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Positional variations of rice protein compositions accumulation within a panicle during the grain filling

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Abstract

Grain protein is a critical quality attribute of rice that influences consumer preferences. However, the spatial variation in protein accumulation within a rice panicle remains poorly understood. This study investigated the dynamics of protein accumulation, including protein components and protein synthesis-related enzymes and genes, among grains located at the top, middle, and bottom primary rachises of a rice panicle during the grain filling. The results revealed significant variations in protein compositions across different rachis positions. The contents of albumin, globulin, prolamin, glutelin, and total protein contents exhibited fluctuations during grain filling. Notably, the grain position had a significant effect on glutelin content, with grains at the bottom primary rachis consistently having higher glutelin level than those at the top and middle rachises, except 17 days after flowering (DAF). A similar trend was observed for total protein content. Grains at the bottom rachis demonstrated dominance in the rate of protein accumulation, initiating rapid accumulation 2.0 d later and 2.2 d earlier than grains at the top and middle rachises, respectively. Furthermore, the duration of active protein accumulation was 1.9 d and 3.4 d shorter for grains at the bottom rachis compared to those at the top and middle rachises, respectively. This phenomenon was attributed to alterations in enzymatic activities. Specifically, the activities of glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate pyruvate transaminase (GPT), and glutamic oxalo-acetic transaminase (GOT) in grains located at the basal rachis exhibited a marked increase from 8 DAF to 17 DAF. These activities were significantly elevated compared to those observed in grains at the top and middle rachis, although they experienced a subsequent sharp decline. The glutelin content and enzymatic activities demonstrated a strong correlation, either positive or negative, at 11 DAF and 20 DAF. These findings suggest that the positional changes of grain protein were closely associated with nitrogen assimilation and glutelin accumulation during the rice grain filling process.

Keywords Rice, Protein content, Protein component, Positional variations, Grain filling

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Introduction

Rice (*Oryza sativa* L.) is extensively cultivated and serves as a staple food for a significant portion of the global population, particularly in Asia, where over 90% of the world's rice is both produced and consumed [1]. As living standards continue to rise, there is an increasing global demand for high-quality rice [2]. Consequently, enhancing grain quality has emerged as a primary objective in rice production, alongside yield improvement [3]. The yield and quality of rice are largely influenced by grain filling, which is closely linked to the grain's position on the rice panicle [4, 5]. Rice panicles are characterized by numerous branches and a large number of spikelets, which vary in grain location and flowering time within the panicle. Grains situated on the apical primary branches typically develop more rapidly and attain greater weight compared to those on the proximal secondary branches [6], significantly impacting the overall quality of the rice panicle. Previous research has documented variations in carbohydrate and starch accumulation among grains on a rice panicle [7]. During the initial and mid-filling stages, the content and rate of starch and amylose accumulation in spikelets decrease in accordance with the flowering sequence. Zhu et al. [5] conducted an investigation on the variations in starch structure, physicochemical properties, and textural characteristics of grains located at different positions within a rice panicle.

Grain protein represents the second most abundant component of rice endosperm after starch, constituting 7–10% of the milled rice [8]. Numerous prior studies have indicated that grain protein content adversely influences the cooking and eating quality of rice. Elevated grain protein content (GPC) is typically associated with increased hardness of cooked rice, which may be considered an undesirable trait in certain regions [9]. Experimental removal and substitution of individual protein fractions have demonstrated that protein composition can impact the texture of cooked rice [10]. Despite this, the significance of protein content in determining rice grain quality has traditionally been underestimated, emerging as a critical factor that impedes the concurrent enhancement of rice productivity and quality.

Previous studies have indicated that elevated temperatures during the grain filling stage can enhance GPC, leading to an increase in the hardness of cooked rice [11]. Similar outcomes have been observed with the application of additional nitrogen fertilizer to rice plants, particularly during the late growth stage [12, 13]. Comparative analyses of rice grain protein content have also been conducted between *indica* and *japonica* rice varieties, revealing significant variations in *indica* rice, ranging from 4.9 to 19.3% [14, 15]. However, research on the impact of grain position within the panicle on protein content in rice endosperm remains relatively scarce. It has been reported that rice GPC is generally higher in the lower part of the panicle compared to the upper and middle sections, and higher in the secondary branch rachis than in the primary branch rachis [7]. Rice grain storage proteins, characterized by their divergent solubility properties, consist of albumin, globulin, prolamin, and glutelin [16]. Liu et al. [4] examined the effect of grain position within the panicle on protein content in japonica rice cultivars, finding considerable variation in grain composition and total protein content among grains located at the top, middle and bottom rachis.

Grain protein represents the final product of nitrogen metabolism during the grain filling phase, with its composition and quantity being critical determinants of rice quality [17]. Inorganic nitrogen is assimilated and transaminated into a series of amino acids within crops, subsequently participating in protein synthesis and accumulation within the grains [18]. Throughout this process, variations in a series of enzymes and genes associated with nitrogen metabolism coordinately control the quantity and composition of proteins that accumulate in the grain endosperm. Ammonium is incorporated into glutamate by the glutamine synthetase/glutamate synthase (GS/GOGAT) cycle [18]. Glutamate pyruvate transaminase (GPT) and glutamic oxalo-acetic transaminase (GOT) involve in the amino transfer of various amino acids [12]. The expression levels of related genes, such as OSNADH-GOGAT, OSFD-GoGAT, OsGS1; 2 and OsGS1; 3 affects nitrogen assimilation and reuse, indirectly regulating protein accumulation in rice [12, 14]. For instance, GS-overexpressed plants had higher total amino acids and nitrogen content in plants compared with wild-type plants, but decreases in rice seeds [19]. OsGS1; 3 was correlated with nitrogen use efficiency in rice, and its expression was significantly up-regulated under limiting nitrogen supply [20]. Additionally, protein accumulation initially increased and then declined during the grain filling, and 10–20 d after flowering is the key period of protein accumulation [13, 21].

This study aims to elucidate the effects of grain positions within a panicle on rice protein content and composition, as well as their relationships with enzymes involved in nitrogen metabolism. This will be achieved by investigating the accumulation dynamics of protein components, enzyme activities, and gene expression related to protein synthesis. The findings will contribute to a deeper understanding of the positional variation of rice protein content within the panicle.

Materials and methods

Experimental site and material

Rice was grown in a micro-plot at the experimental farm of the Anhui Academy of Agricultural Sciences, Hefei,

China (31°86′N, 117°27′E), during the summer of 2022. This region is characterized as a single-season *indica* rice-producing area located in the lower reaches of the Yangtze River. It experiences a subtropical humid monsoon climate, with air temperatures frequently exceeding 35.0 °C from mid to late July through early August. In the year of the experiment, the average daily air temperature during the rice growing season was recorded at 27.1 °C, with total precipitation amounting to 216.0 mm. The study utilized the rice cultivar Yangdao 6, developed by the Lixiahe Agricultural Research Institute of Jiangsu Province, China. This cultivar is extensively cultivated in the middle and lower reaches of the Yangtze River, attributed to its superior field yield performance.

Crop management

The seeds underwent disinfection and soaking prior to being sown in a seedling nursery on April 29. Seedlings possessing 4.0–4.5 leaves were subsequently transplanted on May 28, with two plants per hill, arranged at a spacing of 30.0 cm × 13.3 cm. Fertilization involved the application of 225 kg ha^{-1} of nitrogen (N) in the form of urea, 90 kg ha^{-1} of phosphorus (P_2O_5) as calcium superphosphate, and 135 kg ha^{-1} of potassium (K_2O) as potassium chloride. The nitrogen fertilizer was administered to the rice plants in three phases: 50% as a basal application, 20% during the tillering stage, and 30% at the panicle initiation stage. The phosphorus fertilizer was entirely applied as a basal treatment. Potassium fertilizer was distributed in two applications, at the basal stage and the panicle initiation stage. A floodwater layer of 2–3 cm was maintained seven days post-transplantation, followed by a dry-wet alternating irrigation regime during the grain-filling stage, concluding with final drainage 10 days prior to harvest. Agricultural chemicals were applied as appropriate.

Plant sampling

When approximately 50% of the rice panicles had emerged from the flag leaf sheath, a total of 500 panicles exhibiting similar growth patterns were tagged using white plastic markers. The first day of flowering within the panicle was recorded as the flowering day. Subsequently, the tagged panicles were sampled from 9: 00 and 9: 30 am at intervals of every three days after flowering (DAF) from 8 to 20 DAF (8, 11, 14, 17, and 20 DAF), and then every five days from 20 to 35 DAF (20, 25, 30, and 35 DAF). Each panicle was dissected into three sections: the top (comprising the four primary branches at the apex of the panicle), the bottom (consisting of the four primary branches at the base of the panicle), and the middle (encompassing the remaining primary branches in the central portion of the panicle), as delineated in prior studies [21, 22]. Grains located on the top, middle, and bottom primary branches were designated as Y1, Y2, and Y3, respectively. Half of the grain samples were immediately frozen in liquid nitrogen and stored at − 80 °C for subsequent physiological analyses. The remaining grain samples were subjected to deactivation at 105 °C for one hour, followed by drying at 70 °C until a constant weight was achieved, to ascertain the grain dry weight and protein content. At maturity, panicles exhibiting similar maturity levels were harvested from each replication.

Determine of grain protein fractions

The grain protein content in rice is quantified as the percentage of protein present in the grain on a dry matter basis [23]. Developing rice grains were manually dehusked and subsequently ground into a powder for protein analysis. Mature grains were harvested manually, processed into milled rice (JNMJ3, Zhejiang Food Co., Ltd., China), and then ground into a fine powder to assess protein content. The concentrations of albumin, globulin, prolamin, and glutelin in rice grains were measured according to the methodology outlined by Liu et al. [4]. Sequential extraction of albumins, globulins, prolamins, and glutelins was performed using water, 10% NaCl, 55% n-propanol, and Biuret reagent, respectively. Glutelin content was determined using the Biuret colorimetric method, whereas the other protein components were quantified using the Bradford reagent. All experiments were conducted with three biological replicates for each sample. The total protein content was calculated as the cumulative sum of the four protein fractions.

Grain protein accumulation analysis

Richards' equation has been widely utilized to model material accumulation during rice grain filling [24]. In this study, Richards' equation was employed to examine the relationship between grain protein accumulation and days after anthesis. The amount of protein accumulation (mg $grain^{-1}$) in grains across different days after anthesis was determined by multiplying the grain dry weight (mg $grain^{-1}$) by the corresponding protein content (mg g^{-1}) at the same time point. The primary characteristic parameters include the time reaching the maximum accumulation rate (t_{max}), the average rate of protein accumulation (V_a), the maximum rate of protein accumulation (V_m), and the duration of the active protein accumulation period (T). V_m is obtained by inserting t_{max} into Eq. (2).

$$W = A/\left(1 + Be^{-kt}\right)^{1/N} \quad (1)$$

where W (mg $grain^{-1}$), t (days) and A (mg $grain^{-1}$) are protein accumulation, DAF, and its ultimate limiting value, respectively, and B, k and N are equation parameters.

$$V = AkBe^{-kt}/\left(1 + Be^{-kt}\right)^{(N+1)/N} \quad (2)$$

where V is the rate of protein accumulation in grains.

$$V_a = AK/2\,(N + 2) \quad (3)$$

$$t_{max} = (lnB - lnN)\,/k \quad (4)$$

$$T = 2\,(N + 2)\,/k \quad (5)$$

where V_a, t_{max} and T are the average rate of protein accumulation, the time reaching the maximum accumulation rate and the duration of the fast protein accumulation phase.

Enzyme extraction and analysis

The grain samples collected at 8, 11, 14, 17, 20, 25, and 30 DAF were manually dehulled and subsequently ground into a fine powder using liquid nitrogen. The enzymes included glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate pyruvate transaminase (GPT), and glutamic oxalo-acetic transaminase (GOT) that involves in N assimilation and protein synthesis were analyzed were extracted from approximately 0.5 g samples with 5 mL extract solution (pH7.5, 0.1 mol L^{-1} Tris-HCl, 5 mmol L^{-1} 2-mercaptoethanol and 2 mmol L^{-1} EDTA). The crude enzymes of GS and GOGAT were extracted at 8000 r min^{-1} for 15 min at 4 °C, and measured according to the method of Tang et al. [21]. The supernatant enzymes GPT and GOT were extracted with 5 mL of buffer solution (pH 7.2, 0.2 mol L^{-1} Tris-HCl) and determined as reported by Xi et al. [13]. Samples were analyzed in triplicate, and mean values were used for comparison analysis.

qRT-PCR analysis

Total RNA was isolated from frozen rice spikelets collected 14 days post-flowering using the RNAiso Plus kit (TaKaRa, Dalian, China). Reverse transcription was conducted with the Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). The quantitative real-time polymerase chain reaction (qRT-PCR) was executed with the following thermal cycling conditions: an initial denaturation at 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, 60 °C for 20 s, and 72 °C for 1 min, and concluded with a final extension at 72 °C for 10 min. PCR products were resolved via electrophoresis on a 1.5% agarose gel, stained with GelStain (TransGen Biotech, Beijing, China), and visualized using the HoferMV-25 instrument (Amersham Pharmacia). The Actin gene served as an internal control, and expression levels of the genes OsGS1; 2, OsGS1; 3, OsNADH-GOGAT, and OsFd-GOGAT were quantified using three biological and technical replicates. The $2-^{\Delta\Delta CT}$ method [25] was employed to determine the expression levels of the target genes. Primer sequences are provided in the Supplementary file (Table S1).

Statistical analysis

Microsoft Excel 2010 was used for data processing. Statistical significance of differences in protein content, protein composition and enzymes activities between different grain positions within a rice panicle were determined by using two-way analysis of variance (ANOVA) at $P < 0.05$ level using IBM SPSS Statistics 21.0, and the results were plotted using GraphPad Prism 8.0. Person correlation analysis was performed in the Origin 2018 software to explore the correlations between grain protein, protein components and enzymatic activities.

Results

Positional variations in grain protein and protein components in a rice panicle

We initially conducted a survey of grain weight (GW) and protein content in mature grains within a rice panicle. The influence of grain positions along the primary rachises was prominently observed on GW and protein content, with notable variations among the top, middle, and bottom primary rachis of the panicle (Table 1). The GW of Y3 was significantly greater than that of Y2 and Y1. Rice GPC exhibited considerable variability depending on grain position within the panicle. Specifically, the GPC of Y3 was markedly higher than that of Y1 and Y2, with no significant difference detected between Y1 and Y2. Regarding protein components, glutelin levels varied substantially, exceeding those of other rice storage proteins. No significant differences were found in albumin and globulin contents among grains at different panicle positions. Y3 exhibited significantly higher prolamin levels compared to Y1 and was comparable to Y2. Notably,

Table 1 GPC and storage protein fractions among different positions of the rice panicle

Position	Albumin (%)	Globulin (%)	Prolamin (%)	Glutelin (%)	GPC (%)	GW(g)
Y1	0.15 a	0.20 a	0.37 b	7.16 b	7.87 b	28.9 c
Y2	0.16 a	0.19 a	0.40 a	7.20 b	7.96 b	29.2 b
Y3	0.17 a	0.20 a	0.41 a	7.74 a	8.52 a	29.7 a
Mean	0.16	0.21	0.41	7.73	8.51	29.3

GPC, grain protein content; GW, 1000-grain weight. Y1, Y3 and Y2 mean grains from the four top primary rachis of the panicle, the four bottom primary rachis of the panicle and the remaining middle primary rachis of the panicle. Values are the means of three replications. Different lowercase letters in the same column mean significant differences at $P < 0.05$

the glutelin content of Y3 was significantly higher than that of Y1 and Y2 by 0.58 and 0.54% points, respectively.

Accumulation process of rice grain proteins in a panicle

The positional variation of GPC in a rice panicle was further examined by analyzing the dynamic accumulation of protein compositions during the grain filling (Fig. 1). The findings revealed significant differences among the four protein fractions. Albumin exhibited an irregular pattern, initially increasing and subsequently decreasing, with a notable decline observed at 25 and 35 DAF, although a rebound was detected at 30 DAF (Fig. 1a). A similar trend was observed in globulin (Fig. 1b). Throughout the grain filling process, prolamin accumulated steadily, reaching

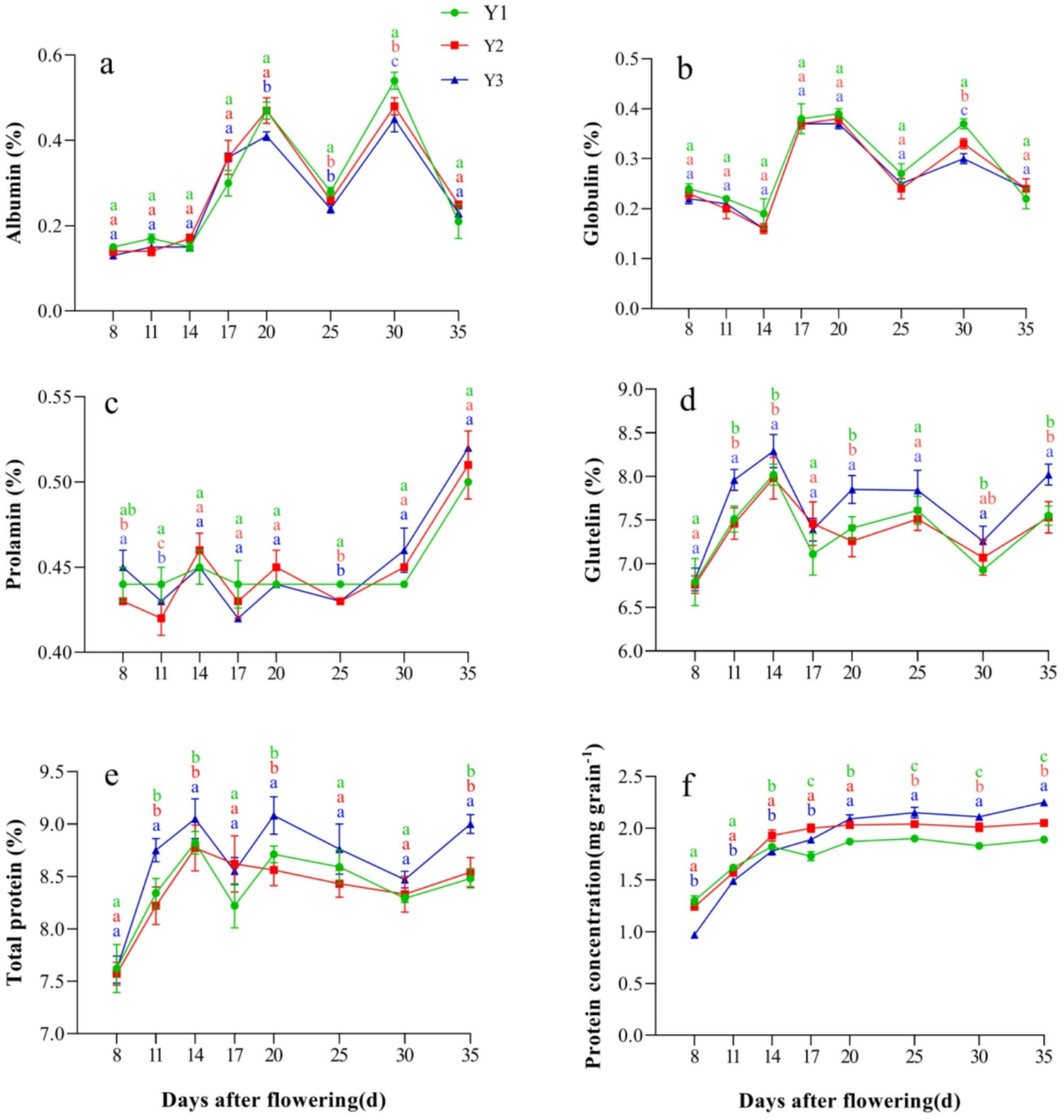

Fig. 1 Dynamics of protein fractions accumulation in grains at different spikelet positions within a rice panicle. **a**, **b**, **c**, **d**, **e** and **f** indicate albumin, globulin, prolamin, glutelin, total protein and protein concentration per grain, respectively. Y1, Y3 and Y2 mean grains from the four top primary rachis of the panicle, the four bottom primary rachis of the panicle and the remaining middle primary rachis of the panicle. Significant differences at each time point are indicated by different letters ($P < 0.05$)

a peak at 35 DAF (Fig. 1c). Among the protein components, glutelin showed the most pronounced changes in accumulation within the rice grain, initially increasing to a maximum at 14 DAF, followed by a decrease, and then rising again at 35 DAF (Fig. 1d).

It was observed that the positional location of rice grains within a panicle significantly influenced protein accumulation. Notably, glutelin accumulation exhibited greater variability across different grain positions within the panicle compared to the other three protein fractions. Throughout the entire grain filling stage, the glutelin content of Y3 consistently exceeded that of Y1 and Y2, particularly at 11, 20, and 35 DAF, with the exception of a slight decrease at 17 DAF. The variations in total protein accumulation were significantly influenced by glutelin accumulation in grains located at different positions on the panicle (Fig. 1e). In contrast to Y1 and Y3, the total protein content of Y2 remained stable during the later stages of grain filling. Furthermore, the protein concentration per grain exhibited an overall increasing trend during grain filling, with the order Y3 > Y2 > Y1 observed after 14 DAF (Fig. 1f).

Dynamic simulation of grain storage protein accumulation in rice

To elucidate the variation in GPC within a rice panicle, this study simulated the dynamics of grain protein accumulation during grain filling (Table 2). Notable differences were observed in the progression of protein accumulation in grains situated at various positions on the panicle. The V_a displayed little variation among three positions, whereas the V_m showed a trend of Y3 > Y1 > Y2. The maximum rate of protein accumulation in Y3 displayed an increase of 7.8% and 27.4% in comparison with those in Y1 and Y2. Moreover, the time of the protein reaching the maximum accumulation rate (t_{max}) of Y3 was later by 2.0 d and earlier by 2.2 d when compared with those of Y1 and Y2. Consequently, the duration of the protein active-accumulation period (T) for Y3 prolonged by 1.9 d and shorted 3.4 d during the grain filling period than those of Y1 and Y2.

Table 2 Dynamic models of protein accumulation in grains at different positions of the panicle

Position	R^2	t_{max} (DAF)	V_a	V_m	T (d)
Y1	0.9551	5.3 c	0.10 a	0.20 a	18.7 c
Y2	0.9943	9.5 a	0.08 a	0.17 b	24.0 a
Y3	0.9943	7.3 b	0.11 a	0.22 a	20.6 b

Y1, Y3 and Y2 mean grains from the four top primary rachis of the panicle, the four bottom primary rachis of the panicle and the remaining middle primary rachis of the panicle. t_{max}, time reaching the maximum accumulation rate; T, duration of the protein active-accumulation period; V_a, average rate of protein accumulation; V_m, maximum rate of protein accumulation; DAF, days after flowering. Different letters in the same column indicate significant difference at $P < 0.05$ probability level

Activities of the enzymes involved in grain N metabolism

During the grain filling, the enzymes GS, GOGAT and two transaminases GOT and GPT play important roles in protein accumulated in the endosperm [13, 14]. The effects of grain position in the panicle on the activities of the enzymes during the grain filling stages were investigated (Fig. 2). These enzymes activities decreased to varying degrees at the early stages of grain filling, and then continued to increase with the process of grain filling until 17 DAF or 20 DAF. The enzymatic activities of GS, GOGAT, GPT, and GOT in Y3 increased sharply after 8 DAF until 17 DAF, and the activities of GS, GOGAT and GPT were significantly higher than those in Y1 and Y2 (Fig. 2a, b, c, d). After 20 DAF, the enzymatic activities of GS, GOGAT and GOT decreased rapidly, showing that the activities were much lower than those in Y1 and Y2 (Fig. 2a, b, d).

qRT-PCR analysis

The GS/GOGAT cycle is the key pathway for inorganic N assimilation and ensures protein synthesis of grain. In this study, we analyzed the main regulatory factors of grain protein synthesis pathway to explore the position changes of protein deposition in rice grains (Fig. 3). The expression level of OsGS1; 2 increased before 11 DAF, with a trend of Y1 > Y2 > Y3, then decreased sharply, and then rebounded at 17 DAF, showing that Y1 was equal to Y2 and significantly lower than Y3 (Fig. 3a). The expression level of OsGS1; 3 increased sharply before 14 DAF with Y1 > Y2 > Y3, and then Y1 and Y2 decreased significantly and their values were equivalent at 17 DAF, while Y3 had a slightly decrease and a higher value than the other two (Fig. 3b). Both OsNADH–GOGAT and OsFd–GOGAT expressions increased significantly at 17 DAF, resulting in the expression levels of Y1 > Y2 > Y3 (Fig. 3c, d).

Correlations of enzyme activities with protein accumulation

A correlation analysis was conducted to examine the relationships between grain protein content, protein composition, and the enzymatic activity involved in nitrogen metabolism at various positions within the rice panicle during the grain filling stage (Fig. 4) (Supplementary file: Table S2). Activities of the enzyme GS and GOGAT at 8 DAF were significantly positively correlated with albumin and globulin, and GOGAT and GOT were negatively correlated with prolamin (Fig. 4a). Enzymatic activities of GS, GOGAT and GPT in the grain at 11 DAF were significantly positively correlated with glutelin and total protein. At 20 DAF, GS, GOGAT, and GOT were significantly negatively correlated with glutelin and total protein, while GPT was significantly positively correlated with glutelin and total protein (Fig. 4b).

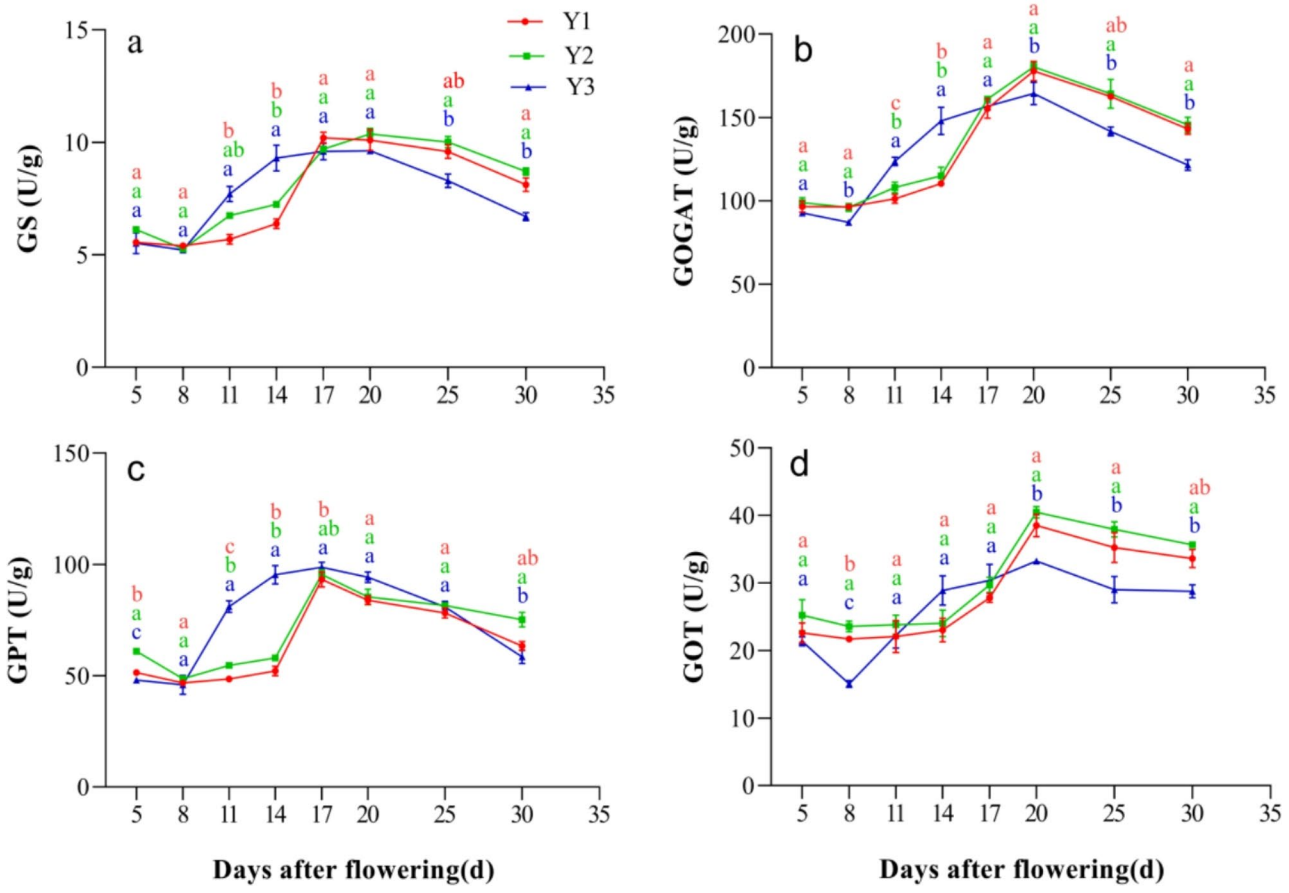

Fig. 2 Dynamics of enzymes actives related to protein analysis in grains at different spikelet positions within a rice panicle. **a**, **b**, **c** and **d** are glutamine synthetase (GS), glutamate synthase (GOGAT), glutamate pyruvate transaminase (GPT) and glutamic oxalo-acetic transaminase (GOT). Y1, Y3 and Y2 mean grains from the four top primary rachis of the panicle, the four bottom primary rachis of the panicle and the remaining middle primary rachis of the panicle. Significant differences at each time point are indicated by different letters ($P < 0.05$)

Discussion

Rice is the most extensively cultivated and produced crop globally [26]. Understanding the impact of grain positions within a rice panicle on rice quality is crucial for improving the uniformity of the grain population through selective breeding and agronomic practices [27, 28]. Grain storage protein is a significant quality attribute of rice [29], and has garnered increased attention in recent years. This study examined the dynamics of protein and protein component accumulation in grains located at various positions on a rice panicle during the grain-filling stage. Our current findings demonstrate a substantial variation in grain protein content and protein composition within a rice panicle, highlighting the importance of minimizing disparities in grain protein content and composition among grains within a panicle.

Grain protein concentration (GPC) significantly influences rice cooking and eating quality, necessitating maintenance within a consumer-acceptable range [30]. Given that protein levels are sensitive to environmental conditions, the effect of grain positions on the panicle on GPC warrants heightened attention, particularly in China. The growth of individual grains is largely dependent on the timing of fertilization within a rice panicle, and grain position influences its growth pattern [31, 32]. In the present study, GPC exhibited significant variation among the top, middle, and bottom primary rachis of the panicle. Grains located at the bottom rachis demonstrated the highest GPC compared to those at the top and middle rachis. This finding aligns with previous reports indicating that earlier-flowering spikelets generally possess lower protein content than later-flowering spikelets [33, 34]. The results of protein fraction analysis further corroborated this observation, revealing that variations in glutelin content substantially contributed to the GPC differences within a rice panicle.

Glutelin, which constitutes 60–80% of the total endosperm protein, is highly sensitive to environmental conditions and plays a pivotal role in determining the overall protein content [15, 35]. Our analysis identified fluctuations in various protein components, including albumin, globulin, prolamin, and glutelin, during grain filling.

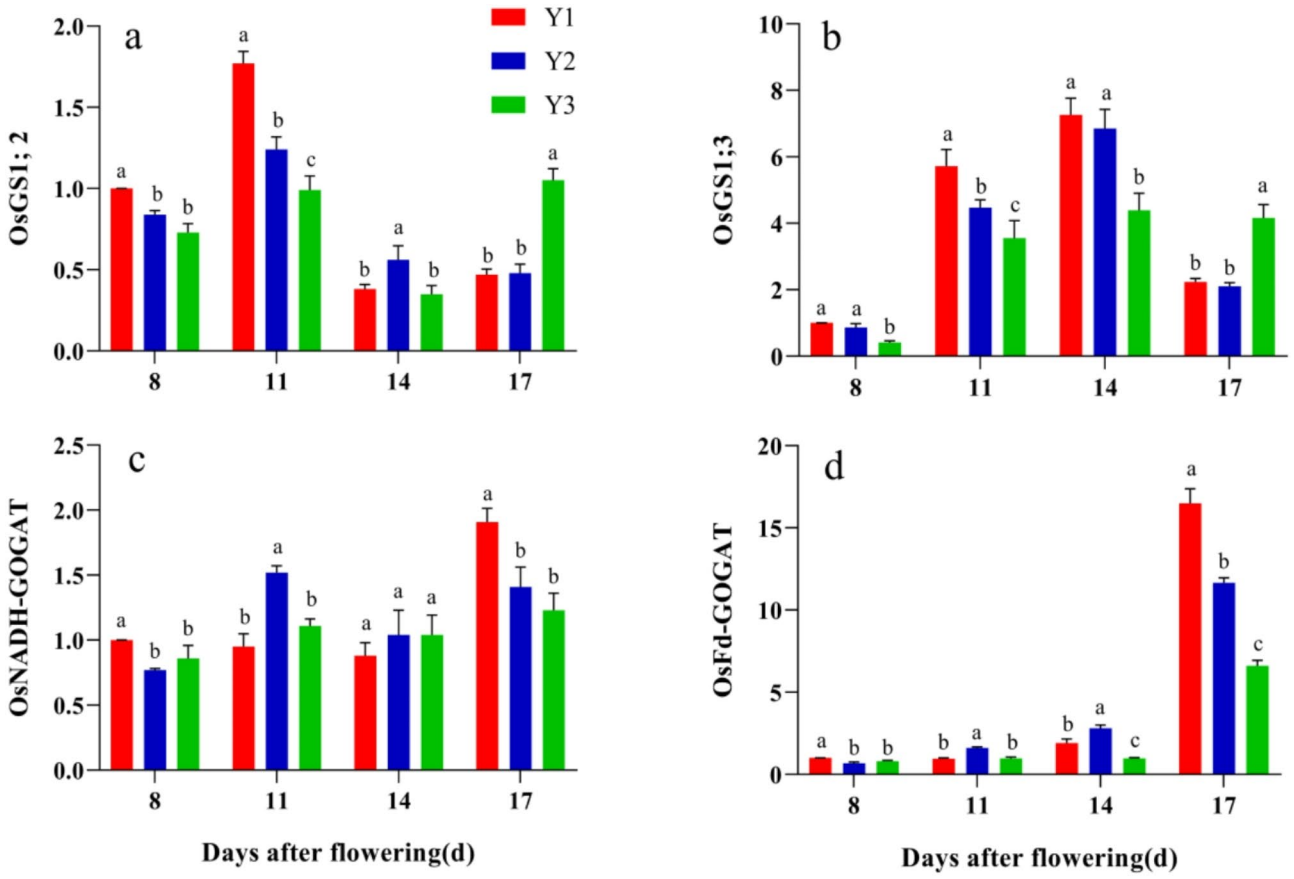

Fig. 3 Gene expression related to N metabolism in grains that located at different positions within a rice panicle. Relative expression levels of genes were analyzed by qRT-PCR using RNA extracted from developing grains at 4, 8, 11 and 17 DAF. Error bars mean the SD of three replicates. Y1, Y3 and Y2 mean grains from the four top primary rachis of the panicle, the four bottom primary rachis of the panicle and the remaining middle primary rachis of the panicle. The error bars indicate the mean ± SD ($n = 3$). Significant differences at each time point are indicated by different letters ($P < 0.05$)

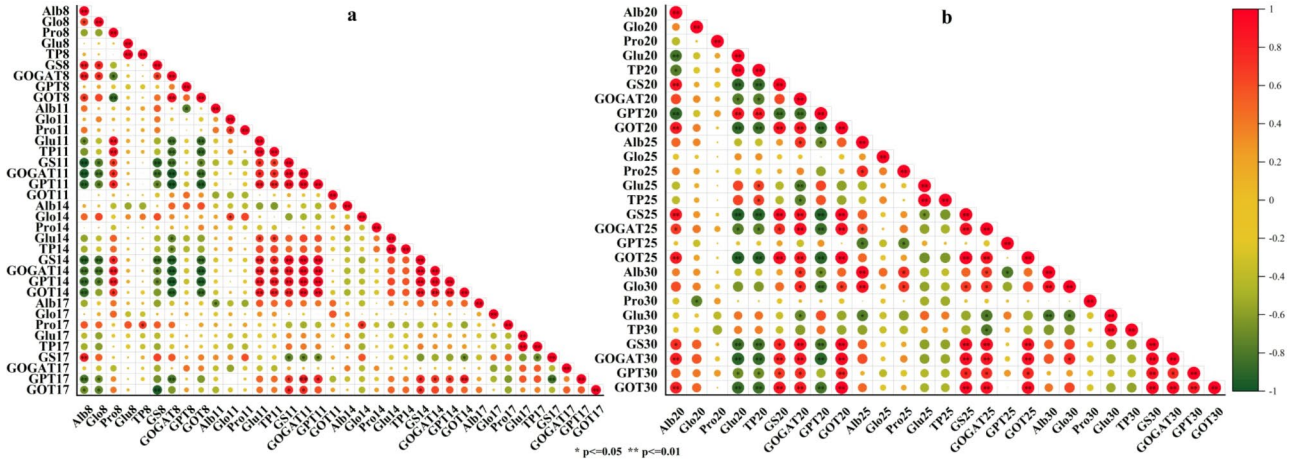

Fig. 4 Relationships of protein content and protein compositions with activities of the enzymes involved in N metabolism during grain filling stages of the rice. (**a**) Their relationships from 8 days to 17days after flowering. (**b**) Their relationships from 20 days to 30 days after flowering. Alb, Albumin; Glo, Globulin; Pro, Prolamin; Glu, Glutelin; TP, Total protein; GS, glutamine synthetase; GOGAT, glutamate synthase; GPT glutamate pyruvate transaminase; GOT, glutamic oxalo-acetic transaminase. The numbers including 8, 11, 14, 17, 20, 25 and 30 mean the days after flowering. * and ** indicate correlation significance at the $P < 0.05$ and $P < 0.01$ levels of probability, respectively

Notably, the accumulation of the glutelin component was more pronounced among grains at different positions on the panicle during grain filling, highlighting a significant effect of grain position The glutelin content in grains located at the basal rachis of the panicle consistently exceeded that of grains at the apical and median rachis during the grain filling period, with the exception of 17 DAF. A similar pattern was observed in the total protein content across grains situated at various positions within the panicle (Fig. 1). These findings underscore the significant contribution of glutelin to the variation in GPC within a rice panicle, highlighting the necessity of considering positional effects in efforts to enhance rice grain quality.

Grain quality is the result of the synergistic effect of optimal allocation of resources in rice panicle and development timing. Protein content levels in grains are contingent upon the grain filling process [21], with the duration and rate of protein accumulation determining the ultimate quantity of storage substances and grain quality [13]. The rate of accumulation of grain storage substances varies significantly with grain position within the rice panicle [7]. In this study, grains at the bottom of the panicle exhibited an accelerated rate of protein accumulation compared to those at the apex and middle, with increases of 7.8% and 27.4%, respectively. The initiation of rapid protein accumulation in grains at the panicle's base occurred between that of the top and middle grains, as did the duration of active protein accumulation. In this context, grains located at the base of the panicle exhibited rapid filling within an appropriate time frame, resulting in the highest protein content among the three sections. The dynamic simulation of storage protein accumulation in grains at the middle part of panicle differed with other two parts, indicating the possible of physiological incompetence. These observations may account for the variation in storage protein content among grains within the same panicle, and suggest that protein accumulation among grains at different positions within a rice panicle during grain filling are not fully correlated with the flowering order in rice.

Rice seed development is regulated by the grain filling process, which not only related to flowering time but also related to gene expression of physiological process [7, 32]. The suboptimal grain filling observed in late-flowering spikelets is likely due to reduced biological activity rather than a limitation in assimilates supply [6, 7]. Consequently, we investigated the relationship between the dynamics of protein component accumulation and the enzymes involved in nitrogen assimilation and protein synthesis during the grain filling process. It is estimated that 95% of the inorganic nitrogen absorbed by plants is assimilated via the GS/GOGAT cycle, subsequently converted into amino acids through the action of transaminase, and ultimately synthesized into proteins [36, 37]. In our study, in grains at the bottom of the panicle, the enzymatic activities of GS, GOGAT, GPT, and GOT enhanced sharply after 8 DAF until 17 DAF, and activities of GS, GOGAT and GPT were significantly higher than those at the top and middle of the panicle (Fig. 3), implying more assimilated nitrogen that used for protein synthesis during this period. In a panicle, the enzymatic activities of GS, GOGAT, and GOT in grains located at the bottom decreased rapidly, exhibiting significantly lower levels than those in other parts after 20 DAF. Previous supported studies have demonstrated that grain protein accumulates rapidly between 10 and 20 days post-anthesis in rice, with nitrogen metabolism-related enzymes influencing protein biosynthesis [13, 21]. Moreover, we found difference in the genes that encoded the enzymes involved in protein synthesis of grain. For instance, the expression level of OsGS1; 3 increased sharply before 14 DAF regardless of grain positions, and the values in grains showed a trend of top > middle > bottom. Afterwards, the expression level of OsGS1; 3 in the top and middle part of the panicle decreased dramatically at 17 DAF, while Y3 decreased slightly with a final higher value than the others two. Above findings indicated that the dynamics of grain protein accumulation are affected by the grain position on the panicle, metabolic enzyme activity and gene expression. The inconsistencies between enzyme activity and gene expression may involve post-translational regulation or enzyme degradation. Further functional analyses, such as gene knockout or overexpression experiments, may be needed in the future to observe phenotypic changes in rice. Thank you very much for your advice. Further correlation analysis between protein content and metabolic enzymes confirmed that these enzymes are involved in regulating protein accumulation and contribute to the final quantitative differences observed among grains in various positions within the rice panicle. Additionally, we observed that glutelin content and enzymatic activities associated with nitrogen metabolism were closely linked, either positively or negatively, at 11 DAF and 20 DAF. Therefore, it can be inferred that these enzymes related to nitrogen metabolism play crucial regulatory roles in the accumulation of protein components, and there is potential for regulating protein quantity by targeting glutelin.

Conclusions

Understanding the impact of grain positions within a rice panicle on rice quality is crucial for improving the uniformity of the grain population. In this study, significant differences were observed in the accumulation of proteins and their compositions among grains at different panicle positions throughout grain filling. Protein components exhibited fluctuations during grain filling, with a notable

grain position effect observed in glutelin. Specifically, glutelin content in grains at the bottom rachis was consistently higher than that in grains at the top and middle rachis during the grain-filling period, except at 17 DAF, a trend also observed in total protein content. Grains located at the bottom rachis demonstrated a superior rate of protein accumulation compared to those at the top and middle rachis. The time reaching the maximum accumulation rate for these grains occurred 2.0 d later and 2.2 d earlier than for grains at the top and middle rachis, respectively, with the duration of active accumulation being 1.9 days and 3.4 days shorter. The enzymatic activities of GS, GOGAT, GPT, and GOT in grains located at the basal rachis exhibited a marked increase from 8 DAF to 17 DAF. These activities were significantly higher compared to those observed in grains at the apical and central rachis, although they subsequently experienced a pronounced decline. A strong correlation, either positive or negative, was observed between glutelin content and enzymatic activities at 11 DAF and 20 DAF. These findings suggest that the spatial variation in protein distribution is intricately linked with nitrogen assimilation and glutelin dynamics during the grain filling.

Abbreviations

GPC	Grain protein content
DAF	Days after flowering
GS	Glutamine synthetase
GOGAT	Glutamate synthase
GPT	Glutamate pyruvate transaminase
GOT	Glutamic oxalo-acetic transaminase
RT	Reverse transcription
qRT-PCR	Quantitative real-time polymerase chain reaction
GW	Grain weight

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions
MX conceived and designed the experiments; MX, SL, YZ, YX, DT, XS and LY performed the experiments; MX, ZL and YZ performed the data analysis; MX and ZL prepared the manuscript. All authors have read and approved the final manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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