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Nitrogen effect on amino acid composition in leaf and grain of japonica rice during grain filling stage

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ABSTRACT

This paper reports the effect of nitrogen on amino acid composition of leaf and grain for japonica rice during the grain filling stage, using pot experiments with three N rate treatments. Dynamic changes of free amino acids (FAA) and protein amino acids (PAA) in leaves and grains were monitored. Results showed that FAA in leaves decreased with the progress of grain filling. FAA in grains increased at first 14 days after anthesis and then decreased to a nearly constant level. PAA in leaves showed a diminishing trend as leaf senesced after anthesis. Conversely, PAA in grain exhibited an increasing trend with the progress of grain filling. N had significantly positive effect on contents of FAA and PAA in leaves and grains. There was no obvious trend of the variation of FAA in leaves and grains with N rates. PAA in leaves was more sensitive to N fertilization. By contrast, PAA in grains was more stable across N rates, especially at the late stage of grain filling. In addition, composition of leaf PAA was different from that of grain, indicating the reconstruction of amino acids during the process of translocation of N compounds from leaves to grains.

1. Introduction

Nitrogen (N) is the element that plants require in largest quantity. It plays a central role in plant metabolism as a constituent of many cell components like proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones, and secondary metabolites (Hawkesford et al., 2012). N has two major functions in the formation of crop yield: (1) establishment and maintenance of photosynthetic capacity and (2) establishment of sink capacity and maintenance of functional sinks (Below, 2001). Addition of N from fertilizer is typically needed to maximize crop yields. However, excessive use of fertilizer N has been implicated in the contamination of ground water and the acidification of soils, representing a potential health hazard to both environments and humans. Therefore, a better understanding of how crops use N will undoubtedly help in adding to information required to improve N use efficiency and thereby minimize the adverse impact of N fertilizer.

The N composition of grains or seeds has important nutritional consequences, since crops are a major source of proteins in the diet of humans and animals (Galili and Amir, 2013). Rice is the most important cereal crop worldwide. Rice quality in terms of nutrition is valuable for its protein content and the balance of essential amino acids (Zhang et al., 2011). Rice protein has an excess of essential sulfur-containing amino acids Cys and Met, but it is deficient in the essential amino acid Lys. Thus, fortifying rice grain with Lys is of great significance for humans especially in developing regions.

The grain filling stage is crucial for both grain yield and quality of rice. N in mature grains is largely derived from the N compounds accumulated in leaves and stems up to the flowering stage. During the grain filling stage, redistribution of N compounds from leaves to harvesting organs (grains) occurs (Osaki et al., 1991). In leaves, the decomposed protein amino acids (PAA) are pooled as free amino acids (FAA). FAA is temporarily stored in leaves and then transported to the pool in the grains through the stems. Thereafter, FAA pooled in the grains is reconstructed to PAA, the storage proteins like prolamin and glutelin. In addition, it was suggested that there was a large difference between the composition of PAA in leaves or harvesting organs and that of FAA in each organ, indicating an

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active exchange of carbon (C) skeletons of amino acids took place between leaves and grains during the grain filling stage.

N fertilizer has a marked influence on grain protein content, with topdressing at the panicle initiation stage showing the larger effect (Ning et al., 2010). There exists disagreement on the changes in amino acid composition of proteins in leaves and grains in response to N fertilizer. Shinano et al. (1991) investigated the variations of FAA and PAA under N treatments. They found some kinds of FAA like Cys in leaves decreased with N level, whereas in the harvesting organs, the composition of FAA did not change appreciably with increase of N level. Further, the differences in the amino acid composition of the proteins (PAA) were negligible regardless of level of nitrogen application. By contrast, Ning et al. (2010) found a significant influence of N on amino acid composition of brown and milled rice, in particular Phe and Tyr. Similarly, Chang et al. (2008) reported that N significantly increased the content of essential amino acids in rice grains. Of note is that the results above are mainly based on analysis of mature grains, little information is available concerning the time course accumulation of FAA and PAA during grain filling. Thus the changes in amino acid compositions of leaves and grains of the rice plant still need to be further investigated.

This study employed pot experiments with three N rate treatments, and dynamic changes of FAA and PAA in leaves and grains sampled during the grain filling stage were monitored. The objectives were to clarify the effect of N on the amino acid composition in leaves and grains, and to discuss its significance with respect to N use efficiency and rice protein quality.

2. Materials and methods

2.1. Plant material and experiment design

Using a japonica rice cultivar, Wuyunjing7, the pot experiment was conducted at Danyang experimental station (31°54′31″N, 119°28′21″E) in 2012. Rice was sown in seedbeds on May 27, transplanted on June 18, and harvested on October 27. The pot was 30 cm in height and 34 cm in diameter, filled with 15 kg fine-grained soil. Each pot had five hills, with the seedling rate being one seedling per hill. The soil type was clay soil, containing 0.83 g/kg total N, 10.72 mg/kg available P, and 69.15 mg/kg exchangeable K. The basal fertilizers were 1.2 g N, 1.2 g P_2O_5, and 1.8 g K_2O/pot, and were applied before transplanting.

Four N topdressing treatments were conducted as follows: (1) CK, no N was applied during the whole panicle development stage; (2) N treatments of low level (LN, 0.6 g N/pot), medium level (MN, 1.2 g N/pot), and high level (HN, 1.8 g N/pot) were performed during the panicle development stage. In addition, N topdressing was split equally at two stages, the panicle initiation stage (4th leaf age in reverse order) and middle development stage (2nd leaf age in reverse order).

2.2. Sampling and chemical analysis

Pooled samples of the top three leaves and grains of three pots for each treatment were collected on the 7th, 14th, 21st, 28th, 35th, 42nd day after anthesis (DAA). Deactivation of enzymes was performed by 105 °C heating for 1 h and 80 °C drying to constant weight. Grain and leaf samples were milled into powder and stored in the refrigerator (4 °C) until analysis.

PAA in power samples was precipitated by 10% sulfosalicylic acid, and the resulting solution was used for FAA analysis with an L-8900 High Speed Amino Acid Analyzer (Hitachi Corp., Japan). Total amino acid (TAA) was measured by hydrolysis of powder samples by 6M HCl by the same analyzer. PAA was calculated by subtraction of FAA from TAA. The content of amino acids was expressed on a dry weight basis. Samples were analyzed in triplicate, and mean values were used for comparisons. Variance analysis was performed using SPSS (Statistical Product and Service Solutions, IBM). During acid hydrolysis, Asn and Gln are deaminated to Asp and Glu, respectively, and Trp is also completely destroyed. In addition, sulfur-containing amino acid Cys is partly damaged. Therefore, only 16 kinds of amino acids can be measured accurately.

3. Results

3.1. Dry matter accumulation in leaves and grains

Generally, dry weight decreased slightly in leaves while it increased markedly in grains with the progress of grain filling (Table 1). N showed significant effect on dry matter accumulation in both leaves and grains from the beginning of grain filling (7 DAA) to maturation (42 DAA), with the high N rate (HN) demonstrating the largest influence. In leaves, dry matter was 1.26 g/plant for CK at 42 DAA, while it increased to 2.72 g/plant by HN at the same time. Similarly, dry weight of grains was 7.98 g/plant for CK at 42 DAA, whereas it was nearly 3 times higher (21.85 g/plant) for HN treatment.

3.2. Contents of FAA and PAA in leaves and grains

FAA in leaves decreased with the progress of grain filling (Table 2). Averaged across N treatments, FAA was 3.79 mg/plant at 7 DAA, then reduced to 2.66 mg/plant at 14 DAA, and finally decreased to 2.11 at 42 DAA. FAA in grains increased at first at 14 DAA, being 16.18 mg/plant, and then decreased to a nearly constant level at 28, 35, and 42 DAA. N affected dramatically the FAA in leaves and grains, with the high N treatment of HN having the largest promoting effect (Table 2).

PAA in leaves showed a diminishing trend as leaf senesced after anthesis (Table 2). It was 282.53 mg/plant at the beginning of leaf senescence at 7 DAA and decreased drastically to 66.21 mg/plant at maturity. Conversely, PAA in grain exhibiting an increasing trend with the progress of grain filling, being lowest (306.51 mg/plant) at

Table 1
Dry matter accumulation of leaves and grains during grain filling stage (g/plant).

Treatments	Leaves							Grains						
	7d[a]	14d	21d	28d	35d	42d	Mean[b]	7d	14d	21d	28d	35d	42d	Mean
CK	1.51	1.40	1.35	1.34	1.28	1.26	1.36d	1.28	3.01	5.70	8.57	7.51	7.98	5.68c
LN	2.26	1.81	1.67	1.56	1.49	1.61	1.73c	3.27	6.79	9.60	12.83	12.60	13.86	9.83b
MN	2.52	2.51	2.19	2.49	2.17	2.35	2.37b	4.66	8.39	13.46	16.75	17.89	18.49	13.27a
HN	3.10	2.51	2.98	2.57	2.58	2.72	2.74a	4.80	8.75	16.98	16.86	21.09	21.85	15.06a

[a] Day after anthesis.
[b] Averages of six sampling times, different letters mean significance at $p < 0.05$ level. CK, no N was applied during the whole panicle development stage. N treatments: LN, low level (0.6 g N/pot); MN, medium level (1.2 g N/pot); HN, high level (1.8 g N/pot).