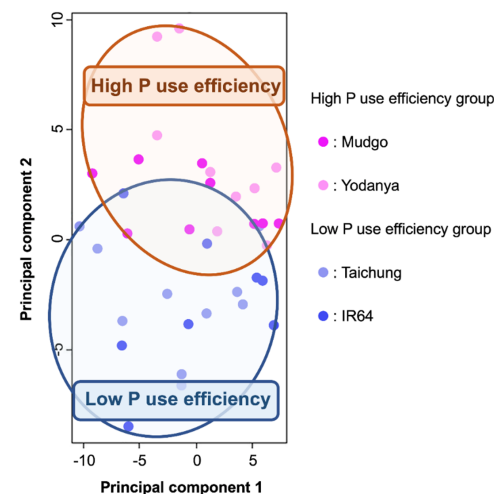


### Key metabolites for estimating phosphorus use efficiency in rice

Phosphorus (P) is an indispensable macronutrient for plants, and it is widely applied as fertilizer to crop fields. However, the overuse of P fertilizers causes problems such as eutrophication of water bodies and depletion of P reserves on earth. In contrast, insufficient amounts of P fertilizers are often applied in developing countries due to their high cost. The development of more P-efficient crop varieties offers one avenue to increase yield in such environments. In our previous study, P use efficiency was investigated in a wide range of rice accessions collected from different regions of the world. This screening identified characteristic *Indica* rice accessions contrasting in P use efficiency, e.g., Taichung and IR64 (one of the major rice varieties in the world) have low P use efficiency, while Mudgo and Yodanya have high P use efficiency. In the current study, we hypothesized that P-efficient varieties have characteristic metabolic profiles that enable efficient use of P. To identify potential metabolites associated with high P use efficiency, plants were grown in a hydroponic culture containing different concentrations of P, and metabolic profiles were analyzed.

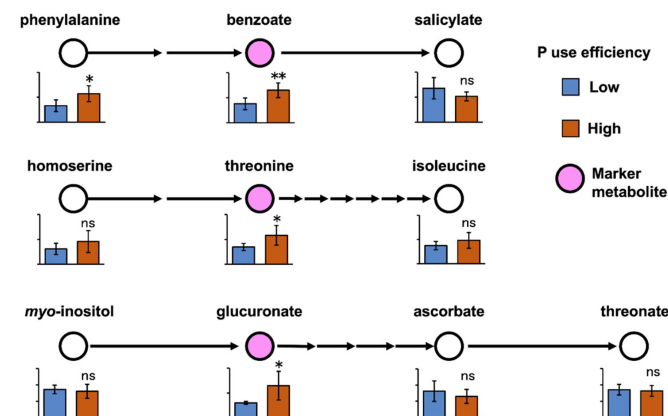
Based on the metabolomics data, a principal component analysis was conducted (Fig. 1). Under the P-deficient condition, metabolite profiles between the two groups (i.e., P-efficient and P-inefficient) were clearly separated, indicating that P-efficient and P-inefficient varieties have characteristic metabolite signatures. We next aimed at identifying key metabolites that distinguishes the P-efficient and P-inefficient variety groups. Our statistical analyses using LIMMA and ANOVA indicated key metabolites whose abundance is significantly different between the two groups. Notably, these metabolites were previously unassociated with P metabolism, implying that identification of these metabolites may shed light on basic molecular mechanisms of P use efficiency in rice. We further adopted a logistic Ridge regression model, which is a multi-variate analysis to predict a binary classification, and demonstrated that these key metabolites are indeed effective in predicting P use efficiency. Based solely on the contents of 14 key metabolites (such as benzoate and threonine; Fig. 2), P use efficiency (high or low) of 7 other rice varieties could be predicted successfully.

This study identified characteristic metabolite signatures for P-efficient varieties, whose usefulness was further validated through logistic regression analysis. Because analyses of P use efficiency from a large number of plants are cumbersome, identification of these key metabolites shall accelerate the selection of P-efficient accessions without the need for actual assessment of P use efficiency, and thus, boost rice breeding targeting efficient use of P. Based on the result of this study, we suggest that 1) metabolites serve as important molecular markers for complex trait that is difficult to assess, and 2) phenotypes can be predicted through combination of metabolite analysis and statistical modeling. In addition, the key metabolites may serve as the basis for further elucidation of the molecular mechanisms for P metabolism and P use efficiency.



**Fig. 1. Metabolite profile of rice varieties contrasting in P use efficiency**

Result of principal component analysis based on the foliar metabolite content of the plants grown under P-deficient condition is shown.



**Fig. 2. Marker metabolites for P use efficiency and related metabolic pathways**

Examples of metabolic pathways involving marker metabolites and their relative contents are shown. \*\* and \* indicate statistical significance at 1% and 5% levels (by *t* test).

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