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Comparative analysis of metabolite changes in two contrasting rice genotypes in response to low-nitrogen stress

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ABSTRACT

Identification of metabolites responsible for tolerance to low nitrogen availability (low-N) will aid in the genetic improvement of rice yield under nitrogen deficiency. In this study, a backcross introgression line (G9) and its recurrent parent Shuhui 527 (SH527), which show differential responses to low-N stress, were used to identify metabolites associated with low-N tolerance in rice. Differences in metabolite contents in the leaves of G9 and SH527 at three growth stages under low-N stress were assessed by gas chromatography–mass spectrometry. Many metabolites, including amino acids and derivatives, were highly enriched in G9 compared with SH527 under the control condition, suggesting that the two genotypes had basal metabolite differences. Low-N stress induced genotype-specific as well as growth stage-dependent metabolite changes. Metabolites induced specifically in G9 that were involved in glycolysis and tricarboxylic acid metabolism were enriched at the tillering and grain filling stages, and metabolites involved in nitrogen and proline metabolism were enriched at the booting stage. Enrichment of pyroglutamate, glutamate, 2-oxoglutarate, sorbose, glycerate-2-P, and phosphoenolpyruvic acid in G9 suggests that these metabolites could be involved in low-N stress tolerance. The results presented here provide valuable information for further elucidation of the molecular mechanisms of low-N tolerance in crops.

1. Introduction

Rice (*Oryza sativa* L.) is a staple food crop worldwide, especially in developing countries. A predicted 26% increase in rice production will be required to feed the expanding population by 2035 without a corresponding increase in the area of agricultural land [1, 2].

Rice cultivation requires higher inputs of nitrogen (N) fertilizers than other agricultural crops [3, 4]. However,

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incorporation of N into agricultural crops rarely exceeds 40% of the amount of applied N, resulting in severe N pollution that is becoming a threat to ecosystems [3, 5–9]. Moreover, nutrient malnutrition is aggravated by the human demand for increased food production, which has led to the development of nutrient-hungry crop cultivars [10]. Therefore, the breeding of novel rice cultivars tolerant of low nitrogen availability (low-N) is urgently needed for sustainable agricultural production.

In higher plants, low-N stress results in physiological, morphological, and molecular changes. Plants show adaptive responses to low N availability, including extensive changes in primary and secondary metabolism, protein synthesis, cellular growth processes, expression of regulatory genes, and other cellular pathways [11]. For example, the plant root system is the most important organ for acquisition of soil N [12] and low-N stress leads to an increased root-to-shoot ratio [10, 13], reduced growth and photosynthesis rates [14, 15], accelerated uptake of N at early growth stages [16], and efficient internal recycling at terminal stages of development [17–19]. Under low-N conditions, a variety of responsive genes with diverse functions are upregulated to support plant survival or maintain grain yield [20, 21]. For example, overexpression of nitrate transporter (*NRT*) genes [22], the *Arabidopsis* ammonium transporter *AtAmt1.1* [23], Dof1 transcription factor [24], NADH-glutamate synthase [25], and the alanine aminotransferase gene (*AlaAT*) [26, 27] may increase plant growth or grain yield under low-N conditions. In addition, the low-N response in rice genotypes varies at different growth stages [10, 20, 28, 29].

Genetic variation in low-N tolerance is associated with adaptation to low-input agriculture [30]. This relationship is complex and depends on the crop and the environmental conditions. Although many physiological, phenotypic, and molecular analyses have been performed, the molecular mechanisms that govern genetic variation in low-N tolerance among cultivars remain poorly understood. To our knowledge, genetic manipulations for nutrient use in rice have been limited to experimental validation of few candidate genes.

Metabolite profiling is increasingly used to investigate metabolic regulation of the systemic response to the environment or to decipher gene function in plants [31–35]. Integration of the results from metabolic profiling and morphological analyses is a powerful strategy for crop improvement [36–39].

As part of our rice breeding program involving introgression of diverse germplasm into elite cultivars [40], an introgression line (G9) that shows high yield under both normal growth and low-N conditions was selected from a backcross population (BC_2F_8) derived from the cross between Shuhui527 (SH527) and Ye-Tuo-Zai. In the present study, a metabolomic analysis was conducted to investigate the impact of low-N stress on metabolite profiles of G9 and SH527 at the tillering, booting, and grain filling stages, with the aim of identifying metabolite differences in response to low-N stress between two genotypes with contrasting low-N tolerance.

2. Materials and methods

2.1. Plant materials and growth conditions

Two rice genotypes, Shuhui 527 (SH527) and G9, were used. G9 is an introgression line selected from a BC_2F_8 backcross population derived from the cross of SH527 (*indica*) and Ye-Tuo-Zai (*indica*). SH527 is the recurrent parent of G9 and is a commercial elite *indica* restorer line in China [41]. The G9 line was selected as a low-N-tolerant line because it showed stably higher grain yields than SH527 under low-N conditions in field experiments over several years. Genotype analysis using SSR markers shows that G9 differs from SH527 in 15 genomic segments from Ye-Tuo-Zai (Fig. S1).

Field experiments were conducted in 2014 and 2015 at the Langfang experimental station of the Chinese Academy of Agricultural Sciences, Hebei province, China. The soil chemical properties were pH 8.1, organic matter content 7.9 g kg^{-1}, total N 0.57 g kg^{-1}, alkali-hydrolyzable nitrogen 37.4 mg kg^{-1}, Olsen-P 7.8 mg kg^{-1}, and available K 70.7 mg kg^{-1}. Forty days after sowing, the rice plants were transplanted into 10-row plots, consisting of 12 plants in each row (120 plants per plot) with spacing of 25 cm × 15 cm and with three replications in 2014 and five replications in 2015. As fertilizer treatment, 307 kg urea, 1029 kg calcium superphosphate, and 239 kg potassium sulfate were applied per hectare for the control (normal growth conditions). In the low-N treatment, 102 kg urea per hectare (30% of the amount applied in the control) was applied and the amounts of P and K fertilizers applied were identical to those in the control.

2.2. Trait evaluation

Grain yield (GY), plant height (PHT), and tiller number per plant (TN) were recorded at plant maturity in 2014 and 2015. N concentration analysis was performed in 2015. The leaves and stems from three plants at maturity in each replicate were collected and dried in an oven for three days at 80 °C and the N concentration in each dried sample was then measured following Lu [42].

The metabolite characteristics under low-N stress in G9 and SH527 were systematically evaluated at the tillering (65 days after sowing), booting (95 days after sowing), and grain filling (123 days after sowing) stages in 2015. The five topmost leaves of G9 and SH527 plants were collected in each replicate. All samples with five biological replicates were frozen in liquid nitrogen and stored at –70 °C until metabolite extraction. Metabolite extraction followed Bowne et al. [43] and Zhao et al. [44]. The extracted samples were derivatized and analyzed by gas chromatography–triple quadrupole mass spectrometry (GCMS-TQ8040, Shimadzu Corporation, Japan). Chromatograms and mass spectra were processed using the search algorithm implemented in GC–MS Postrun Analysis software. Specific mass spectral fragments were detected in defined retention-time windows using the mass spectral libraries of the Smart Metabolites Database (Shimadzu Corporation). The gas chromatograph was equipped with a capillary column (SGE, BPX-5/30 m × 0.25 mm × 0.25 μm). The temperature program started at 60 °C, held for 2 min,