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In-season assessment of grain protein and amylose content in rice using critical nitrogen dilution curve

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ABSTRACT

Effective nitrogen (N) management strategies ensure optimal N status in rice (*Oryza sativa* L.) plants for improving crop growth and grain quality with optimal N use efficiency. In-season plant N status affects rice grain quality. The critical N (Nc) dilution curves have been applied for predicting in-season nitrogen requirement (NR) and grain yield in rice, however, its application for estimating rice grain quality at harvest is yet to be tested. This research was endeavored to establish the quantitative relationships of protein content (PC) and amylose content (AC) with nitrogen nutrition index (NNI), accumulated nitrogen deficit (AND), and NR at various growth stages during vegetative growth period of rice and to validate these relationships in *Japonica* and *Indica* rice ecotypes. Five multi-locational field trials with five rice cultivars and varied N application rates were carried out in eastern China. The quantitative relationships of PC and AC with NNI, AND, and NR at various growth stages in both rice ecotypes were highly significant (for *Japonica* and *Indica*, $R^2 > 0.88$ and 0.85 for PC, $R^2 > 0.85$ and 0.81 for AC, respectively). The strongest relations were observed for both rice ecotypes at later vegetative growth stages and periods. The validation of the developed quantitative relationships with the independent dataset revealed a solid model performance, especially during later vegetative growth period ($R^2 > 0.90$, root mean square error < 18%) and confirmed their robustness as reliable predictors for assessing in-season grain quality in rice. The projected results can be used for estimating in-season grain quality and precision N management for rice.

1. Introduction

Rice (*Oryza sativa* L.) is a major food crop for almost half of the world's inhabitants. In China, rice crop accounts for approximately 28 and 43% of the total area under grain cultivation and production, respectively. With an ever increasing population and reducing cropland, China requires almost 20% higher rice production by 2030 (Peng et al., 2009). Therefore, N fertilizer, a key crop nutrient, is often applied in excess to maximize rice production in the country (Yuan et al., 2016; Ata-Ul-Karim et al., 2016a). Higher rice yields in Chinese agricultural systems during past few decades have been associated with excessive N fertilizer applications. China accounts for 37% of the global N consumed in rice production, and N applications rates in rice cultivation are as high as 387 kg N ha^{-1} in Jiangsu province of east China (Chen et al., 2011a,b). Adequate N application is essential for optimum crop production, however supra-optimal N application results in excessive vegetative growth, thus lodging, delayed maturity (Ata-Ul-Karim et al., 2017a), as well as reduced grain yield and poor quality (Yoon et al., 2001). Therefore, effective N regulation/management during crop growth period is key to efficient crop production, as well as to enhance crop yield and quality (Peng et al., 1996; Zhao et al., 2017).

Crop yield potential and grain quality are of utmost importance (Gu et al., 2015), however; negative reciprocity between them is a challenge for rice scientists and growers (Chandel et al., 2010). Rice grain quality is determined by several factors such as genetic differences in cultivars, environmental factors and agronomic management practices (Tsukaguchi et al., 2016). Therefore, improving rice grain quality is the most difficult and vital task in breeding and agronomic research.

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Modern agronomic management strategies require an improved understanding of the determinants governing changes in rice grain yield and quality to meet the increasing global demands. Additionally, the capability to access the grain quality prior to harvest is not only advantageous for rice farmers to increase price premiums by segregating rice grains on quality basis but also to retrospectively evaluate the efficiency of N management approaches (Gu et al., 2015). Recently grain quality parameters such as protein content (PC) and amylose content (AC) have attained considerable attention by researchers, merchants, and consumers. Protein and amylose contents generally differ among and within the rice field, depending on the external factors such as fertilization, irrigation, and meteorology (Ozturk and Aydin, 2004; Blumenthal et al., 1998).

Due to substantial effects of N fertilizer on caryopsis development and grain quality, the formation of grain protein and amylose are physically dependent on plant N uptake during the vegetative growth period and its translocation to grain during the grain filling stage (Chen et al., 2011a,b; Zhao et al., 2005; Onoyama et al., 2011). Prolamin and glutelin are two major storage proteins accumulated in protein bodies of rice seed endosperm to serve as an N source during the germination. Prolamin and glutelin are stored in protein body type-I (PB-I) and protein body type-II (PB-II), respectively (Song et al., 2012). The prolamin and glutelin have contrasting effects on protein and amylose related quality traits of rice grain due to variation in their relative proportions which is largely determined by N, and a higher ratio of PB-I to PB-II results in higher accumulation of AC as compared to PC and vice versa (Ning et al., 2010). Moreover, cereals endosperm contains two different groups of starch granules (A-types and B-types). A-type granules contain higher AC than B-type granules (Van Hung and Morita, 2005). Judicious N application can maintain the proportion of these granules to achieve good grain quality as previous studies reported that higher N application rates decrease the proportion of A-type granules which results in lowering the AC and vice versa (Kaufman et al., 2013). Therefore in-season N management (the amount and timing of N application) are imperative to optimize grain quality in rice (Thu et al., 2014). Consequently, it is indispensable to assess crop NR at critical growth stages is to ensure optimum crop N uptake required for higher yield and good quality in rice (Xue et al., 2007; Ata-Ul-Karim et al., 2016a).

The ability to determine the in-season N status of crop plants and to relate it with N translocation in the grains opens the possibility of predicting grain quality in cereals using various N diagnostic tools. Previous studies attempted to predict grain yield and quality of cereal crops using rapid and non-destructive measurements acquired from chlorophyll meter and remote sensing techniques (Xue et al., 2007; Basnet et al., 2003). The results of previous studies indicated that the quantitative relationships established between chlorophyll meter and canopy spectral measurements against plant N concentration and PC provided in-season estimates of grain yield, grain N uptake, and total N uptake in cereals, however; these relationships were not consistent across locations and years (Hansen et al., 2002; Mistele and Schmidhalter 2008; Zhao et al., 2005; Onoyama et al., 2011). Consequently, the grain PC prediction in cereals is still challenging. Moreover, no attempt has been made for in-season estimation of grain AC in any crop including rice.

Recently, the concept of critical N (Nc) has also been extensively used to reliably quantify and forecast crop growth performances (Ata-Ul-Karim et al., 2016a,b, 2017a). The Nc dilution curves have been developed in various agronomic crops including *Japonica* and *Indica* rice on plant dry matter (Sheehy et al., 1998; Ata-Ul-Karim et al., 2013, 2017b; He et al., 2017) as well as on leaf area index and specific organ DM basis (Ata-Ul-Karim et al., 2014a,b, 2017b; Yao et al., 2014). Additionally, this concept has been successfully used for in-season evaluation of N requirement, grain yield and setting yield targets according to varied N rates in both rice ecotypes (Ata-Ul-Karim et al., 2016a, 2017a). Therefore, the grain quality parameters such as PC and AC can also be possibly predicted indirectly using this approach. However, no attempts have been yet reported on quantitative estimation of grain PC and AC using this approach. Therefore, it is imperative to test the applicability of this approach for estimating rice grain quality.

The current study aimed to substantiate quantitative relationships of grain protein and amylose contents with Nc dilution curve based NNI, AND and NR, during growth periods of *Japonica* and *Indica* ecotypes and to forecast in-season grain quality parameters. The results of this study will help to improve the rice grain quality with in-season precision N management.

2. Materials and methods

2.1. Experimental sites and designs

This study was conducted in five experimental fields at Yizheng (32°16′ North, 119° 10′ East) and Rugao (32°23′ North, 120° 33′ East), situated in eastern China. The area containing these sites has a subtropical-temperate climate with hot summer and cold winter, and is appropriate for planting different ecotypes of rice. The rice-wheat cropping system is widely practiced in the region. Experiment 1, and 2 were performed at Yizheng while Experiment 3, 4, and 5 were performed at Rugao. The main soil type at both sites was same (Ultisoles). The averaged soil pH, organic matter, total N, available phosphorous (P) and available potassium (K) at Yizheng and Rugao were 6.3 and 6.2, 16.5 and 17.3 g kg^{-1}, 1.45 and 1.32 g kg^{-1}, 40.5 and 36 mg kg^{-1}, and 87.5 and 90 mg kg^{-1}, respectively.

Experiment 1 was conducted in 2010 with five N treatments (0, 80, 160, 240, 320 kg N ha^{-1}), which were applied as: 50% at pre-planting (PP), 10% at active tillering (AT), 20% at panicle initiation (PI), and 20% at booting (BT) stage. The *Japonica* rice cultivars Wuxiangjing14 and Lingxiangyou18 were planted.

Experiment 2 was conducted in 2011 with five N treatments (0, 90, 180, 270, 360 kg N ha^{-1}), which were applied as: 50% at PP, 10% at AT, 20% at PI, and 20% at BT stage. The *Japonica* rice cultivars Wuxiangjing14 and Lingxiangyou18 were planted.

Experiment 3 was conducted in 2012 with five N treatments (0, 70, 170, 270, 370 kg N ha^{-1}), which were applied as: 50% at PP, 10% at AT, 20% at PI, and 20% at BT stage. The *Indica* rice cultivar Shanyou63 was planted.

Experiment 4 was conducted in 2013 with six N treatments (0, 75, 150, 225, 300, 375 kg N ha^{-1}), which were applied as: 40% at PP, 10% at AT, 20% at PI, and 30% at BT stage. The *Japonica* rice Wuxiangjing14 and *Indica* rice Shanyou63 cultivars were planted.

Experiment 5 was conducted in 2014 with five N treatments (0, 150, 225, 300, 375 kg N ha^{-1}), which were applied as: 40% at PP, 10% at AT, 20% at PI, and 30% at BT stage. The *Japonica* rice Wuyunjing14 and *Indica* rice YLiangyou1 cultivars were planted.

All the five experiments were laid in a randomized complete block design with three replications. The N was applied as urea and winter wheat was the preceding crop in all experiments. In all the experiments, P and K fertilizers (59 and 158 kg ha^{-1}) as P_2O_5 and K_2O, respectively, were incorporated in each plot before transplantation. Sowing dates, seeding densities, and the other management practices were chosen according to the best possible management strategy to ensure that N fertilizer was the only limiting experimental factor.

2.2. Measurement

In all five experiments, plants from an area of 0.23 m^2 were harvested at various crop growth stages such as AT, mid tillering (MT), stem elongation (SE), PI, BT, heading (HD), and maturity for analysis of plant DM (t ha^{-1}) and plant N concentration (%). Plants were fractionated into different organs (leaves, stems, and panicles). The fractionated samples after oven drying and weighing were grounded for N determination by the standard Kjeldahl method (Bremner and