

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2026): 5.29
IJABR 2026; SP-10(1): 814-821
www.biochemjournal.com
Received: 23-11-2025
Accepted: 26-12-2025

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Pup1 QTL enhances Pi acquisition, internal Pi remobilization, and increases phosphorus use efficiency and grain yield in rice

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i1Sj.7082>

Abstract

Phosphorus (P) deficiency is a major constraint limiting rice productivity in P-deficient soils worldwide. Phosphorous Uptake 1 (*Pup1*) quantitative trait locus (QTL) has been identified as a key genetic determinant conferring tolerance to low P availability. To evaluate the impact of P-deficiency on plant physio-biochemical and agronomic performance, two contrasting rice genotypes were used: Pusa-44 (Pi-deficiency-sensitive) and its near-isogenic line NIL-23 (Pi-deficiency-tolerant due to the introgression of *Pup1*). Plants were grown under Pi-deficient (4 ppm), optimum (16 ppm) conditions in PusaRicH hydroponic medium. Physio-biochemical analyses revealed that NIL-23 exhibited pronounced plasticity in root system architecture under Pi deficiency, with significant increases in total root length, root tip number, root surface area, and root volume, enhancing soil exploration and Pi acquisition. Consistently, intrinsic and secreted acid phosphatase activities were markedly higher in NIL-23, indicating improved extracellular Pi scavenging and internal Pi remobilization under low-Pi availability. Despite reduced Pi availability, NIL-23 displayed improved agronomic performance, with a higher grain yield per plant compared with Pusa-44. Enhanced P uptake and utilization in NIL-23 were further evidenced by higher P accumulation in roots, leaves, and seeds across developmental stages, along with more efficient remobilization of P from vegetative tissues to grains. NIL-23 also showed significantly greater shoot and root biomass, higher total P uptake, increased grain P content, and a consistently higher phosphorus harvest index under both Pi regimes. Collectively Our findings demonstrate that the *Pup1* QTL enhances P deficiency tolerance by coordinately regulating root architecture, phosphatase activity, photosynthesis, biomass production, P uptake, remobilization, and grain P partitioning. The enhanced phosphorus use efficiency and sustained productivity of NIL-23 highlight the potential of *Pup1* as a valuable genetic resource for developing P-efficient rice cultivars suited for sustainable agriculture in P-limited agroecosystems.

Keywords: *Oryza sativa*, *Pup1* QTL, phosphorous stress, agronomical performance, phosphorus use efficiency

Introduction

Phosphorus (P) is an essential macronutrient required for plant growth, development, reproduction, and overall health [1]. Although soils may contain sufficient total P, only a small fraction is available to plants as inorganic phosphate (Pi). Limited Pi availability is largely due to its poor solubility and mobility, as well as its tendency to become fixed or immobilized through adsorption, precipitation, or conversion into organic forms [2, 3]. In rice, P-deficiency typically reduces plant growth, produces smaller, erect leaves often with purple pigmentation, and results in fewer tillers and panicles, substantially lowering yield potential [4].

Plants employ a range of morpho-physiological, biochemical, and molecular strategies to cope with P-deficiency at different developmental stages. Among these, modifications in root system architecture (RSA), including increased root length and number of root tips, are significant for improving P-uptake efficiency [5, 6]. Additionally, secretion and release of acid phosphatases are another fundamental strategy to mobilize organic P sources in the rhizosphere and recycle intracellular phosphate [7-8].

P-deficiency-tolerant ancestral rice landrace Kasalath (*aus* subgroup) enabled the identification of a major quantitative trait locus (QTL), *Pup1* (Phosphorus uptake 1), on chromosome 12 [9, 10].

The *Pup1* region contains a limited number of coding genes, including *Phosphate Starvation Tolerance 1 (PSTOL1)*, which encodes a serine-threonine kinase. Under P-deficiency condition, this kinase stimulates early root growth, enhancing P acquisition from the soil [11]. Recent studies indicate that *Pup1* enhances P-acquisition during early vegetative growth [12, 13]. Introgression of *Pup1* into elite rice cultivars has therefore been proposed as an effective strategy to enhance phosphorus use efficiency (PUE).

Although the beneficial effects of *Pup1* QTL on root growth and yield under P-deficiency have been reported, a comprehensive understanding of how *Pup1* coordinates morphological, physiological, and biochemical responses at the reproductive stage of crop growth remains limited. In particular, integrated analyses linking RSA modifications, phosphatase activity, photosynthetic performance, biomass production, P uptake and remobilization, and grain P partitioning are still lacking. Moreover, the influence of *Pup1* on seed phosphorus content and phytate accumulation has not been thoroughly examined.

To address these gaps, the current study was conducted as a comparative analysis of a *Pup1*-introgressed near-isogenic line (NIL-23) and its recurrent parent, Pusa-44, under contrasting Pi regimes [sufficient (16 ppm) and deficient (4 ppm)] in a hydroponic medium. This study provides a holistic understanding of how *Pup1* mediates P-deficiency tolerance in rice by enhancing phosphorus acquisition, utilization, and remobilization. These findings offer valuable insights into the genetic and physiological basis of P use efficiency and also highlight the role of *Pup1* QTL for developing P-efficient rice cultivars for sustainable production in P-limited agroecosystems.

Materials and Methods

Plant materials and growing conditions

Two contrasting rice (*Oryza sativa* L.) genotypes were used in this study: Pusa-44, a high-yielding indica cultivar sensitive to Pi-deficiency, and its near-isogenic line (NIL)-23, Pi-deficiency tolerant genotype. The near-isogenic line NIL-23 is a BC₃F₆ line developed through a marker-assisted backcross breeding strategy to introgress the *Pup1* QTL into the genetic background of the high-yielding indica rice cultivar Pusa-44. The *Pup1* donor parent was the *aus* rice landrace Kasalath (tolerance to Pi-deficient condition). Fully mature seeds of both genotypes were surface-disinfected using 0.5% (w/v) mercuric chloride and subsequently rinsed three times with sterile double-distilled water to remove residual disinfectant. Sterilized seeds were soaked in autoclaved water and placed on moist germination paper in Petri dishes. Germination was carried out in darkness at 37 °C for 48 h to achieve uniform seedling emergence. After germination, seedlings were arranged on moist germination paper with the coleorhiza oriented downward and the coleoptile upward, rolled into cylindrical “cigar rolls” as described previously [1]. These rolls were placed in jam bottles containing PusaRicH hydroponic medium supplemented with Pi-deficient (4 ppm), optimum Pi (16 ppm) levels. The pH of the nutrient solution was adjusted to ~5.5 before use. Seedlings were maintained for one week under ambient conditions (35-37 °C; 80-85% relative humidity). Twelve-day-old seedlings were transferred individually to jam bottles containing the medium supplemented with different Pi concentrations. To minimize

pH drift, nutrient depletion, and ion imbalance, the entire hydroponic medium was replaced once every three days throughout the growth period until maturity.

Experimental Design

The experiment was conducted in a factorial completely randomized design (CRD) with two factors: genotype and phosphorus (Pi) level. The genotypes included NIL-23 (Pi-deficiency tolerant) and Pusa-44 (Pi-deficiency sensitive), while Pi was supplied at two different concentrations: 4 ppm (Pi-deficiency, treatment) and 16 ppm (Pi-deficiency sufficient, control). Each genotype × Pi level combination was replicated 15 times, with each plant grown independently in an individual jam bottle. Thus, each Pi level comprised 30 plants (15 per genotype). All treatments were assigned completely at random and maintained under natural environmental conditions in a net house at the Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi.

Analysis of changes in root system architecture

Root system architecture traits were measured using a WinRHIZO Pro LA2400 root scanning system (Regent Instruments Inc., Montreal, Canada) with three independent biological replicates. At the reproductive stage (panicle initiation to early flowering stage), plants were taken, and roots were carefully separated from shoots and thoroughly washed. Cleaned roots were evenly arranged in an acrylic scanning tray measuring 40 cm × 30 cm, filled with water for scanning. Root images were captured in grayscale mode at 600 dpi and processed using WinRHIZO Pro software (v2009). RSA (total root length, number of root tips, total root surface area, and total root volume) was quantified on a per-plant basis.

Estimation of intrinsic phosphatase activity

Intrinsic acid phosphatase activity in root and panicle tissues was determined at the reproductive stage by following the protocol described by [14]. Approximately 100 mg of fresh tissue was finely homogenized in 5 mL of ice-cold citrate buffer (0.1 mol L⁻¹, pH 5.2). The homogenate was centrifuged at 8,000 × g for 15 min at 4 °C, and the resulting supernatant was used as a source of crude enzyme extract. For the assay, a total reaction volume of 1 mL was prepared by combining 0.1 mL enzyme extract, 0.5 mL *p*-nitrophenyl phosphate (10 mmol L⁻¹, pH 5.2), and 0.4 mL citrate buffer. After incubation at room temperature for 10 min, the reaction was stopped with 2 mL of Na₂CO₃. Absorbance was measured at 405 nm, and enzyme activity was quantified using a *p*-nitrophenol standard curve (10-100 mmol L⁻¹). Results were expressed as $\mu\text{mol } p\text{-NP released min}^{-1} \text{ g}^{-1}$ of fresh tissue weight.

Estimation of secreted acid phosphatase activity

Secreted acid phosphatase activity in roots was analysed by immersing the roots in a jam bottle containing a buffer composed of EDTA (1 mM), NaCl (1 mM), and sodium acetate (50 mM), and incubating for 3 h under light conditions. The buffer was then collected and stored at 4 °C. Enzyme assays were performed using *p*-nitrophenyl phosphate (*p*-NPP) as substrate, and activity of root-secreted phosphatase was expressed as $\mu\text{mol } p\text{-NP released per plant}$.

Measurement of photosynthetic activity in the leaf and yield

A portable photosynthesis system (LI-6400, LI-COR Inc., USA) was used to measure the net photosynthetic rate at the reproductive stage. Gas-exchange measurements were taken at a constant airflow rate of $500 \text{ cm}^3 \text{ s}^{-1}$, with the leaf chamber maintained at 25°C and an ambient CO_2 concentration of $400 \mu\text{mol mol}^{-1}$. Photosynthetic photon flux density (PPFD) was fixed at a saturating level during measurements. Data were collected from five plants per treatment, with three independent biological replicates. Agronomic traits, grain yield per plant, were recorded for plants grown under different Pi regimes in hydroponic culture.

Estimation of total phosphorus content in root, leaf, and seed

Total phosphorus content was quantified in root, leaf, and mature seed samples using the method described previously^[1]. Approximately 1.0 g of oven-dried plant materials was acid-digested using a mix of HClO_4 (3.5 mL) and HNO_3 (10 mL) by continuous heating for >2 h at 200°C . The resulting extract was filtered using Whatman No. 1 paper, and the volume was adjusted with distilled water to a final volume of 100 mL. An aliquot of 25 mL from the digested sample was mixed with 10 mL of Vanadate-molybdate solution, and the final volume was made to 50 mL using double-distilled water. This solution was mixed thoroughly and incubated for color development at 25°C for 10 min. The absorbance of the reaction mix was measured at 420 nm with the help of a spectrophotometer. A standard curve for different P concentrations ranging from 1.0 to 5.0 mg was prepared by recording the absorbance at 420 nm and plotting a graph for OD (A420) value (on the Y-axis) against P concentration (on the X-axis). P concentration in plant tissues was calculated from a standard curve and expressed as mg/g dry weight, based on the mean of three biological replications. Total phosphorus uptake by the plant and phosphorus harvest index (PHI) were computed using the following formula:

$$\text{P uptake by grain (mg/plant)} = \text{Grain yield (g/plant)} \times \text{grain P concentration (mg/g)},$$

$$\text{Total P uptake by plant (mg/plant)} = \text{P uptake by grain} + \text{P uptake by straw} + \text{P uptake by roots},$$

$$\text{P harvest index (PHI)} = [\text{Total P taken up by grains} \div (\text{Total P taken up by straw} + \text{Total P taken up by roots})] \times 100$$

Estimation of phytate content in mature seeds

Phytate content in mature seeds was determined using finely powdered seed materials. Approximately 0.5 g of ground sample was transferred to a screw cap tube, to which 10 mL of hydrochloric acid (0.66 M) was added. The mixture was agitated at 150–200 rpm and kept at 25°C overnight for efficient extraction. Following the extraction, 1.0 mL aliquot was taken and centrifuged at 13,000 rpm for 10 min, and

0.5 mL of clear supernatant was collected and neutralised using 0.75 M sodium hydroxide. For enzymatic hydrolysis, 50 μL of an aliquot from the neutralised extract was mixed with 620 μL of double-distilled water, 200 μL of 50 mmol sodium acetate buffer, and 20 μL of phytase enzyme. The content was mixed thoroughly by vortexing and incubated at 40°C for 10 min in a water bath. The reaction was initiated by adding 20 μL alkaline phosphatase and 0.2 mmol glycine buffers, followed by incubation at 40°C for 15 min in a water bath. A control reaction set was prepared in parallel without addition of phytase and alkaline phosphatase. The enzymatic reactions were terminated by adding 300 μL of 50% (w/v) TCA solution and centrifuged at 13000 rpm for 10 min. The resultant supernatant obtained was used for the colorimetric estimation. For color development, 1.0 mL of the supernatant was mixed with 500 μL of coloring reagent [sulfuric acid (1.0 mM), ascorbic acid (10% w/v), and ammonium molybdate (2.5% w/v) in a 3:1:1 ratio]. The mixture was incubated in a water bath at 40°C for 1 h, after which absorbance was measured at 655 nm. Phytate content was calculated based on the difference between total phosphorus and free phosphorus content, and expressed as $\text{g } 100 \text{ g}^{-1}$ seed.

Statistical analysis

All experiments were conducted with three biological replicates (with each biological replicate representing three independent plants). Data were analyzed using analysis of variance (ANOVA). When ANOVA indicated significant treatment effects ($p \leq 0.05$), mean separation was performed based on Tukey's test for conservative pairwise comparison, and, where appropriate, Duncan's multiple range test (DMRT) was performed to resolve differences among treatment means. Mean values with different lowercase letters indicate significant differences. Error bars represent standard deviation ($\pm \text{SD}$).

Results and Discussion

Changes in the root system architecture

RSA is one of the primary determinants of Pi acquisition, particularly under Pi-limited conditions. Quantitative evaluation of RSA traits such as total root length, number of root tips, total root surface area, and total root volume—demonstrated pronounced variation under both Pi-deficient and optimum conditions. Even under Pi-deficient condition, NIL-23 exhibited a pronounced improvement in root traits relative to Pusa-44, with increases of 62.11% in total root length, 22.35% in the number of root tips, 27.02% in root surface area, and 5.06% in total root volume (Figure 1). Collectively, these findings suggest that introgression of the *Pup1* QTL substantially enhanced root architectural plasticity and responsiveness under Pi-deficient condition, thereby enhancing soil exploration and Pi uptake under low-P stress. Such responses are consistent with earlier reports showing that *OsPSTOL1*, located on the *Pup1* QTL, stimulates early root growth and biomass accumulation, thereby improving Pi acquisition efficiency^[11].

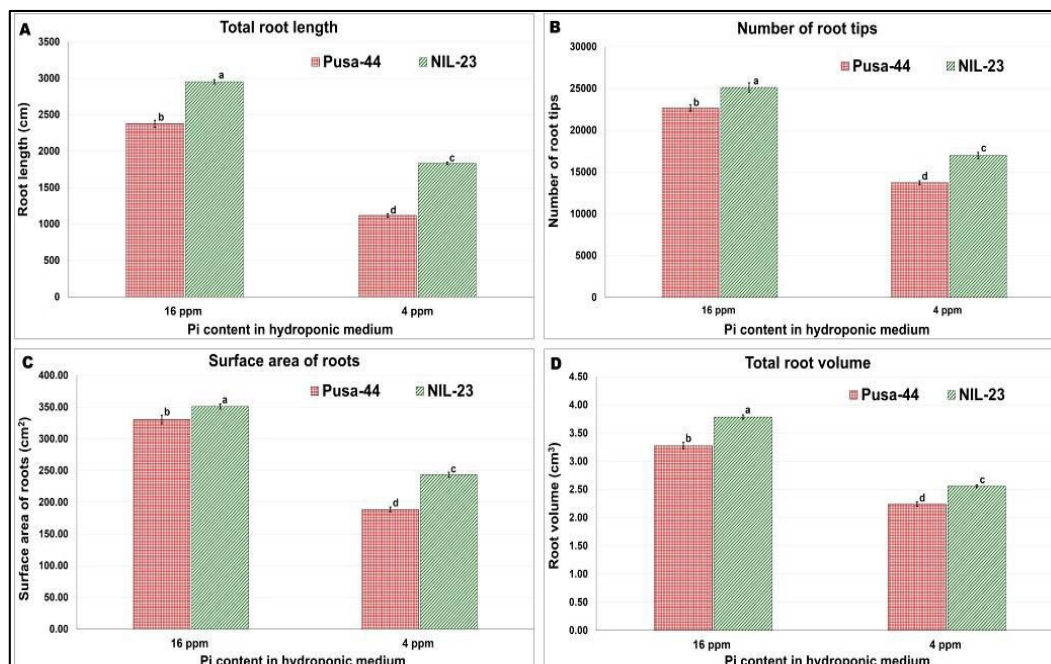


Fig 1: Effect of Pi availability on root system architecture of two contrasting rice genotypes [Pusa-44 (P deficiency sensitive) and NIL-23 (P deficiency tolerant)] grown hydroponically under Pi-deficient (4 ppm, treatment), optimum (16 ppm, control) conditions. (A) Total root length, (B) number of root tips, (C) surface area of root, and (D) total volume of root. The data represent the mean of three replicates, and the bars marked with different lower-case alphabets indicate statistically significant differences at $p < 0.05$. Error bars represent standard deviation ($\pm$ SD).

Changes in intrinsic and secreted acid phosphatase activity

Acid phosphatase plays a key role in plant adaptation under Pi-deficient conditions by facilitating both extracellular Pi scavenging and intracellular Pi remobilization. Our results showed, that under Pi-deficient condition, intrinsic acid phosphatase activity in root and panicle tissues was consistently higher in NIL-23 than in Pusa-44. NIL-23 showed a 22.37% increase in intrinsic acid phosphatase activity in roots and a 17.69% increase in panicles compared with the corresponding tissues of Pusa-44 (Figure 2A, B). In addition, secreted acid phosphatase activity from roots was

significantly elevated in NIL-23, exhibiting a 49.93% increase relative to Pusa-44 under Pi-deficient condition (Figure 2C). These results collectively indicate that the presence of the *Pup1* locus in NIL-23 enhances Pi acquisition and utilization efficiency under Pi-deficient condition. These findings align with earlier studies reporting increased APase activity in Pi-efficient genotypes and *Pup1*-introgressed rice lines under P deficiency [9, 10]. Elevated acid phosphatase activity in NIL-23 likely enhances its capacity to mobilize organic P sources and recycle internal Pi pools, thereby maintaining metabolic activity under low Pi availability.

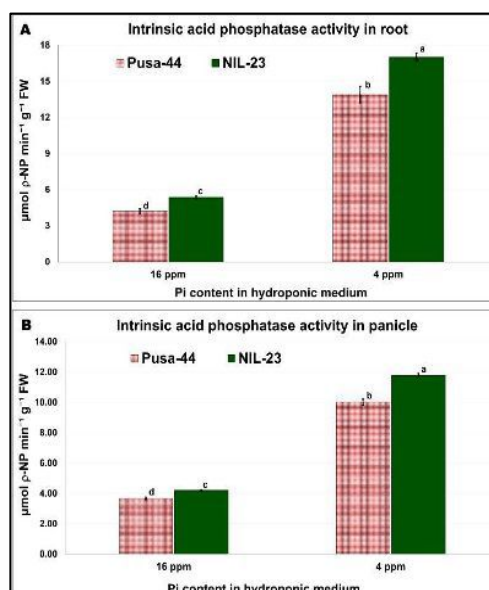


Fig 2: Changes in intrinsic acid phosphatase activity of two contrasting rice genotypes [Pusa-44 (Pi-deficiency sensitive) and NIL-23 (Pi-deficiency tolerant)] grown hydroponically under varying Pi regimes [deficient (4 ppm), optimum (16 ppm)] conditions. (A) Intrinsic acid phosphatase activity in roots, (B) intrinsic acid phosphatase activity in panicle. The data represent the mean of three replicates, and the bars marked with different lower-case alphabets indicate statistically significant differences at $p < 0.05$. Error bars represent standard deviation ($\pm$ SD).

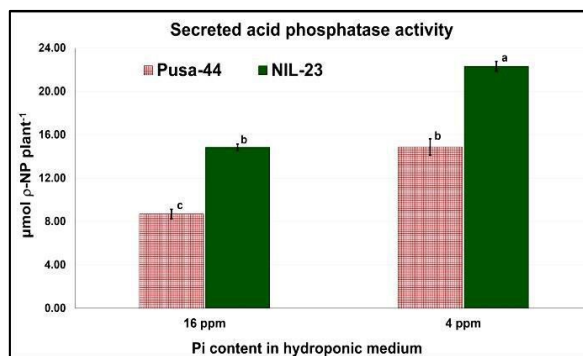


Fig 3: Changes in secreted acid phosphatase activity of two contrasting rice genotypes [Pusa-44 (Pi-deficiency sensitive) and NIL-23 (Pi-deficiency tolerant)] grown hydroponically under varying Pi regimes [deficient (4 ppm), optimum (16 ppm)] conditions. The data represent the mean of three replicates, and the bars marked with different lower-case alphabets indicate statistically significant differences at $p < 0.05$. Error bars represent standard deviation ($\pm$ SD).

Photosynthetic activity in the leaf and yield

In NIL-23, the highest values of net photosynthetic rate ($21.51 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($0.27 \text{ mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and intercellular CO_2 concentration ($258.29 \mu\text{mol CO}_2 \text{ mol}^{-1}$) were observed under optimum Pi availability (16 ppm) (Figure 4A). We observed a significant reduction in net photosynthetic rate under Pi-deficient (4 ppm Pi) condition compared to sufficient Pi condition in both genotypes. However, the level of reduction was lower in NIL-23 compared to Pusa-44. These findings clearly demonstrate the superior photosynthetic efficiency of

NIL-23 even under Pi deficient condition compared with Pusa-44. Grain yield per plant followed a similar pattern, with NIL-23 consistently outperforming Pusa-44, even under Pi deficiency (Figure 4B). These results underscore the regulatory role of the *Pup1* locus in conferring Pi deficiency tolerance and sustaining improved agronomic performance. These findings corroborate the earlier reports that phosphorus availability directly regulates photosynthesis by controlling cellular energy status, carbon assimilation, and source-sink balance [15-18].

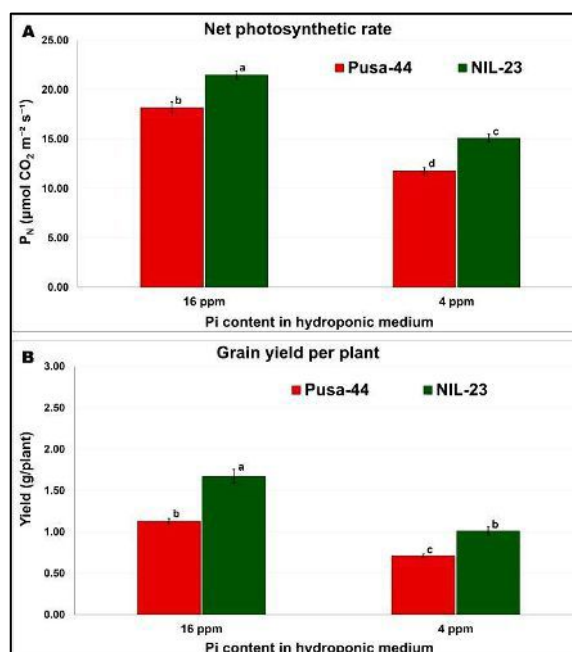


Fig 4: Changes in net photosynthetic yield and grain yield of two contrasting rice genotypes [Pusa-44 (Pi-deficiency sensitive) and NIL-23 (Pi-deficiency tolerant)] grown hydroponically under Pi-deficient (4 ppm) and optimum (16 ppm) conditions. (A) Net photosynthetic yield (B) Grain yield per plant. The data represent the mean of three biological replicates, and the bars marked with different lower-case alphabets indicate a statistically significant difference at $p < 0.05$. Error bars represent standard deviation ($\pm$ SD).

Estimation of total phosphorus content in root, leaf, and seed

A substantial increase in P content was observed in root, leaf, and seed with increasing Pi supply (from 4 to 16 ppm) across all developmental stages (Figure 5). At both 45 and 90 days after sowing, P content in leaves was consistently higher than that in roots in both genotypes. Under Pi-deficient condition, the tolerant genotype accumulated higher content of P in both root and leaf compared to the sensitive genotype, indicating its better P uptake. By 120

days, P content in root and leaf was declined but increased in seed in both genotypes, suggesting efficient remobilization of P from vegetative tissues to developing seeds. Notably, seed P concentration was significantly higher in NIL-23 than in Pusa-44 under even under Pi-deficient condition. Overall, higher P concentration in root, leaf, and seed of NIL-23 across growth stages supports enhanced Pi acquisition, translocation, and remobilization efficiency conferred by the introgressed *Pup1* QTL.

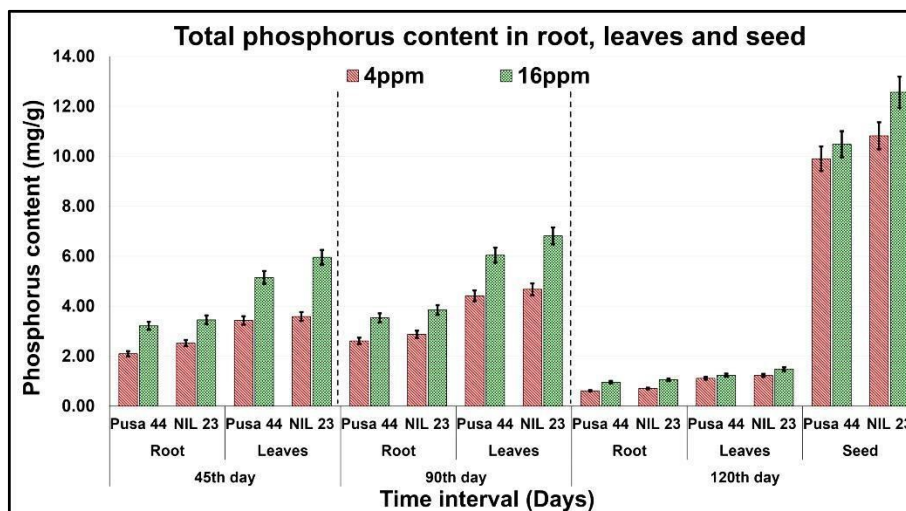


Fig 5: Total phosphorus content in root, leaf and seed of two contrasting rice genotypes [Pusa-44 (Pi-deficiency sensitive) and NIL-23 (Pi-deficiency tolerant)] grown hydroponically under Pi-deficient (4 ppm) and optimum (16 ppm) conditions.

Biomass production and phosphorus harvest index

Shoot and root dry biomass were significantly influenced by Pi availability (Figure 6). Under optimum Pi availability (16 ppm), NIL-23 exhibited significantly higher shoot and root dry biomass than Pusa-44, indicating its superior growth

(Figure 6). Under Pi-deficient condition, we observed significant decline in shoot and root biomass in both genotypes; however, NIL-23 maintained a significantly higher shoot dry weight compared with the sensitive genotype.

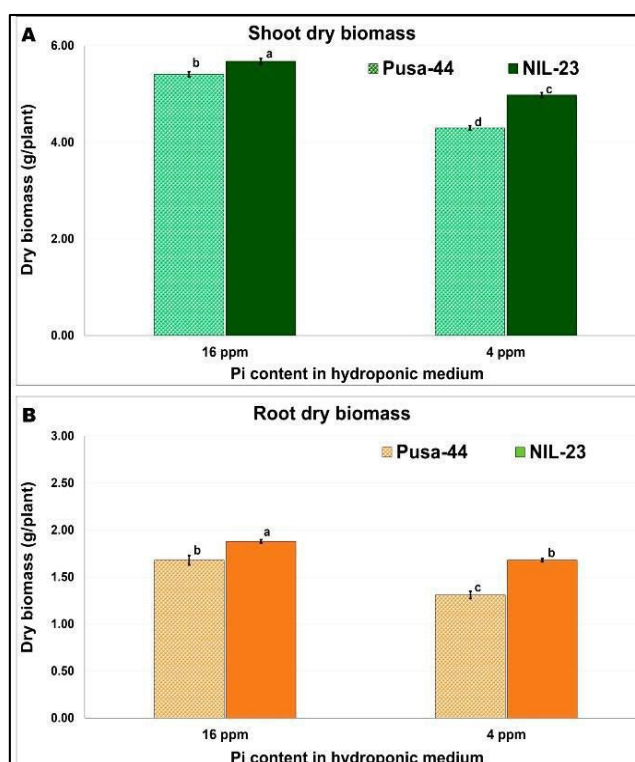


Fig 6: Dry biomass content of two contrasting rice genotypes [Pusa-44 (Pi-deficiency sensitive) and NIL-23 (Pi-deficiency tolerant)] grown hydroponically under Pi-deficient (4 ppm) and optimum (16 ppm) conditions. (A) Shoot dry biomass (B) root dry biomass per plant. The data represent the mean of three biological replicates, and the bars marked with different lower-case alphabets indicate a statistically significant difference at $p < 0.05$. Error bars represent standard deviation ($\pm$ SD).

Total P uptake was significantly higher in the NIL-23 (tolerant genotype) than in the Pusa-44 (sensitive genotype) at both Pi levels. Under optimum Pi availability (16 ppm), NIL-23 accumulated the highest total P uptake (~ 29.47 g plant $^{-1}$), whereas Pusa-44 recorded a comparatively lower uptake (~ 18.54 g plant $^{-1}$) (Figure 7). A similar trend was observed under Pi-deficient condition (4 ppm), with NIL-23 maintaining superior P uptake over Pusa-44. Even under low Pi availability, NIL-23 exhibited significantly higher

grain P content than the sensitive genotype. Furthermore, the phosphorus harvest index (PHI) was consistently higher in NIL-23 across Pi regimes. Notably, NIL-23 maintained a higher PHI even under Pi-deficient conditions, indicating more efficient translocation and partitioning of absorbed P into grains. Collectively, these results demonstrate enhanced P uptake, utilization, and remobilization efficiency in NIL-23.

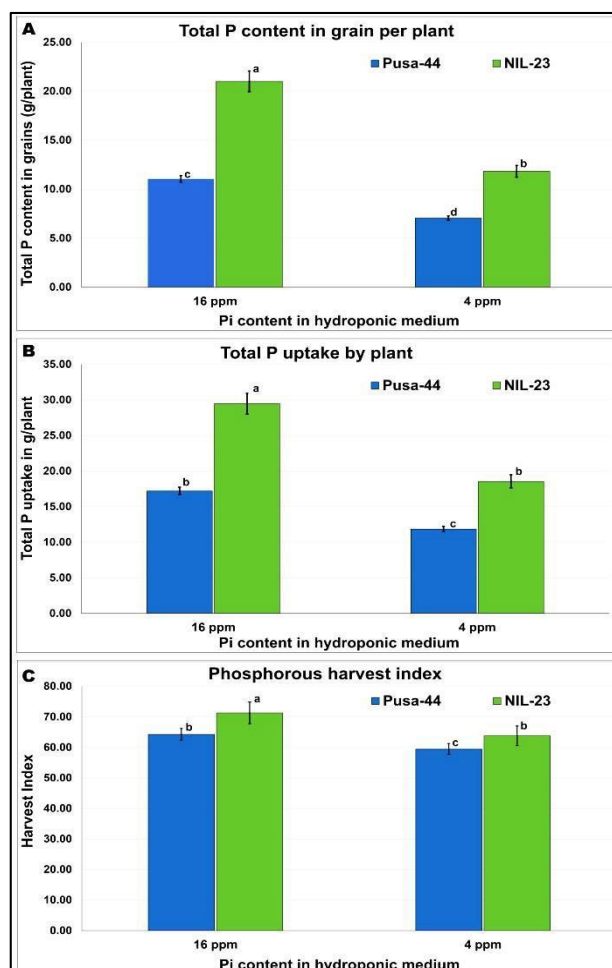


Fig 7: Phosphorus use efficiency of rice genotypes grown under Pi-deficient (4 ppm Pi) and sufficient (16 ppm Pi) conditions. (A) Total P content in grains per plant, (B) total P uptake by plant, and (C) phosphorus harvest index (PHI). Values represent the mean of three biological replications. Bars with different lowercase letters indicate statistically significant differences at $p < 0.05$. Error bars denote standard deviation ($\pm$ SD).

Estimation of phytate content in mature seeds

Phytic acid is the major storage form of P in cereal grains, accounting for a large proportion of total seed P [19, 20]. In this study, seed phytic acid content increased progressively with rising Pi availability in the growth medium. Even under low Pi availability, NIL-23 accumulated approximately 40% higher phytate levels in seeds compared with Pusa-44, reflecting its enhanced P uptake and translocation capacity.

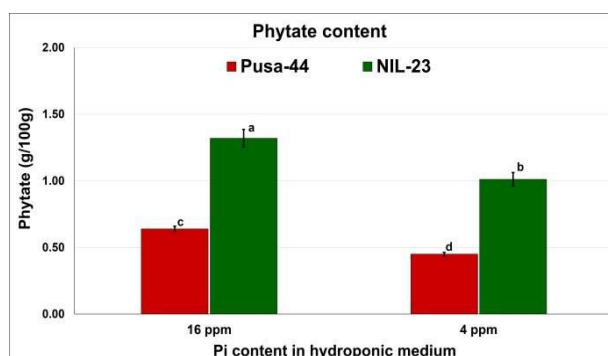


Fig 8: Phytate content in seeds of rice genotypes grown under Pi-deficient (4 ppm Pi) and sufficient (16 ppm Pi) conditions. Values represent the mean of three biological replications. Bars with different lowercase letters indicate statistically significant differences at $p < 0.05$. Error bars denote standard deviation ($\pm$ SD).

Thus, our study demonstrated that introgression of the *Pup1* QTL confers a comprehensive adaptive advantage to rice under Pi-deficient conditions by coordinately regulating root architecture, phosphorus acquisition, internal remobilization, and grain allocation. The improved phosphorus use efficiency, sustained productivity, and enhanced grain P partitioning observed in NIL-23 highlight the significant potential of *Pup1* for developing P-efficient rice cultivars to sustainable crop production under P-limited agroecosystems. Moreover, elucidating the regulatory functions of *Pup1* (particularly epigenetic regulation) might advance our understanding of *Pup1*-mediated low-P tolerance. Such understanding is a prerequisite for epigenetic tools (including epigenome editing) to improve the performance of rice [21] for sustainable agricultural productivity in P-deficient soils.

Acknowledgments

TS acknowledge financial support in the form of a DBT-JRF fellowship. The authors also acknowledge support for the research by the Indian Council of Agricultural Research, Department of Agricultural Research and Education, Government of India.

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