Journal of Cereal Science 50 (2009) 49–55

Contents lists available at ScienceDirect

Journal of Cereal Science

journal homepage: www.elsevier.com/locate/jcs

Effect of nitrogen fertilizer application on grain phytic acid and protein concentrations in japonica rice and its variations with genotypes

Huifeng Ning [a,b], Zhenghui Liu [a,b], Qiangsheng Wang [a,b], Zhaomiao Lin [a,b], Shujian Chen [a,b], Ganghua Li [a,b], Shaohua Wang [a,b], Yanfeng Ding [a,b,*]

[a] *Key Laboratory of Crop Physiology & Ecology in South China, Ministry of Agriculture, China*
[b] *College of Agronomy, Nanjing Agricultural University, Weigang 1, Nanjing 210095, China*

ARTICLE INFO

Article history:
Received 11 November 2008
Received in revised form
16 February 2009
Accepted 19 February 2009

Keywords:
Phytic acid
Storage proteins
Nitrogen
Japonica rice

ABSTRACT

Thirty-one japonica cultivars with contrasting agronomic traits were used and 5 nitrogen (N) fertilizer treatments of level and timing were performed to determine the effects of N application on grain phytic acid and proteins including albumin, globulin, prolamin, and glutelin. Variance analysis showed a large effect of N and a smaller effect of cultivar on grain phytic acid and proteins. With increased N level, grain phytic acid concentrations progressively reduced whereas concentrations of the 4 proteins and ratios of glutelin to total protein increased, indicating that N level has a beneficial effect on rice nutritional quality. In addition, substantial genotypic differences in response of grain phytic acid and proteins to N treatments were detected. Some cultivars such as Xiushui09, Zhendao10, and Yanjing5 exhibited more stability of grain proteins under contrasting N treatments, and the significance of this stability is discussed in regard to its use in rice quality improvement.

1. Introduction

Rice (*Oryza sativa* L.) is one of world's most widely grown cereals and plays a critical role in food security especially in South Asia and East Asia. A continuous improvement in rice production is a challenging task for crop scientists to keep abreast of the world's population growth. Nitrogen (N) is one of the most important elements required for obtaining high rice yields. However, the current irrigated rice system is characterized as low fertilizer N use efficiency due to overuse of N fertilizers, which also poses potential adverse environmental and health concerns (Jing et al., 2007; Samonte et al., 2006). Recently, a new method called heavy panicle fertilization, i.e. reducing the N level as basal before transplanting whereas increasing the topdressing level at panicle initiation stage, has been recommended mainly in China, due to its positive effect of increasing grain yield while reducing N loss and improving N-fertilizer recovery efficiency (Jiang et al., 2004; Perez et al., 1996; Xue and Yang, 2008).

Quality of grain, next to yield, is the most important factor for rice production, and among its key components, nutritional quality has historically been considered as one of the main objectives for rice improvement. Rice serves as an important source of dietary protein for half the world population, contributing approximately 29.1% of protein for human consumption in developing countries (Sautter et al., 2006). Rice grain proteins were empirically classified into albumin, globulin, prolamin, and glutelin based on their solubility. Grain albumin and globulin are mainly stored in the outer layers, while glutelin and prolamin are concentrated in the endosperm (Yamagata et al., 1982). Rice proteins are of better nutritional quality because the majority of them are glutelins, which are nutritionally more important than prolamins due to their greater digestibility by humans and higher lysine concentration (Cagampang et al., 1966; Islam et al., 1996; Zhang et al., 2008). On the other hand, some albumins and globulins were identified as allergenic proteins for patients with kidney disease, and breeding programs have been performed for hypoallergenic rice cultivars (Zhang et al., 2008).

In rice grains, Phosphorus (P) is stored mainly in the form of phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate), which consists of about 70% of grain total P (Lott, 1984). Phytic acid is viewed as an anti-nutritional factor because of its ability to complex with proteins and some nutritionally important micronutrients like iron and zinc, resulting in a marked reduction in the bioavailability of these nutrients (Raboy, 2001). Thus, it is important to improve rice nutritional

Abbreviations: CK, control of fertilizer treatments; CV, coefficient of variation; HN, high nitrogen level; K, potassium; MN, moderate nitrogen level; N, nitrogen; P, phosphorus.

* Corresponding author. College of Agronomy, Nanjing Agricultural University, Weigang 1, Nanjing 210095, China. Tel./fax: +86 25 84395033.
E-mail address: dingyf@njau.edu.cn (Y. Ding).

0733-5210/$ – see front matter
doi:10.1016/j.jcs.2009.02.005

quality by increasing the protein concentration and/or decreasing concentrations of the anti-nutritional components like phytic acid.

To date, great efforts have been made to improve rice protein quality by agricultural approaches including conventional plant breeding (Tsuzuki and Kuroda, 1989) and genetic engineering (Sautter et al., 2006). In addition, agronomical researches have been conducted to develop efficient N management protocols to achieve higher protein production. It was found that concentrations of grain crude protein and its four components both increased with added N level, with N topdressing at panicle development stage playing a major role in determining the protein concentrations of rice grain (Islam et al., 1996; Perez et al., 1996). Further, N application showed a favorable effect on milling quality by modifying protein distribution in rice grains (Leesawatwong et al., 2005).

As for phytic acid, mutants with low phytic acid concentration were characterized and evaluated for their nutritional significance (Larson et al., 2000; Liu et al., 2007; Ren et al., 2007). However, little information is available on how N fertilizer affects grain phytic acid accumulation.

Jiangsu Province is one of the main rice production areas of China, with the average grain yield being about 8.0 t/ha. However, this high yield is associated with high N input, being about 330.0 kg/ha provincially which resulted in low N use efficiency and high N loss to the aqueous environment (Xue and Yang, 2008).

The present study was conducted in Nanjing, Jiangsu Province. According to our previous investigations and the results of Jing et al. (2007), two treatments of N level, 187.5 kg/ha as moderate level and 300.0 kg/ha as high level, were conducted, and 31 japonica rice cultivars with contrasting agronomical traits were used in this study. The objectives were: (1) to determine the effect of N-fertilizer level and timing on grain proteins and phytic acid; (2) to investigate the genotypic differences in response of grain protein and phytic acid to N application; and (3) to study the relationship between proteins and phytic acid under contrasting N levels and thereby discuss the possibility of simultaneous improvement of the two components.

2. Materials and methods

Thirty-one japonica rice cultivars grown in Jiangsu Province were used in this study. The field experiment was performed at the experimental station farm of Nanjing Agricultural University (Nanjing, China; 31°56′39″N, 118°59′13″E) in 2007. The soil type is periodical water-logging paddy soil, having 1.36 g/kg total N, 10.36 mg/kg available P, and 82.6 mg/kg exchangeable K. The experiment was arranged in split plot design with three replicates. N treatment and cultivar were arranged as main plot and sub-plot, respectively. The size of the main plot was $5.0\ m \times 8.0\ m$, and a ridge covered plastic film was constructed between plots to avoid seepage. Rice was sown in seedbeds on May 20, and transplanted on June 20 at the level of two seedlings per hill, spaced 13.2 cm within rows and 30.0 cm between rows. For all treatments, about 140 kg P_2O_5/ha (as Ca $(H_2PO_4)_2$) and 186 kg K/ha (as KCl) were incorporated into the soil before transplanting.

Five N treatments were performed: (1) CK, no N fertilizer was applied during the whole growth stage, except for the P and K fertilizer applied before transplanting; (2) MN82, moderate N level (187.5 kg/ha) according to our previous investigations and related reports, 80% applied as basal before transplanting and 20% as topdressing at panicle initiation stage; (3) MN55, moderate N level and applied equally as basal and as topdressing; (4) HN82, high N level (300.0 kg/ha), 80% applied as basal and 20% as topdressing; (5) HN55, high N level applied equally as basal and topdressing. Of note is that the basal N level of MN82 was the same as that of HN55. Thus effect of panicle fertilizer N level can be also investigated in this experiment.

At maturity, about 60 panicles with similar maturity were harvested in each replication. The samples were naturally dried and dehulled. Brown grains were ground by a stainless grinder for 3 min and the resulting powders were used for chemical analysis. All the data were expressed on a wet mass basis, with the moisture being about 14.0%.

Grain phytic acid was extracted by 0.2 M HCl and measured by the Fe-precipitation method (Liu et al., 2005a). The protein fractions were separated and analyzed according to the method reported by Liu et al. (2005a). Four protein fractions were sequentially extracted in the order of albumin, globulin, prolamin, and glutelin. Glutelin concentrations were determined by the Biuret method (Holme and Peck, 1998), while the concentrations of the other three fractions were determined using the Bradford reagent according to Bollag and Edelstein (1990). Total protein concentration is calculated as the sum of the 4 proteins.

Samples were analyzed in triplicate and mean values were used in comparisons and correlation analysis. Variance analysis was performed using the Data Processing System (DPS, Institution of Agricultural Entomology, Zhejiang University), with N treatment and cultivar being treated as random effects. Means were compared by the least significant difference (LSD) test ($P \leq 0.05$). Correlation analysis was also performed by DPS.

3. Results and analysis

3.1. Yield performance

As shown in Table 1, both N fertilizer and cultivar showed significant effect on grain yield. Significant genotypic variations were detected among the cultivars examined. Averaged grain yield across the 5 N treatments was highest for Wu9522 and lowest for Xudao3, with a range of 5713.5–9851.4 kg/ha (Tables 2–4). N treatment had a larger effect on grain yield than that of cultivar (Table 1). Generally, grain yield increased progressively with N level (Table 2). For HN55 treatment, average yield of the 31 cultivars was 7672.2 kg/ha, being 2.29 times higher than that of the CK (3954.0 kg/ha).

Heavy panicle fertilizer increased grain yield under the moderate N treatment, with MN55 producing higher yield than MN82 did. However, it didn't show a significant effect on grain yield when N level was high, with no significant difference detected between HN82 and HN55. Importantly, moderate N treatment of MN55 produced comparable grain yield to those of the two high N treatments. These results suggest that the combination of moderate N level with heavy panicle fertilizer should be useful for promoting rice production while increasing the N use efficiency and thereby protecting the water environment in Jiangsu Province.

In addition, genotypic differences in yield performance response to N treatments existed. Wuyunjing3, the well-known rice cultivar for its good cooking and eating quality, was less sensitive to N treatments, with a coefficient of variation (CV) being 23.3% across the 5 N levels. By contrast, Zhendao10, the new-released high yield cultivar, was more affected by N fertilizer, showing the highest CV of 40.8% among the 31 cultivars. This is partially associated with the lower yield under no N-fertilizer treatment (CK) and the relatively higher yield when N fertilizer applied for Zhendao10.

3.2. Effect of N-fertilizer application and genotype on grain phytic acid and proteins

N-fertilizer application exhibited significant influence on grain phytic acid and proteins, and its effects were generally larger than those of cultivar (Table 1).