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ARTICLE

Accumulation Characteristics of Protein and Non-Protein Components and Their Correlations with Protein Concentration in Rice Grains

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ABSTRACT

Protein in rice grains is an important source of nutrition for rice consumers. This study mainly aimed to identify the critical factors that determine grain protein concentration in rice. Accumulation parameters, including mean accumulation rate (R_{mean}) and active accumulation duration (D_{active}), for protein and non-protein components and their correlations with protein concentration in rice grains were investigated in field experiments conducted over two years with six rice cultivars. Results showed that grain protein concentration ranged from 9.6% to 11.9% across cultivars and years. Accumulation processes of protein and non-protein components were well fitted by the logistic equation for all six rice cultivars in both years, and the ratio of protein to non-protein for R_{mean} and D_{active} ranged from 0.08 to 0.12 and 1.01 to 1.33, respectively. Grain protein concentration was significantly correlated with protein to non-protein ratio for R_{mean}. This study suggests that grain protein concentration is not solely determined by the accumulation of protein or non-protein component, but by the coordination of protein and non-protein accumulation.

KEYWORDS

Grain protein concentration; logistic regression; non-protein accumulation; protein accumulation; rice

1 Introduction

Rice is the staple food for over half of the world's population [1]. Many rice consumers are among the world's poorest people and have diets that are largely restricted to rice because it is filling and is the most accessible and affordable food [2]. Rice is not only the major source of dietary energy for these consumers but is also an important source of nutrition, especially for those living in poverty [1,2]. Rice provides about 20% of global human per capita energy and approximately 15% of per capita protein [3]. Hence, it is significant to improve protein concentration in rice grains.

Grain protein concentration in rice varies with cultivar and environment [4], and a fundamental understanding of the determinants of variation in grain protein concentration can provide helpful

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information for its improvement. Grain protein concentration is the percentage of protein weight accounting for grain weight, and the grain weight can be divided into protein and non-protein weight. Therefore, the variation in grain protein concentration may be related to a change in protein and/or non-protein accumulation in rice grains, but studies are required to establish this point.

Logistic regression analysis is a commonly used tool to fit the grain-filling process in rice, and parameters deduced from the regression equations (e.g., grain-filling rate and duration) are useful for understanding the factors that determine grain weight [5,6]. The logistic regression equation is also suitable for fitting the accumulation process of protein fractions including glutelin and prolamin in rice grains [7].

In this study, field experiments were conducted over two years with six rice cultivars to investigate the accumulation characteristics of protein and non-protein components in rice, based on logistic regression analysis, and their correlations with protein concentration in rice grains. The main objective of this study was to identify the critical factors that determine grain protein concentration in rice.

2 Materials and Methods

2.1 Data Collection

Field experiments were conducted in Yongan Town (28°09′N, 113°37′E, 43 m asl), Hunan Province, China in the early rice-growing season in 2018 and 2019. Average daily mean temperature during the grain-filling period was 28.3°C and 27.1°C in 2018 and 2019, respectively (Fig. 1). The daily mean temperature was recorded by an on-site weather station (Vantage Pro2, Davis Instruments Corp., Hayward, CA, USA). The soil of the experimental field was a clay (Ultisol, USDA taxonomy) with the following properties: pH = 6.25, organic matter = 37.3 g kg^{-1}, available N = 172 mg kg^{-1}, available P = 18.2 mg kg^{-1}, and available K = 80.7 mg kg^{-1}. The soil test was based on samples taken from the upper 20 cm layer before the experiment began in 2018.

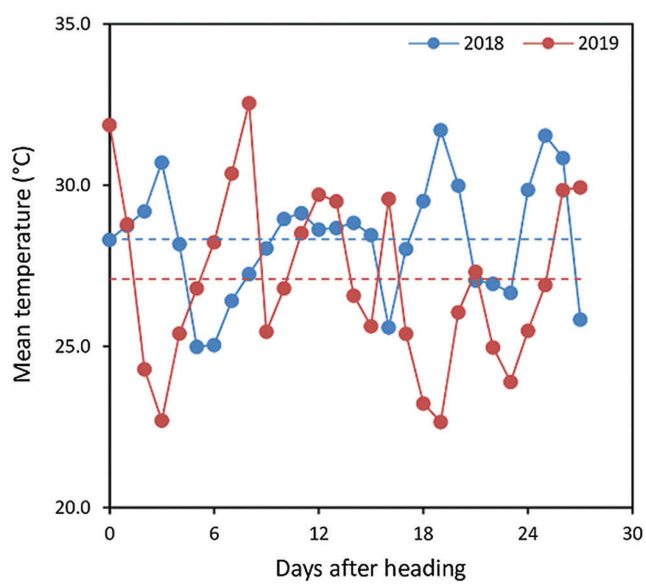

Figure 1: Daily mean temperatures during the grain-filling period in the early rice-growing seasons in 2018 and 2019. Dashed lines represent the average daily mean temperature

Six rice cultivars, Luliangyou 996 (L996), Xiangzaoxian 24 (X24), Xiangzaoxian 32 (X32), Xiangzaoxian 42 (X42), Zhuliangyou 189 (Z189), and Zhuliangyou 819 (Z819), were used in this study.

These cultivars were selected because: (1) they have different grain protein concentrations (8.8–13.0%) registered in the cultivar information of the Seed Management Office of Hunan Province, and (2) they have been widely grown by rice growers in the study region.

The cultivars were arranged in a randomized complete-block design with three replicates and a plot size of 40 m^2. Seedlings were raised in trays for 25 days and then transplanted with a high-speed rice transplanter (PZ80-25, Dongfeng Iseki Agricultural Machinery Co., Ltd., Xiangyang, China). Transplanting was done at a spacing of 25 cm × 11 cm with two seedlings per hill. Missing plants were replanted by hand at 7 days after transplanting to ensure a uniform plant population. N fertilizer was applied in three splits: 67.5 kg N ha^{-1} as basal fertilizer (1 day before transplanting), 27 kg N ha^{-1} at early-tillering (7 days after transplanting), and 40.5 kg N ha^{-1} at panicle initiation. P fertilizer (67.5 kg P_2O_5 ha^{-1}) was applied as basal fertilizer and K fertilizer (135 kg K_2O ha^{-1}) was split equally as basal fertilizer and at panicle initiation. The experimental field was kept flooded from transplanting until 7 days before maturity. Weeds, insects, and diseases were intensively controlled by chemicals to avoid yield losses. Briefly, weeds were controlled by applying a 25 mg penoxsulam ml^{-1} suspension concentrate at 900 ml ha^{-1}. Striped rice borers, planthoppers, and leaf rollers were controlled with 20% triazophos emulsion concentrate at 750 ml ha^{-1}, 25% buprofezin wettable powder at 750 g ha^{-1}, and 45% phoxim emulsion concentrate at 900 ml ha^{-1}, respectively. Rice sheath blight and false smut were controlled by applying 5% validamycin aqueous solution at 2625 ml ha^{-1}.

Approximately 150 main-stem panicles that headed on the same day were tagged in each plot. Seven tagged panicles were sampled randomly from each plot, starting at 3 days after tagging and continuing at a 3-day interval until maturity. The grains in the middle portion of each sampled panicle were hand threshed, hulled, and oven-dried at 70°C to a constant weight. Grain weight was calculated by dividing the total grain weight by number of grains. The dried grains were ground into fine powder and 0.5 g of the powder was digested in a H_2SO_4–H_2O_2 solution. Total N concentration in the digested solution was measured using a segmented flow analyzer (Skalar SAN Plus, Skalar Inc., Breda, The Netherlands) to determine grain N concentration. Grain protein concentration was calculated by multiplying the grain N concentration by the factor 5.95 [8]. The amount of protein per grain was calculated by multiplying the grain protein concentration by the grain weight. The amount of non-protein weight per grain was calculated by subtracting the protein weight from the grain weight. The grain, protein, and non-protein weights were expressed on a dry-weight basis.

The accumulation process (i.e., changes in weight with days after heading) of protein and non-protein components was fitted using the logistic equation, i.e., y = k/(1 + EXP(a–bx)), where y is weight, x is days after heading, k is estimate of final weight, and a and b are fitting parameters of the equation. Accumulation parameters of protein and non-protein components including mean accumulation rate (R_{mean}) and active accumulation duration (D_{active}) were estimated with y at 95% of k using the following equations modified from Huang et al. [5]: R_{mean} = kb/(a–ln(100/95–1)); and D_{active} = 95%k/R_{mean}. Protein to non-protein ratio for a given accumulation parameter was calculated by dividing the value of the parameter for the protein component by that for the non-protein component.

2.2 Statistical Analysis

Data of grain weight and protein concentration at maturity were analyzed by analysis of variance (ANOVA), followed by the least significant difference test (LSD) at the 0.05 probability level. The statistical model for the ANOVA included replication, cultivar, year, and the interaction between cultivar and year. The correlations between grain protein concentration at maturity and accumulation parameters (i.e., R_{mean} and D_{active}) for protein and non-protein components were evaluated by Pearson's coefficient analysis.

3 Results

The main effects of cultivar and year on grain weight were significant ($P < 0.01$), while the interactive effect between cultivar and year on grain weight was not significant ($P > 0.05$) (Fig. 2A). Z189 had the highest grain weight, followed by L996, Z819, X42, X32, and X24. Average grain weight across six cultivars was 5% higher in 2018 than in 2019. Grain protein concentration was significantly affected by cultivar ($P < 0.01$), year ($P < 0.01$), and their interaction ($P < 0.01$), ranging from 9.6% to 11.9% (Fig. 2B). The maximum grain protein concentration occurred in L996 in 2019, while the minimum grain protein concentration was recorded in Z189 in 2018.

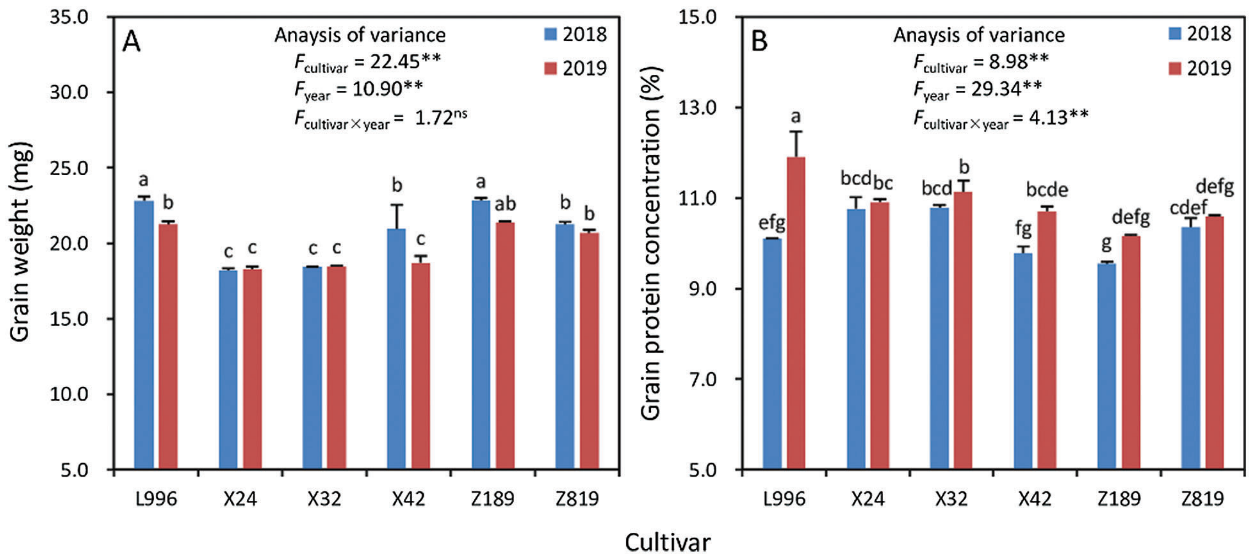

Figure 2: Grain weight (A) and protein concentration (B) at maturity in six rice cultivars (L996, Luliangyou 996; X24, Xiangzaoxian 24; X32, Xiangzaoxian 32; X42, Xiangzaoxian 42; Z189, Zhuliangyou 189; Z819, Zhuliangyou 819) in 2018 and 2019. Data are means of three replicates, and error bars represent standard errors. ** denotes significance at the 0.01 probability level. ns denotes non-significance at the 0.05 probability level. Bars not sharing any letter are significantly different by the LSD test at the 0.05 probability level

Accumulation processes of protein and non-protein components were well fitted by the logistic equation for all six rice cultivars in both years, showing coefficients of determination (R^2) of 0.956–0.996 ($P < 0.01$; Figs. 3A–3D). Accumulation parameters for protein and non-protein components, including R_{mean} and D_{active}, varied with cultivar and year (Tab. 1). For protein, R_{mean} and D_{active} ranged from 0.090 to 0.141 mg grain^{-1} d^{-1} and 13.8 to 19.9 d, respectively. For non-protein, R_{mean} and D_{active} ranged from 1.002 to 1.364 mg grain^{-1} d^{-1} and 12.9 to 16.0 d, respectively. Protein to non-protein ratio for R_{mean} and D_{active} ranged from 0.08 to 0.12 and 1.01 to 1.33, respectively.

There were no significant correlations between grain protein concentration with R_{mean} and D_{active} of protein and non-protein components ($P > 0.05$; Fig. 4). Grain protein concentration was significantly positively correlated with protein to non-protein ratio for R_{mean} ($P < 0.05$), while its correlation with protein to non-protein ratio for D_{active} was not significant ($P > 0.05$).

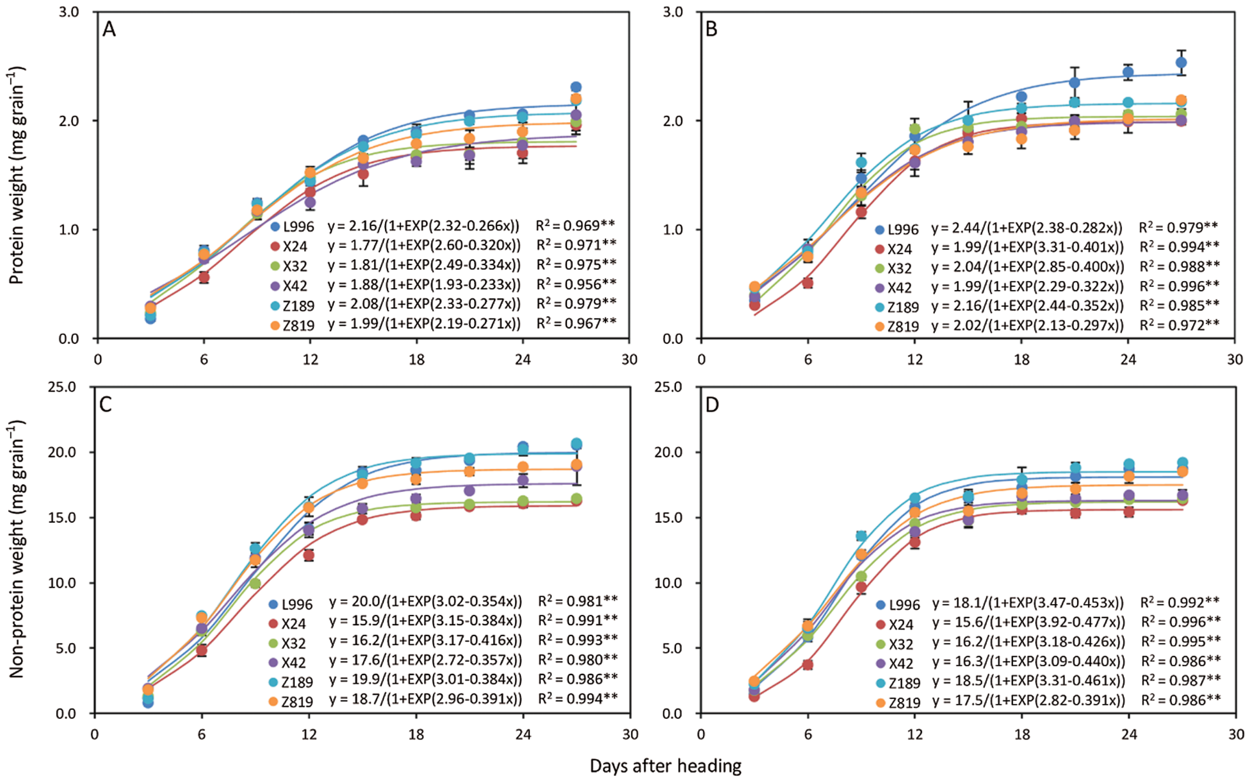

Figure 3: Accumulation processes of protein (A, B) and non-protein (C, D) components in grains fitted by the logistic equation for six rice cultivars (L996, Luliangyou 996; X24, Xiangzaoxian 24; X32, Xiangzaoxian 32; X42, Xiangzaoxian 42; Z189, Zhuliangyou 189; Z819, Zhuliangyou 819) in 2018 (a, c) and 2019 (b, d). Data (circles) are means of three replicates, and error bars represent standard errors. ** denotes significance at the 0.01 probability level

Table 1: Accumulation parameters for protein and non-protein components in grains of six rice cultivars in 2018 and 2019

Cultivar[a]	Protein[b]		Non-protein		Protein to non-protein ratio	
	R_{mean}	D_{active}	R_{mean}	D_{active}	R_{mean}	D_{active}
2018						
L996	0.109	18.8	1.187	16.0	0.09	1.17
X24	0.102	16.5	1.002	15.1	0.10	1.09
X32	0.111	15.5	1.102	14.0	0.10	1.11
X42	0.090	19.9	1.109	15.1	0.08	1.32
Z189	0.109	18.1	1.283	14.7	0.09	1.23
Z819	0.105	18.0	1.238	14.3	0.08	1.25

(Continued)

Table 1 (continued).

Cultivar[a]	Protein[b]		Non-protein		Protein to non-protein ratio	
	R_{mean}	D_{active}	R_{mean}	D_{active}	R_{mean}	D_{active}
2019						
L996	0.129	17.9	1.278	13.5	0.10	1.33
X24	0.128	14.8	1.084	13.7	0.12	1.08
X32	0.141	13.8	1.127	13.7	0.12	1.01
X42	0.122	15.4	1.189	13.0	0.10	1.19
Z189	0.141	14.5	1.364	12.9	0.10	1.13
Z819	0.118	16.2	1.187	14.0	0.10	1.16

Note: [a]L996, Luliangyou 996; X24, Xiangzaoxian 24; X32, Xiangzaoxian 32; X42, Xiangzaoxian 42; Z189, Zhuliangyou 189; Z819, Zhuliangyou 819. [b]R_{mean}, mean accumulation rate (mg grain^{-1} d^{-1}); D_{active}, active accumulation duration (d).

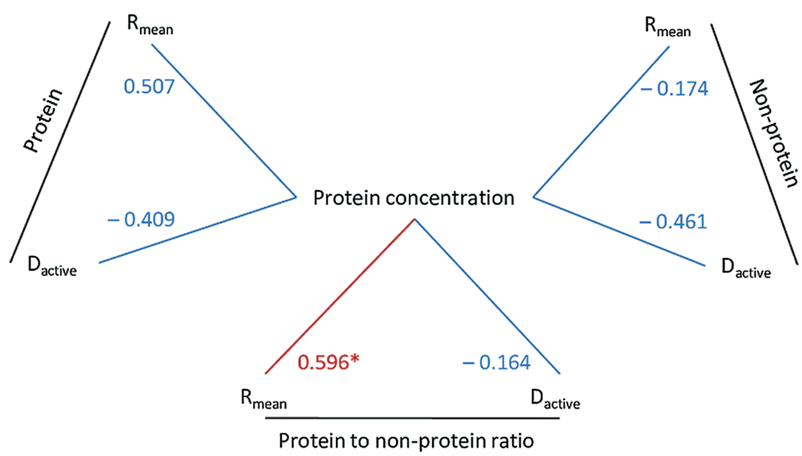

Figure 4: Pearson's coefficients between protein concentration and accumulation parameters for protein and non-protein components in rice grains. The data used for analysis are presented in Fig. 1 and Tab. 1. R_{mean} and D_{active} are mean accumulation rate and active accumulation duration, respectively. * denotes significance at the 0.05 probability level (n = 12)

4 Discussion

In this study, one of the main reasons for selecting the tested cultivars was that they have different grain protein concentrations registered in the cultivar information of the local seed management office (see Section 2). Although the tested cultivars also showed different grain protein concentrations in the present study, the magnitude of the variation in grain protein concentration (2.3%) in the present study is smaller than that (4.2%) recorded in the cultivar information. This is because grain protein concentration in rice does not only depend on the cultivar but also on the environment [4]. This explanation can also be supported by the results of this study, which indicated that grain protein concentration was not only significantly affected by the cultivar but also by the year and the interaction between cultivar and year. In this regard, it has been reported that changes in growth environments such as light can lead to changes in N concentrations in rice plants [9], which are positively related to protein concentrations in rice grains [10]. This indicates that more experimentation should be done on different environments with various rice cultivars showing a larger variation in the grain protein concentration to obtain reliable results.

There have been reports on the use of the logistic equation in modeling the grain-filling and the accumulation processes of grain protein fractions (e.g., glutelin and prolamin) in rice [5–7]. However, very limited modeling studies have been performed on the accumulation processes of protein and non-protein components in rice grains. In the present study, we used logistic equation to fit protein and non-protein weight per grain onto days after heading in six rice cultivars in two years; a very high coefficient of determination of more than 0.95 was obtained for all cultivars in both years. This finding suggests that use of the logistic equation is a reliable method to fit the accumulation processes of protein and non-protein components in rice grains. Furthermore, according to the accumulation parameters deduced from the regression equations in this study, the main difference between protein and non-protein accumulation in rice grains is that protein accumulation is much slower but of somewhat longer active duration than non-protein accumulation.

In addition, and most importantly, the Pearson's coefficient analysis between grain protein concentration and accumulation parameters (i.e., R_{mean} and D_{active}) for protein and non-protein components in the present study provides useful information for identifying the critical factors that determine grain protein concentration in rice. In this sense, grain protein concentration was correlated with protein to non-protein ratio for R_{mean}. This information indicates that grain protein concentration is not solely determined by the accumulation of protein or non-protein component, but by the coordination of protein and non-protein accumulation. This finding suggests that future investigations on the physiological mechanisms regulating grain protein concentration should focus on the coordination of grain N and C metabolisms in rice.

5 Conclusions

Accumulation processes of protein and non-protein components in rice grains are in accordance with the logistic curve. Protein accumulation is much slower but of somewhat longer active duration than non-protein accumulation in rice grains. Grain protein concentration is determined by the coordination of protein and non-protein accumulation in rice.

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Conflicts of Interest: The authors declare that they have no conflict of interest to report regarding the present study.

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