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## **A multi-layered screening model for computational identification of leaf colour-related genes in rice plant**

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Rice field art is a large-scale art form in which people design rice fields using various kinds of ornamental rice plants with different leaf colours. Leaf colour-related genes play an important role in the study of chlorophyll biosynthesis, chloroplast structure and function, and anthocyanin biosynthesis. We performed whole-genome resequencing and transcriptomic analysis of regulatory patterns and genetic diversity among different rice cultivars to discover new genetic mechanisms that promote enhanced levels of various leaf colours. We resequenced the genomes of 10 rice leaf-colour varieties to an average depth of  $40\times$  depth and  $>95\%$  coverage and performed 30 RNA-seq experiments using the 10 rice varieties sampled at three developmental stages. The sequencing results yielded a total of  $1,814 \times 10^6$  reads and identified an average of 713,114 SNPs per rice variety. Based on our analysis of the DNA variation and gene expression, we selected 47 candidate genes. We used an integrated analysis of the whole-genome resequencing data and the RNA-seq data to divide the candidate genes into two groups: genes related to macronutrient (i.e., magnesium and sulfur) transport and genes related to flavonoid pathways including anthocyanidin biosynthesis. We verified the candidate genes with quantitative RT-PCR using transgenic T-DNA insertion mutants. Our study demonstrates the potential of integrated screening methods combined with genetic-variation and transcriptomic data to isolate genes involved in complex biosynthetic networks and pathways.

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