

## Unmasking novel loci for internal phosphorus utilization efficiency in rice germplasm through Genome-Wide Association Analysis

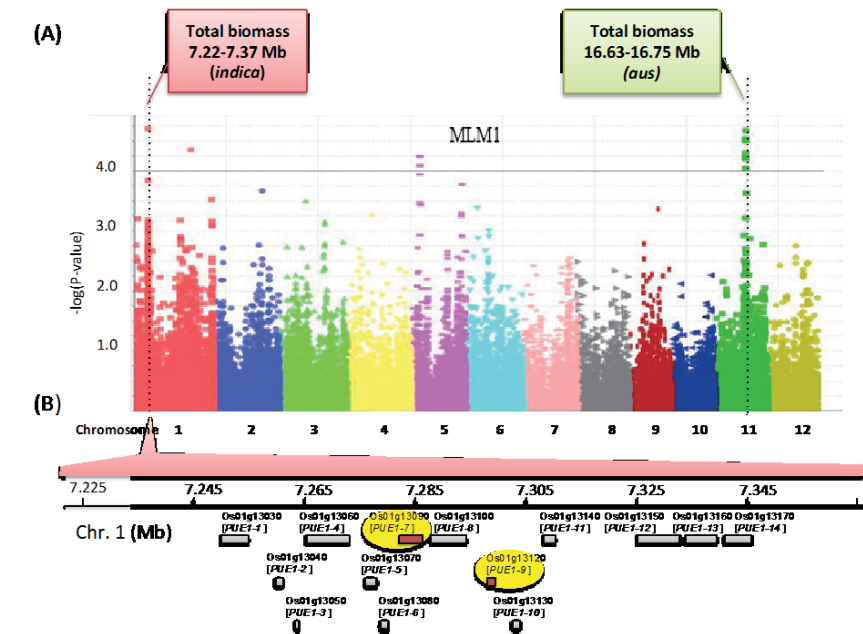
Phosphorus (P) is an essential element needed for plant growth, but it is deficient in many cropping systems worldwide, leading to low grain yields. In addition, the depletion of non-renewable rock phosphate reserves and increase of P fertilizer prices have renewed interest in breeding P-efficient cultivars. Internal P utilization efficiency (PUE), or how efficiently the P that is taken up is utilized to accumulate either grain yield or vegetative biomass, is of prime interest because there has been no progress to date in breeding for high PUE.

We have characterized the genotypic variation for PUE present within the rice gene pool by using a hydroponic system that assured equal plant P uptake. This experiment was performed with a single dose of 800  $\mu\text{g}$  P as the low-P treatment. All genotypes therefore had comparable P content and PUE was estimated as total biomass per amount of P available (P supplied and seed P).

Genotypes Mudgo, Yodanya, Santhi Sufaid, and DJ123 had higher estimated PUE values, while modern varieties such as IR64, Taichung, and Koshihikari had lower values. The loci controlling PUE were mapped via a Genome-Wide Association Study (GWAS), which included 292 diverse *Oryza sativa* accessions from 82 countries. The main loci associated with PUE were mapped on chromosomes 1 and 11 (Figure 1A). The highest PUE was associated with a minor *indica*-specific haplotype on chromosome 1 and a rare *aus*-specific haplotype on chromosome 11 (Figure 1A). Comparative variant and expression analysis for genes contained within the chromosome 1 haplotype identified two high priority candidate genes: *PUE1-7* and *PUE1-9* (Figure 1B). In terms of *PUE1-7*, there is no difference in gene expression pattern, while differences in coding regions between genotypes of contrasting haplotypes suggest that functional alterations for two predicted proteins are likely causative for the observed differences in PUE.

The loci reported here are the first identified for PUE in any crop that is not confounded by differential P uptake among genotypes. Importantly, modern rice varieties were found to lack haplotypes associated with superior PUE and would thus benefit from targeted introgressions of these loci from traditional donors to improve plant growth in phosphorus-limited cropping systems.

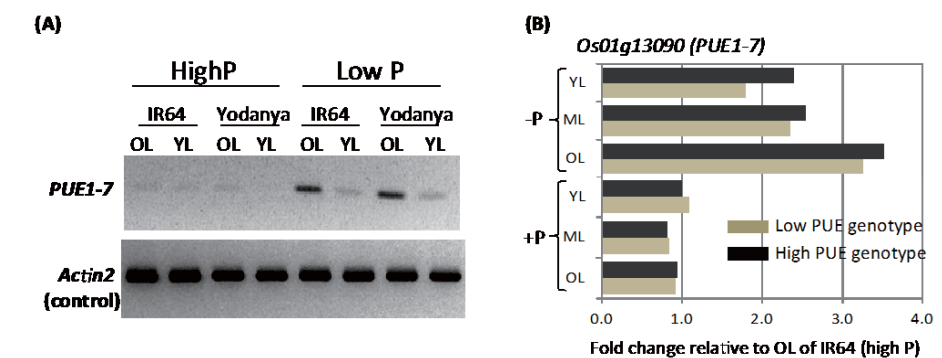
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**Fig. 1. Manhattan plots for PUE**

(A) Dotted lines indicate two major QTL of PUE on chromosome 1 and 11. They are involved in PUE for total biomass (shoot and root). The peak of chromosome 5 is associated with PUE for root biomass.

(B) *PUE* loci on chromosome 1 (7.247-7.343 Mb). The two candidate genes showed different gene expression patterns in low P conditions.



**Fig. 2. Gene expression analysis for candidate gene (*PUE1-7*) of the PUE locus on chromosome 1**

(A) Semi-quantitative RT-PCR (B) qPCR for candidate gene *PUE1-7*. Gene expression was compared in old leaves (OL, beginning to senesce), intermediate leaves (ML, typically the two leaves below the youngest fully expanded leaf) and young leaves (YL, >50% developed).