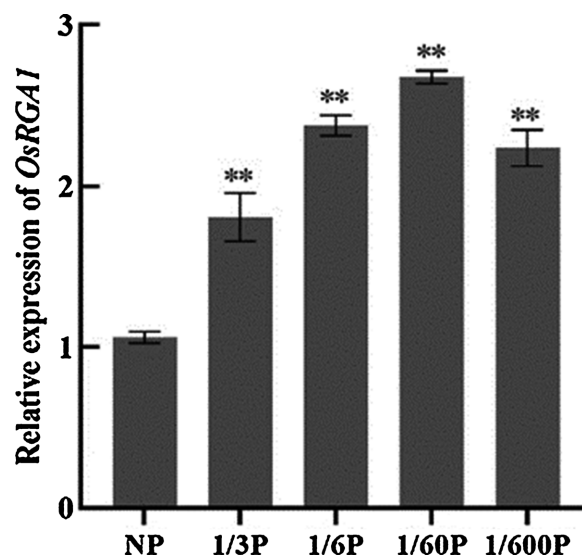
4. Results

4.1. OsRGA1 Expression under Low P Stress

To determine the responsiveness of OsRGA1 to phosphorus availability, Nipponbare (NIP) seedlings were grown under normal phosphorus (NP, 300 μM KH_2PO_4) and a gradient of low phosphorus (LP) concentrations: 100 μM , 50 μM , 5 μM , and 0.5 μM KH_2PO_4 . After 10 days of treatment, leaf samples were collected for RNA extraction and quantitative real-time PCR analysis.

Results showed that OsRGA1 expression (**Figure 1**) was significantly upregulated under LP conditions compared to NP. The highest transcript level was observed at 5 μM phosphorus, where expression peaked before declining at the most severe deficiency (0.5 μM). This indicates that OsRGA1 responds dynamically to phosphorus stress, with maximum induction occurring under moderate deficiency.

The observed expression pattern suggests a potential regulatory threshold, where OsRGA1 activation is optimized to enhance adaptive responses such as root elongation, antioxidant defense, and nutrient transporter gene expression. These findings provided the basis for selecting 5 μM KH_2PO_4 as the LP treatment for subsequent morphological, physiological, and molecular analyses, ensuring consistent assessment of OsRGA1 function under conditions that strongly elicit its transcriptional response.



Note: “**” indicates a significant level of difference between treatments ($P < 0.01$).

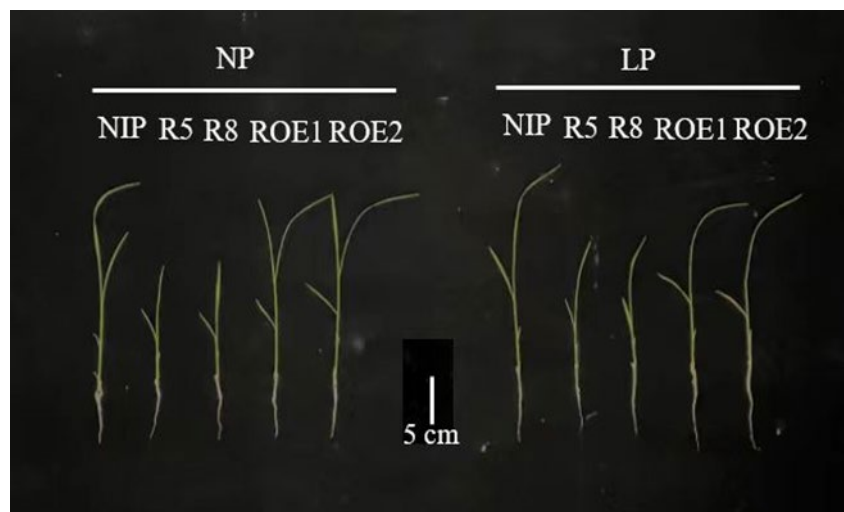
Figure 1. Expression of OsRGA1 in rice leaves under different concentrations of phosphorus.

4.2. Rice Seedling Phenotype

Under conventional phosphorus conditions, the seedlings of R-5 and R-8 are higher than those of the wild type, and the seedlings of ROE1 and ROE2 were not significantly different from those of the wild type; the [16] main root trend of each genotype of seedlings is consistent. Low phosphorus stress treatment has no significant impact on the growth of the upper part of the rice field, but it significantly promotes the elongation of the main root. The main root length of NIP, R-5, R-8, ROE1, and ROE2 increased significantly by 46.18%, 23.73%, 21.14%, 48.98%, and 56.14% respectively. Among them, the growth rate of the main root length of the mutant was significantly lower than that of the wild type, and the growth rate of the main root length of type (Figure 2). The above results show that low phosphorus stress has no significant impact on the seedling height of each genotype, significantly increasing the main root length, and OsRGA1 enhances the rice main root elongation process promoted by low phosphorus stress.

4.3. Morphological and Physiological Indexes of Rice Seedlings

Comp According to NP, primary root elongation under LP increased by 46.18% in NIP, 23.73% in R-5, 21.14% in R-8, 48.98% in ROE1, and 56.14% in ROE2. The more pronounced increase in overexpression lines, especially ROE2, and the reduced response in knockout mutants, suggest that OsRGA1 positively regulates LP-induced root elongation.



Notes: NP: 300 μM KH_2PO_4 , LP: 5 μM KH_2PO_4 . The same as below.

Figure 2. Phenotypes of rice seedlings under low phosphorus treatment.

Other morphological parameters, including root number, shoot and root dry biomass, and root-shoot ratio, were also affected. LP [17] reduced biomass accumulation in all genotypes, with mutants showing the greatest decline. Root oxidation capacity decreased under LP in all lines, but the reduction was smaller in overexpression lines and greater in mutants than in NIP.

These results indicate that OsRGA1 enhances morphological adaptability to LP stress, primarily by promoting primary root elongation and moderating reductions in root physiological performance, thereby improving potential phosphorus acquisition efficiency under nutrient-limited conditions. There was no significant difference between the morphological indicators of ROE1 and the wild type (**Table 3**). It shows that the low phosphorus stress conditions significantly reduce the morphological indicators of rice seedlings, and OsRGA1 weakens the inhibition effect of low phosphorus stress on various morphological indicators.

4.3.1. Antioxidant and Acid Phosphatase Activity

Low phosphorus (LP) Stress significantly enhanced antioxidant enzyme activities—superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) in leaves and roots [18] across all genotypes. In NIP, LP increased SOD, POD, and CAT activities by 19.49% & 113.20%, 35.60% & 123.76%, and 16.42% & 12.60% (leaves & roots), respectively. Overexpression lines, particularly ROE2, showed greater increases, whereas knockout mutants R-5 and R-8 exhibited smaller gains, indicating OsRGA1's role in enhancing antioxidant defenses under LP conditions. Its increased activity is one of the important signs of plant phosphorus deficiency. Under conventional phosphorus conditions, there was no significant difference in the activity of acid phosphatase in rice leaves and roots of various genotypes (**Figure 3**).

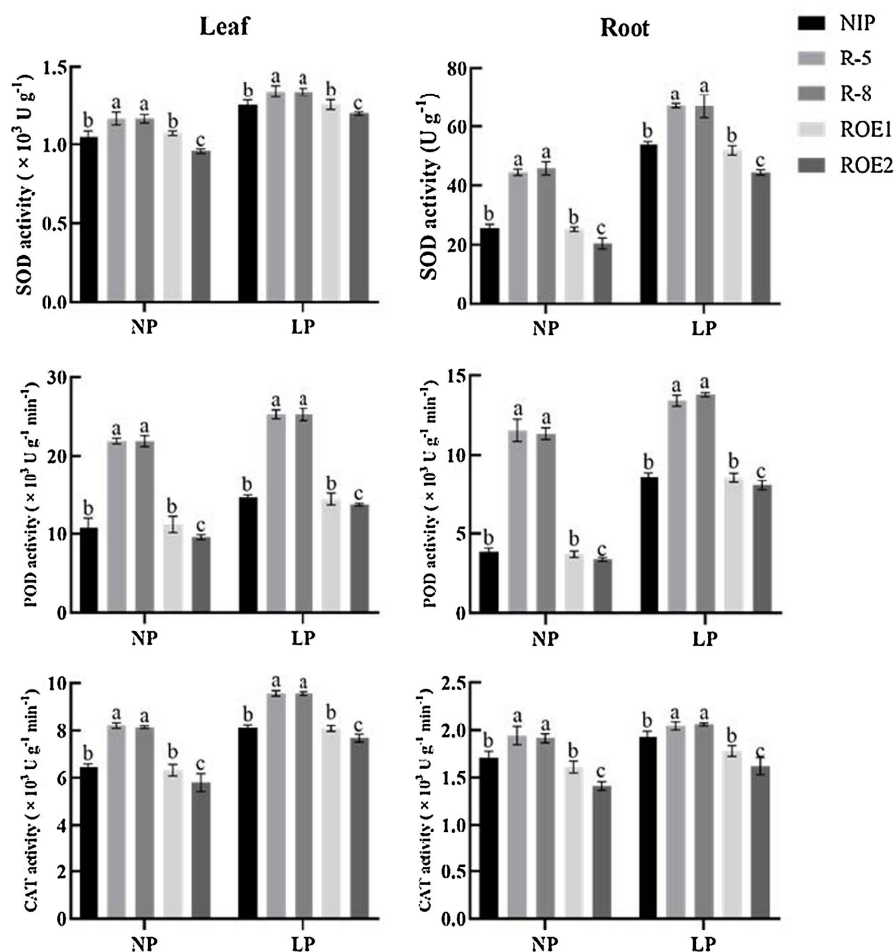
4.3.2. Acid Phosphatase Activity

Acid phosphatase activity, a key indicator of phosphorus deficiency adaptation, also rose markedly under LP in both leaves and roots [19]. This enzyme facilitates the conversion of organic phosphorus into inorganic forms available for uptake.

Table 3. The change in morphological and physiological traits of rice seedlings' phosphorus treatment.

Treatment	Lines	Seedling height (cm)	The dry weight of the shoots (g)	Length of primary root (cm)	Number of roots	The dry weight of the roots (g)	Root shoot ratio	Roots oxidation force ($\mu\text{g}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$)
NP	NIP	16.50 ^a	0.59 ^a	8.15 ^c	12.31 ^a	0.18 ^a	0.31 ^b	81.19 ^d
	R-5	10.43 ^b	0.32 ^c	8.30 ^c	7.57 ^c	0.09 ^c	0.28 ^c	66.67 ^c
	R-8	10.40 ^b	0.58 ^a	8.43 ^c	7.44 ^c	0.08 ^c	0.25 ^c	66.82 ^c
	ROE1	16.30 ^a	0.58 ^a	8.33 ^c	12.06 ^a	0.17 ^a	0.29 ^b	81.52 ^b
	ROE2	16.85 ^b	0.56 ^a	8.30 ^c	12.39 ^b	0.17 ^a	0.30 ^b	95.18 ^a
LP	NIP	15.71 ^a	0.45 ^b	12.44 ^a	10.91 ^b	0.11 ^b	0.24 ^c	56.10 ^d
	R-5	9.74 ^b	0.13 ^d	10.27 ^b	6.48 ^d	0.05 ^d	0.38 ^a	36.65 ^e
	R-8	9.96 ^b	0.12 ^d	10.20 ^b	6.23 ^d	0.04 ^d	0.33 ^a	36.91 ^e
	ROE1	15.56 ^a	0.44 ^b	12.41 ^a	10.65 ^b	0.12 ^d	0.27 ^c	59.10 ^d
	ROE2	15.9 ^a	0.45 ^b	12.46 ^a	10.98 ^b	0.11	0.24	69.13

Note: Different lower cases in the same column indicate significant differences among lines ($P < 0.05$).



Note: Different lower cases indicate significant differences among lines ($P < 0.05$). The same as below.

Figure 3. Antioxidant enzyme activities of rice seedlings.

While all genotypes showed increased activity, ROE2 displayed the highest enhancement, comparable to or superior to NIP, whereas R-5 and R-8 recorded lower increases. The coordinated rise in antioxidant and acid phosphatase activities under LP suggests that OsRGA1 contributes to both oxidative stress mitigation and improved phosphorus mobilization. By strengthening these physiological defenses, OsRGA1 likely supports sustained root function and nutrient acquisition efficiency in phosphorus-deficient environments, reinforcing its role as a positive regulator in LP stress adaptation. The increase in acid phosphatase activity was greater than that of the wild type (Figure 4) [20]. It shows that low phosphorus stress can significantly increase the acid phosphatase activity of rice seedlings' leaves and roots, and OsRGA1 enhances the effect of low phosphorus stress on the acid phosphatase activity of rice seedlings.

4.3.3. Content of Strigolactone

Strigolactone (SL) is a new plant hormone that can regulate various processes of plant growth and development. Among them, 2'-epi-5-deoxystrigol (5DS) is a typical endogenous monodactone [21]. Under conventional phosphorus conditions,

the content of 5DS in R-5 and R-8 was significantly lower than that of the wild type; the content of 5DS in ROE1 was not significantly different from that of the wild type; the content of 5DS in ROE2 was significantly higher than that of the wild type (**Figure 4** & **Figure 5**). Low phosphorus stress treatment can significantly increase the content of 5DS in rice leaves of various genotypes. The growth rate of NIP, R-5, R-8, ROE1, and ROE2 is 190.44%, 181.25%, 153.77%, 155.15%, and 152.71% respectively (**Figure 5**). It shows that OsRGA1 was involved in the process of monopod lactone response to low phosphorus stress [22].

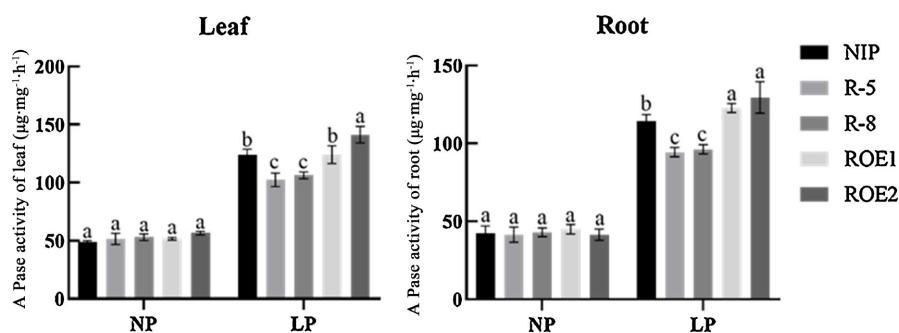


Figure 4. Acids phosphates activities of rice seedlings.

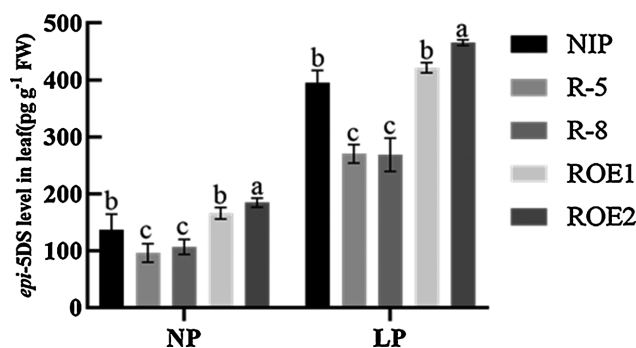


Figure 5. The concentration of strigolactone.

4.4. Phosphorus Uptake and Transport Gene Expression

Low phosphorus (LP) stress significantly upregulated the expression of key phosphorus transporter genes OsPT2, OsPT6, and OsPT8 in all rice genotypes, enhancing the plant's capacity for phosphorus acquisition. In wild-type NIP, transcript levels of these genes increased markedly under LP conditions compared to normal phosphorus (NP) conditions. Overexpression lines, especially ROE2, exhibited even greater induction, whereas knockout mutants R-5 and R-8 showed smaller increases, indicating a diminished transcriptional response. Low phosphorus stress treatment significantly increases the expression of OsPT2, OsPT6, and OsPT8 in rice leaves of various genotypes, but the increase in the expression of OsPT2, OsPT6, and OsPT8 in R-5 and R-8 leaves was lower than that of wild types, and the increase of gene expression in ROE1 leaves and wild There was no significant difference in type, and the increase of gene expression in ROE2 leaves was greater than that of wild type (**Figure 6**). This genotype-dependent variation suggests that OsRGA1

positively influences the activation of phosphorus transporter genes during LP stress, likely improving phosphorus uptake efficiency. The enhanced transporter expression in overexpression lines aligns with their superior root elongation and physiological resilience, while the reduced response in mutants corresponds with weaker adaptive performance.

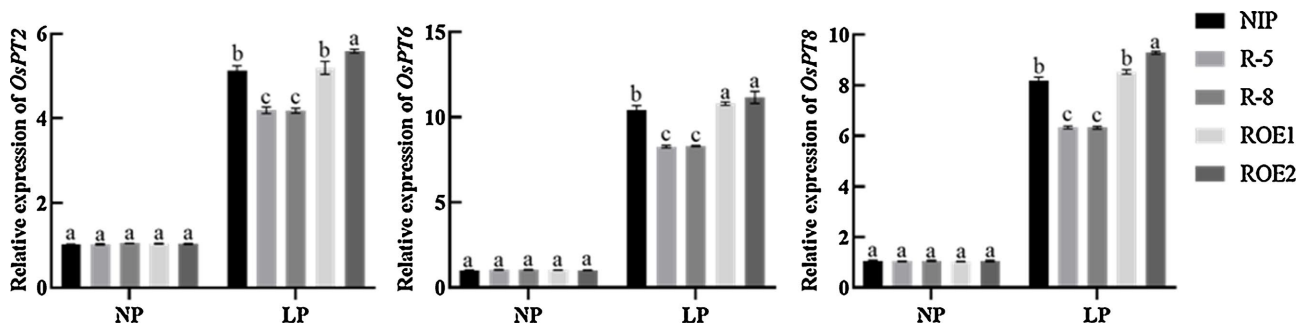


Figure 6. Relative expression of phosphorus-transporting genes in rice leaves

These findings indicate that OsRGA1 functions as a regulatory component linking LP perception to molecular mechanisms of nutrient acquisition. By promoting the expression of high-affinity phosphorus transporters, OsRGA1 may help maintain internal phosphorus homeostasis under nutrient-limited conditions, supporting growth and metabolic activity. This role positions OsRGA1 as a valuable target for breeding rice varieties with improved phosphorus-use efficiency in low-input agricultural systems.

5. Discussion

5.1. OsRGA1 as a Regulator of Low P Response

The results of this study demonstrate that OsRGA1 plays a positive regulatory role in rice adaptation to low phosphorus (LP) stress. Elevated OsRGA1 expression under LP conditions, particularly at 5 μ M phosphorus, coincided with morphological, physiological, and molecular adjustments that enhance phosphorus acquisition. Overexpression lines (especially ROE2) showed superior primary root elongation, higher antioxidant enzyme activities, greater acid phosphatase activity, and stronger induction of phosphorus transporter genes compared to the wild type, while knockout mutants exhibited weaker responses in all parameters.

These findings suggest that OsRGA1 integrates multiple adaptive pathways under LP stress. It appears to coordinate root architectural changes that improve soil exploration, boost antioxidant defenses to mitigate oxidative damage, and promote acid phosphatase-mediated phosphorus mobilization. Furthermore, its role in upregulating high-affinity phosphorus transporter genes indicates involvement in optimizing nutrient uptake at the molecular level [23]. There was no significant difference between the morphological indicators of ROE1 and the wild type (Table 3). It shows that the low phosphorus stress conditions significantly reduce the morphological indicators of rice seedlings, and OsRGA1 weakens the inhibition

effect of low phosphorus stress on various morphological indicators. Taken together, the evidence positions OsRGA1 as a central component in LP stress signaling and adaptation. Its regulatory influence spans root development, stress physiology, and nutrient transport, making it a promising genetic target for breeding rice varieties with enhanced phosphorus-use efficiency in P-deficient environments. The content of 5DS in rice leaves of different genotypes can be dramatically increased by low phosphorus stress treatment. **Figure 5** shows that the respective growth rates of NIP, R-5, R-8, ROE1, and ROE2 are 190.44%, 181.25%, 153.77%, 155.15%, and 152.71%. It demonstrates how OsRGA1 contributes to the monopod lactone response to low phosphorus stress.

5.2. Mechanisms of Root and Antioxidant Adaptation

Under low phosphorus (LP) stress, rice plants exhibited coordinated root architectural changes and enhanced antioxidant defenses, both of which were influenced by OsRGA1 activity. Primary root elongation was the most prominent morphological adaptation, enabling deeper soil exploration for phosphorus acquisition. Overexpression lines, particularly ROE2, showed greater root extension than wild-type plants, while knockout mutants had reduced responses, indicating that OsRGA1 promotes LP-induced root growth [24].

Parallel to structural changes, antioxidant enzyme activities, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), increased significantly in leaves and roots under LP stress. These enzymes mitigate reactive oxygen species (ROS) accumulation, protecting cellular structures and maintaining root functionality in nutrient-limited environments. Overexpression lines displayed stronger antioxidant responses, suggesting OsRGA1 enhances ROS-scavenging capacity, while mutants showed diminished activity and potentially higher oxidative stress.

This dual role, stimulating root elongation and reinforcing antioxidant systems, suggests that OsRGA1 enables rice plants to maintain growth and physiological stability during phosphorus scarcity. By integrating morphological and biochemical adaptations, OsRGA1 contributes to improved resilience and phosphorus-use efficiency, supporting its potential as a breeding target for enhancing crop performance in low-input agricultural systems.

5.3. Breeding and Agriculture Implications

The positive regulatory role of OsRGA1 in low phosphorus (LP) adaptation highlights its potential as a valuable target for rice improvement. By promoting primary root elongation, enhancing antioxidant defense, increasing acid phosphatase activity, and upregulating phosphorus transporter genes, OsRGA1 contributes to more efficient phosphorus acquisition and utilization under nutrient-limited conditions. These traits are particularly important for rice production in P-deficient soils, where fertilizer inputs are limited or costly [25].

Incorporating OsRGA1 into breeding programs through marker-assisted selection,

transgenic approaches, or gene editing could accelerate the development of rice varieties with enhanced phosphorus-use efficiency (PUE). Such varieties would require less phosphate fertilizer, reduce production costs, and mitigate environmental impacts such as eutrophication from fertilizer runoff.

Furthermore, the integration of OsRGA1-based improvements with other nutrient-efficient traits could create multi-stress-resilient cultivars suited for low-input and sustainable agricultural systems. This approach aligns with global goals of improving food security while preserving natural resources, making OsRGA1 an important candidate gene for modern crop breeding strategies aimed at climate-resilient and resource-efficient rice production.

6. Conclusions

This study provides comprehensive evidence that OsRGA1, encoding the heterotrimeric G protein α -subunit, is a positive regulator of rice adaptation to low phosphorus (LP) stress, functioning through coordinated morphological, physiological, and molecular mechanisms. Our findings demonstrate that OsRGA1 expression is highly responsive to phosphorus availability, peaking at 5 μM KH_2PO_4 , which was identified as the optimal LP condition for eliciting its regulatory effects.

Under LP stress, overexpression lines, particularly ROE2, exhibited significantly greater primary root elongation compared to wild-type Nipponbare (NIP), while knockout mutants (R-5 and R-8) showed reduced elongation. This enhanced root growth in overexpression lines likely improves soil exploration and phosphorus acquisition. In parallel, OsRGA1 influenced key physiological responses: antioxidant enzyme activities (SOD, POD, CAT) were strongly induced in overexpression lines, helping to mitigate oxidative stress, while knockout mutants showed weaker antioxidant responses. Acid phosphatase activity, essential for mobilizing organic phosphorus, also increased more in overexpression lines than in wild-type or mutant plants.

Molecular analysis revealed that OsRGA1 positively regulates the expression of phosphorus transporter genes (OsPT2, OsPT6, OsPT8) under LP stress, enhancing the plant's capacity for high-affinity phosphorus uptake. Additionally, LP treatment elevated strigolactone (5DS) content, with a stronger response in overexpression lines, suggesting hormonal cross-talk between OsRGA1 and phytohormone pathways in modulating root architecture and nutrient signaling.

The combined data indicate that OsRGA1 serves as a central signaling hub integrating LP perception, hormonal regulation, root morphological adaptation, and nutrient transporter activation. Its multifaceted role makes it a promising genetic target for developing rice cultivars with improved phosphorus-use efficiency (PUE).

From an agricultural perspective, exploiting OsRGA1 in breeding programs via marker-assisted selection, transgenic modification, or genome editing offers potential to reduce reliance on phosphate fertilizers, lower production costs, and minimize environmental impacts. These advances could contribute to sustainable rice

cultivation in phosphorus-deficient regions, supporting global food security and environmental conservation.

Data Availability

All data generated and analyzed in this study are included within the article. Experimental datasets, including raw measurements of morphological traits, physiological parameters, biochemical assays, and gene expression levels, are available from the corresponding author upon reasonable request.

No proprietary datasets or third-party restrictions apply beyond standard academic sharing policies.

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Conflicts of Interest

The authors declare no conflicts of interest.

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