

Dissecting of the Deterioration in Eating Quality for Erect Panicle (Ep) Type High Yield *Japonica* Super Rice in Northeast China

Sibo Chen

Shenyang Agricultural University, Ministry of Education

Shuangjie Chen

Shenyang Agricultural University, Ministry of Education

Yihui Jiang

Shenyang Agricultural University, Ministry of Education

Qing Lu

Shenyang Agricultural University, Ministry of Education

Zhongyuan Liu

Shenyang Agricultural University, Ministry of Education

Wanying Liu

Shenyang Agricultural University, Ministry of Education

Xuhong Wang

Shenyang Agricultural University, Ministry of Education

Wenhua Shi

Shenyang Agricultural University, Ministry of Education

Quan Xu

Shenyang Agricultural University, Ministry of Education

Jian Sun

Shenyang Agricultural University, Ministry of Education

Fan Zhang

Chinese Academy of Agricultural Sciences

Liang Tang (✉ tangliang@syau.edu.cn)

Shenyang Agricultural University, Ministry of Education

Research Article

Keywords: Erect panicle, Eating quality, Nitrogen metabolism, Grain filling, Grain protein

Posted Date: December 9th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1146132/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Rice on March 8th, 2022. See the published version at <https://doi.org/10.1186/s12284-022-00561-9>.

Abstract

Ep type is an important morphological improvement (following dwarf breeding and ideal plant type) to adapt to super high yield breeding of rice, which shows a pleiotropic effect in increasing grain yield and nitrogen use efficiency (NUE) in rice. Nevertheless, it remains unclear whether Ep has adverse effects on eating quality and its regulatory of increasing nitrogen uptake and assimilation. In this study, we developed a pair of near-isogenic lines (NILs) of *dep1* (NIL-Ep, NIL-Non Ep) in the Liaogeng 5 (LG5) and Akihikari (AKI) backgrounds. Here, we report that rice plants NIL-Ep have more grain numbers per panicle in middle to bottom spike positions than plants NIL-non Ep. This part of increased grain not only is the key factor to increase the yield, but also is the reason to reduce the eating quality. The content of prolamin and glutelin in the grain increased significantly, which resulted in higher hardness and worse viscosity of rice after cooking. Additionally, the activity of several essential enzymes catalyzing nitrogen metabolism is higher in the NIL-Ep line than in NIL-non Ep line, especially from the mid to late grain filling stage. Based on these results, we conclude that Ep positively regulates grain protein accumulation primarily through enhance the activity of enzyme enroll nitrogen assimilation and redistribution during the mid to late grain-filling stage, resulting in excessive accumulation of grain protein and decreased the quality of eating.

Introduction

Rice is a dietary staple for more than half of China's population, so improving rice production is crucial for ensuring food security (Godfray and Garnett 2014). Over the past half century, rice yields have been dramatically improved by the tireless efforts of breeders and the use of high-yielding varieties (Chen et al. 2001). In particular, the release of a series of super rice varieties has pushed rice production to a new peak in Northern China (Tang et al. 2017). Since 1980, with the popularization and use of a series of high-yielding Ep varieties, the cultivated area (from 2.8 million hectares to 9.8 million hectares) and yield (from 4,013.2kg/hm² to 7,429.5kg/hm²) of *japonica* rice have been promoted rapidly. In 2019, the total cultivated area of *japonica* rice in China was 9.8 million hectares, of which the area of *japonica* rice in the dominant area of northeast China had reached 5.3 million hectares, accounting for 53.7% of the total area of *japonica* rice (Tang et al. 2021).

After entering the 21st century, China's per capita consumption of *japonica* rice continued to rise, the average annual consumption increased from 37.8 kg to 55.4 kg, resulting in a sustained growth in the domestic rice market demand for high-quality *japonica* rice (Tang et al. 2021). Although the Ep type plays an important role in promoting the yield of northern *japonica* rice, there is room for improvement in eating quality (EQ) compared with Japanese high-quality rice (Xu et al. 2005). In recent years, breeders have paid more and more attention to the eating quality of rice. High yield with inferior quality have become the main problems to be solved in this rice-cultivated area.

The eating quality of rice is mainly determined by its components. The starch and protein contents and components have significant effects on the hardness, elasticity and viscosity of rice (Crofts et al. 2017; Wu et al. 2021). In the pasting process, protein will affect the swelling and water absorption of starch granules, so its content is negatively correlated with EQ (Balindong et al. 2018; Zhu et al. 2020). Our previous studies have done a lot of research on the effect of Ep type on quality, but there is no systematic explanation for EQ due to material limitations (Wang et al. 2019; Fei et al. 2019). The main gene *dep1* controlling panicle type in Ep type super rice varieties is also a nitrogen efficient gene (Sun et al. 2014). This high-efficiency nitrogen utilization plays a very important role in improving rice yield, but there is still no clear explanation for whether it will cause excessive protein enrichment in grains and reduce the EQ to form high yield with inferior EQ. In this study, LG5, a Ep with high yield variety in northern China, AKI, a non Ep with high quality rice variety in Japan, and near-isogenic lines constructed with LG5 and AKI as parents were used as experimental materials. The effects of Ep type on yield and quality were deeply analyzed from the physiological point of view under the two N fertilizer management modes of high nitrogen with high yield and low nitrogen with high quality, and the main reasons for high yield with poor EQ were explained. This conclusion provides a theoretical basis for achieving EQ improvement under the premise of high yield.

Results

Multi-year phenotypic identification of yield and taste quality of different panicle types

To investigate the effects of Ep on the grain yield and taste quality under different N fertilizer treatments, we set two N fertilizer treatments as low (L) and high (H) respectively. The state of plants under high nitrogen condition was shown in Fig. 2A. The yield and taste quality traits of tested materials in 4 years were investigated as shown in Fig 1. Under L treatment, LG5 yield had no significant difference with AKI yield in 2020 (Fig. 1C), while LG5 yield was significantly higher than AKI yield in 2018, 2019, and 2021 (Fig. 1A, B and D). However, no significant difference was observed in near-isogenic line materials in L treatment (Fig. 1E-H). LG5 yield in H treatment was significantly higher than AKI in 4 years (Fig. 1A-D), near-isogenic lines showed the same pattern as their parents (Fig. 1E-H).

As for taste quality, in L treatment, there was no significant difference of LG5 and AKI. While in H treatment, the taste quality of LG5 was significantly lower than that of AKI, and AKI did not decrease with the increase of N fertilizer (Fig. 1I-L). The taste quality of near-isogenic lines showed the same pattern as that of their parents under the same treatment. However, compared with L treatment, under H treatment NIL-non Ep also showed a significant decrease in taste quality (Fig. 1M-P).

Yield and yield components

There was no significant difference in yield under L treatment, but there was a significant difference in yield and its components in H treatment (Table 1). Under different treatments, the yield and yield component traits of NILs were consistent with their parents AKI and LG5. The average PNP and GNP in NIL-Ep was 19.9% and 29.5% higher than that of NIL-non Ep (Fig. 2B). In order to clarify the sources of differences in GNP, we divided rice panicles into 24 parts from

1-1 to 12-2 according to the origin positions of branches, the results showed that the significant increase of SGN from 5th spike to 12th spike explained the difference in GNP (Fig. 2D). Subsequently the panicle is divided into 3 parts namely top (top)(from 1-1 to 4-2 panicle locations), middle (mid)(from 5-1 to 8-2 panicle locations) and bottom (bot) (from 9-1 to 12-2 panicle locations) respectively. Results demonstrated that compared with the NIL-non Ep, grain number significant increased mainly in mid to bot locations (Fig. 2E). The panicle weight ratio of each part was changed, and the panicle weight ratio in the mid to bot part increased by 21.5 % and 18.7 % (Fig. 2F). Although Ep showed a low TWG because grain length decreased significantly (Fig. 2C, G), the extremely significant increase of GNP and PNP was the key factor leading to the significant increase of yield, the contribution rates of the three factors were -7.1%, 29.5% and 19.9% respectively.

Table 1 Performance of grain yield related traits for LG5, AKI, NIL-Ep, NIL-non Ep.

Treatment	Panicle type	HD	PH(cm)	PL(cm)	PNP	GNP	FGN	PBN	PGN	SBN
L	LG5	115	109.82±2.91	14.97±0.63	448.10±32.71	89.50±2.74	76.50±2.74	10.50±1.05	57.67±2.42	11.33±1.21
	AKI	114	118.77±1.37	17.22±0.86	433.29±36.51	90.83±6.49	78.17±10.76	10.67±0.82	59.33±4.18	11.67±1.75
	p	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	NIL-Ep	110	92.58±0.90	13.74±0.55	474.46±39.23	92.94±5.80	79.94±5.80	10.71±1.21	61.53±6.25	11.53±0.87
	NIL-non Ep	111	118.29±2.04	16.87±1.01	464.75±28.82	90.50±6.62	79.00±11.45	10.69±1.08	60.25±5.37	11.19±1.64
	p	n.s.	***	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
H	LG5	118	111.52±1.43	15.82±0.48	497.73±30.14	147.00±6.42	126.00±6.51	12.60±1.02	71.40±3.61	25.40±1.62
	AKI	116	127.43±4.27	18.25±0.86	427.41±44.11	120.76±9.32	108.71±12.45	12.00±0.61	68.71±3.85	18.41±2.94
	p	n.s.	***	**	**	***	***	n.s.	n.s.	***
	NIL-Ep	113	94.24±1.83	16.28±0.45	524.39±25.33	167.60±5.50	141.80±6.26	12.60±0.55	60.60±3.58	29.00±2.00
	NIL-non Ep	114	115.22±4.80	19.16±0.96	437.08±24.36	129.47±6.22	114.53±7.62	12.06±0.90	69.41±2.76	19.76±2.49
	p	n.s.	***	**	***	***	***	n.s.	**	***

HD, heading date; PH, plant height; PL, panicle length; PNP, panicle number per square meter; GNP, grain number per panicle; FGN, filled grain number per panicle; SBN, secondary branches number; SGN, secondary grain number; TGW, thousand-grain weight; GY, grain yield . The *, ** and *** denote significance of Student's t test at p < .05, p < .01, and p < .001, respectively.

Eating quality and protein content in different panicle positions

The EQ is a complex sensory trait affected by the hardness, viscosity, elasticity and other indicators of rice. In order to accurately and objectively measure the EQ of the tested materials, we adopted two sets of evaluation systems: artificial tasting and machine evaluation. The two evaluation systems showed the same results. Under L treatment, there was no significant difference in the EQ between near isogenic lines, and the eating quality of NIL-Ep was significantly lower than that of NIL-non Ep under H treatment (Fig. 3A). The texture of rice showed that the hardness increased significantly while the viscosity and elasticity decreased significantly (Fig. 3E-G).

Then we identified the EQ of the top, mid and bot parts, and the results showed that the EQ of the mid to the bot part of NIL-Ep was significantly decreased, which was the key factor affecting the overall EQ (Fig. 3B). For RVA characteristics, mid and bot locations exhibited significantly lower breakdown, higher final viscosity and setback values than those of NIL-Ep (Additional file 1: Table S1). Previous studies demonstrated that rice with high palatability has a higher breakdown and a lower final viscosity and setback than low-palatable varieties (Ma et al, 2017). Therefore, it also proves that the eating quality of mid-bot position grains is lower compared with NIL-non Ep.

Starch and protein account for 70–80 and 7–10% of the components in the rice endosperm, respectively, and were considered to be the main factors that affecting eating quality (Chen et al. 2021). Therefore, the amylose content and protein components of different panicle locations in H treatment were detected to analyze the key factors causing the decline of eating quality. The results showed that there was no significant difference in amylose content among different panicle positions, but there was a significant difference in nitrogen content of mid and bot grains, which was significantly higher than that of NIL-non Ep (Fig. 3C, D).

Then we tested the protein content of 24 parts of panicle, and the results were shown that under H treatment, the grain protein content in the mid-bot panicle of NIL-EP was significantly higher than top part, while there was no significant difference among panicle sites of NIL-non Ep (Fig. 3 H, I). Subsequent analysis of the protein components showed that the difference in nitrogen accumulation was due to the significant increase in the prolamin and glutelin contents (Table 2).

Table 2 Comparison of protein content traits for NIL-Ep and NIL-non Ep in different positions of panicle

Locus	Panicle type	Accumulation amount(mg grain ⁻¹)				Relative content(%)				
		ALB	GLO	PRO	GLU	ALB	GLO	PRO	GLU	Total protein
TOP	NIL-Ep	0.196±0.010	0.225±0.009	0.099±0.003	1.440±0.101	0.75±0.04	0.85±0.04	0.38±0.02	5.48±0.27	7.45±0.37
	NIL-non Ep	0.193±0.015	0.227±0.011	0.105±0.005	1.453±0.073	0.71±0.02	0.83±0.02	0.38±0.01	5.30±0.16	7.22±0.22
	p	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
MID	NIL-Ep	0.182±0.013	0.214±0.017	0.154±0.005	1.935±0.116	0.70±0.03	0.81±0.04	0.59±0.03	7.64±0.38	9.75±0.69
	NIL-non Ep	0.184±0.009	0.219±0.011	0.092±0.006	1.426±0.057	0.68±0.02	0.80±0.02	0.34±0.01	5.29±0.16	7.11±0.21
	p	n.s.	n.s.	**	**	n.s.	n.s.	***	***	**
BOT	NIL-Ep	0.184±0.015	0.219±0.018	0.130±0.010	1.677±0.134	0.72±0.06	0.83±0.07	0.51±0.04	6.61±0.53	8.68±0.49
	NIL-non Ep	0.197±0.010	0.228±0.011	0.105±0.005	1.410±0.070	0.72±0.04	0.83±0.04	0.39±0.02	5.18±0.26	7.12±0.36
	p	n.s.	n.s.	**	**	n.s.	n.s.	**	**	**

ALB, Albumin; GLO, Globulin; PRO, Prolamin; GLU, Glutelin.
The *, ** and *** denote significance of Student's t test at $p < .05$, $p < .01$, and $p < .001$, respectively.

Nitrogen use efficiency and grain protein accumulation

Previous studies have shown that *dep1* is considered the major gene controlling nitrogen-use efficiency. In the two years of repeated experiments (Supplementary file 2: Table S2), under the high nitrogen condition, the yield of Ep increased by 30.6 and 50.4% than non Ep in 2019 and 2020. In H nitrogen treatment, the nitrogen recovery efficiency and physiological nitrogen use efficiency were significantly higher compared with non Ep. There was no significant difference in the yield and nitrogen use efficiency under L nitrogen treatment.

Then we examined the nitrogen transport in different organs of harvest stage under the condition of high nitrogen. The results showed that the nitrogen accumulation of NIL-Ep was significantly higher than that of NIL-non Ep in all organs (Fig. 4A, F, H, J). So that the total nitrogen accumulation increased significantly in the harvest period (Fig. 4O). From booting to full heading stage, nitrogen content in leaves still maintained an upward trend in NIL-Ep, while in NIL-non Ep, this phenomenon only occurred in flag leaves and 2nd leaves. In addition, it is interesting that 80-100 days after transplanting, the leaf nitrogen content of NIL-Ep showed a sharp downward trend (Fig. 4B-E), and the same trend also appeared in stem and sheath organs (Fig. 4G, I). We further analyzed the dynamic changes of the glutelin and prolamin content of the two genotypes during grain filling stage, and the results showed that there were significant differences in the contents of two protein components between different panicle locations in NIL-Ep compared with NIL-non Ep (Fig. 4K-N).

Nitrogen metabolism processes are in fact a series of reactions including inorganic nitrogen and organic nitrogen inter-conversion and protein biosynthesis and are highly regulated by both genetic and environmental factors. Many enzymes involve to catalyze these reactions, including glutamine synthetase (GS), glutamate synthase (GOGAT), asparagine synthetase (AS), glutamate dehydrogenase (GDH), etc., and all of them are well-known to play key roles in the regulation of nitrogen metabolism. We also measured the activities of enzymes encoded by these genes of the two genotypes during grain filling. As shown in Fig. 4P-S, significant differences in GS, NADH-GAGOT activity between two genotypes occurred at the whole grain filling stages, and the activity of AS and GDH increased significantly in 4-28 and 22-44 day after flowering. Origin of nitrogen in grains from various organs are shown that the ability of NIL-Ep to absorb nitrogen was significantly higher than that of NIL-non Ep in filling stage under high nitrogen condition (Fig. 4T).

Discussion

Proposed from ideal plant type breeding, Ep is widely used in super rice breeding, such as Shennong 265, a typical variety, which not only has higher yield potential, but also has better performance in lodging resistance and disease resistance, thereby replacing Japanese *japonica* rice (e.g. Toyonishiki, Akihikari and Akitakomachi) as the main type of *japonica* rice grown in Northern China (Xu et al. 1996). Previous studies have shown that *dep1* expression is positively regulated by nitrogen fertilizer (Palme et al. 2014). Under high nitrogen condition (120 kg/hm²), compared with the non Ep varieties, the EP varieties significantly improve plant type and yield-related traits. Under low nitrogen condition (60 kg/hm²), the yield of the EP varieties is greatly reduced, and even lower than that of the non Ep ones (Tang et al. 2017). This means that EP varieties cannot reach their full production potential given a limited nitrogen supply. Our experiment reached the same conclusion (Fig. 1A-H, Table 1). In 4 years of field trials, the parents and near-isogenic lines showed similar patterns in yield. Under L treatment, Ep showed no obvious yield advantage in yield. However, the yield was significantly increased under H treatment. As for non Ep type, there was no significant difference in yield between the two fertility treatments. In the analysis of yield components, the increase of grain number in the mid to bot part had the highest contribution to yield (Fig. 2E, F).

Rice is the main food crop for more than half of the world's population and its eating quality is the main index to determine its value (Zhu et al. 2020). The quality of cooked rice is a complex trait, not only affected by the inherent characteristics of the kernel, but also the components in rice (Vidal et al. 2007). There is a common view that the protein content has a positive correlation with the hardness of cooked rice (Amagliani et al. 2017). The viscosity profiles of rice flour with similar starch properties supported that protein negatively correlated with gelatinization temperature and peak viscosity (Bornhorst et al. 2013; Fitzgerald et al. 2003). The formation of protein-starch matrixes showed that protein inhibited starch maximum swelling, and restricted its ability to absorb water (Derycke et al. 2005; Saleh. 2017). Our research institution has conducted research on the quality and composition of Ep in grains, but due to the limitation of genetically modified materials on eating, there is no clear conclusion about its influence on EQ (Fei et al. 2020). In this study, Ep decrease the EQ through enhancing protein content of grains in the mid and bot panicle locations (Fig. 3). The significant increase of prolamin and glutelin content was the main factor leading to the increase of grain protein content (Table 2).

The nitrogen utilization of rice is a complicated physiological and biochemical process that contains multiple processes of absorption, transport, assimilation, remobilization and allocation (Chen et al. 2021). *dep1* regulates nitrogen uptake and metabolism by affecting *OsAMT1;1* that is associated with ammonium uptake (Sun et al. 2014). It enhances the ability of the root system to absorb ammonia nitrogen, allowing the plants to accumulate more nitrogen accumulation, and then improves the utilization efficiency of nitrogen (Xu et al. 2016). In addition, *dep1* overexpressed lines have higher expressions of *GS* and *GOGAT* genes, and thus showing higher nitrogen metabolic activity than the wild type under high nitrogen conditions (Zhao et al. 2019). In our research, under H treatment, Ep had higher nitrogen use efficiency than non Ep (Table S2), and nitrogen accumulation in all organs increased significantly (Fig. 4). During filling period, Ep stimulates nitrogen absorption and redistribution through enhancing the nitrogen metabolism-related enzymes activity (Fig. 4P-S). At the same time, the absorption and utilization ratio of exogenous nitrogen in Ep was significantly higher than that in non Ep (Fig. 4T).

Researchers have conducted extensive studies on the effect of Ep on yield and nitrogen use efficiency, but there is no clear conclusion on the effect of Ep on EQ. Combined with previous studies and the results of this experiment, we used a simplified model to explain its impact on EQ from the perspective of nitrogen assimilation and redistribution (Fig. 5). Under high nitrogen condition, at the vegetative stage the Ep type significantly increased the nitrogen accumulation by enhancing the absorption of roots and the assimilation of leaves, which provided the necessary guarantee for the increase of panicle number and grain number per panicle. When the filling stage begins, nitrogen is remobilized by nitrogen metabolism related enzymes to accumulate in grains. Especially in the middle to late stages of grain filling, the rapid transfer of nitrogen content in organs led to a significant increase in protein content in the mid to bot grains (mainly glutelin and prolamin protein). As reported in previous studies that protein mainly competed to absorb water and restricted the swelling of starch granules, which in turn affected texture of the cooked rice (Fitzgerald et al. 2003; Saleh 2017). The kernel of rice is a heterogeneous assemblage of the distinct components (Cai, et al. 2014). In fully maturity rice kernel, the protein bodies(PB) were tightly gathered along the cell walls and surrounded by the starch granules (Zhu et al. 2020). The steric hindrance effect of protein and starch-protein interactions was the main factors influencing the starch gelatinization. The surround structure of protein or the bond between starch and protein, preventing more water penetrate into the starch granules. This led to the reduction of water and space required for gelatinization, thereby inhibiting the extension of starch granules (Fitzgerald et al. 2003). In addition, protein has good thermal stability and hydrophobicity, which makes it difficult to change its conformation during cooking. Therefore, compared with the non Ep type, the excessive protein content in grains is one of the key reasons for the decrease of rice EQ.

In addition, our study proved that Ep has a positive effect on yield and a negative effect on EQ under high nitrogen input conditions. So how to achieve EQ improvement under the premise of guaranteeing yield has become the aim of many agricultural researchers' unremitting efforts. From the breeding point of view: (1) cultivating the Ep type with higher percentage of grains on top position to reduce the adverse effect of mid and bot grains on EQ (Supplementary file 3: Fig. S1). (2) introducing dominant genes controlling grain length such as *GS3* (Fan et al. 2006), *GL3.1* (Qi et al. 2012; Zhang et al. 2012), *GLW7* (Si et al. 2016) and *GS2* (Che et al. 2015; Hu et al. 2015) in order to increase yield by improving grain weight rather than increasing the number of weak grains. (3) to enrich more EP haplotypes more natural variations can be explored, in another way gene editing can be used to generating a greater number of *dep1* alleles, such like *Wx* and *SD1* gene did (Huant et al. 2021; Hu et al. 2018). (4) in order to delay the excessive transfer of N during grain filling, gene editing technology can be used to reduce the expression of genes controlling N metabolism-related enzyme activities (*OsGS*, *OsNA-GAGOT*, *OsGDH*) and fine-tune grain protein content in rice. From the cultivation point of view: according to the characteristics of different varieties, reasonable regulation of fertilization mode to explore the balance of yield and quality of nitrogen fertilizer operation mode to achieve the premise of guaranteeing yield to achieve quality improvement. These will be the key research directions that need to be further studied and clarified in the future work, and will also provide an important theoretical basis for the realization of EQ improvement under the premise of super-high yield.

Conclusion

In conclusion, under high N conditions, Ep significantly increased yield by increasing the effective panicle number and the number of grains in the middle and lower part of the panicle, but it was these increased grains that led to the decrease of the overall EQ. The protein content of these grains increased significantly (mainly prolamin and glutelin), which significantly reduced the characteristic values of EQ and thus decreased the taste. At the same time, EP type showed high nitrogen use efficiency. At the filling stage, the key enzyme activities of nitrogen metabolism in EP flag leaves were significantly increased, which promoted the process of nitrogen reassimilation. This also provides conditions for the rapid increase of protein content in grains, which is one of the key factors affecting grain EQ.

Materials And Methods

Plant material and experimental site

In this study, we constructed a pair of rice (*Oryza sativa* L.) denoted as NIL-Ep, NIL-non-Ep, in the LG5 and AKI backgrounds (Fig. 1A). In order to further determine the genome composition of the two NIL lines, we performed high-throughput sequencing analysis on both. First, the raw paired-end sequence data

were generated by Illumina HiSeq4000 for each sample. Then, raw fastq files were filtered by using fastp software with default settings. Clean reads were mapped to rice reference genome Nipponbare-IRGSP v1.0 through BWA software. The sequence variants between NIL-Ep and NIL-non Ep were detected by Samtools and GATK4 software for joint genotyping. Then 504,474 SNPs and 88,169 indels in genome-wide were identified after filtered using vcftools software according to threshold: $-\text{min-alleles } 2$ $-\text{max-alleles } 2$ $-\text{max-missing } 1$ $-\text{minDP } 3$ $-\text{minQ } 30$. Finally 2,048 SNPs and 1,322 indels were obtained if only consider the polymorphism between NIL-Ep and NIL-non Ep, thus the genetic identity of the two materials was 99.43%. The distribution of this 3,370 variants was visualized as Supplementary file 3: Fig. S2, in which the genomic region of *DEP1* exhibit strong genetic differentiation.

The experiment was carried out at the farm of Shenyang Agricultural University, Shenyang (41.8°N; 123.4°E), China during the rice growing seasons in 2018 and 2021. The germinated seeds were grown in the paddy field, and seedlings raised in the field with the sowing date on April 24 were transplanted on May 24 at a spacing of 0.30 m between rows and 0.15 m between plants with one seedling per hill. The materials were arranged in a randomized block design with three replicates, and each replicate was grown at least 800 plants. Set up 3 nitrogen treatments, respectively 0 kg/hm² nitrogen in control check (CK) area, 11.25 kg/hm² in low (L) (model of high quality cultivation in Japan) nitrogen area and 22.5 kg/hm² in high (H) (model of high yield cultivation in northern China) nitrogen area, P, K input and production management are the same as conventional production methods. Potted treatment and varieties are the same as the field, with two plants in each pot.

Evaluation of yield and yield components

At the heading stage, plant height (PH, in cm) and heading date were recorded when 50% of the plants emerged panicle material. At maturity, plants from a 4 m² area in each plot were harvested for grain yield (GY) based on 14% moisture content after being air-dried. Panicles selected from 6 main stems per hill, repeated 3 times, eighteen plants were sampled for trait evaluation, including panicle number per plant (PN), panicle length (PL), panicle number per plant (PNP), grain number per panicle (GNP), filled grain number per panicle (FGN) primary grain number (PGN), secondary grain number (SGN), and thousand-grain weight (TGW).

N content and N utilization efficiency

The N content of the samples were analyzed with the vario MACRO cube (Elementar Co., Hanau, Germany), which is based on the Dumas combustion method. Operation process and parameter setting according to Tang et al. (2019). NUEs of agronomic N use efficiency and apparent N recovery efficiency were calculated according to Chen et al. (2018).

Eating quality evaluation

The assays of taste and palatability in cooked rice were conducted on an STA1A rice taste analyzer (STA1A; SATAKE) using the method of Lai et al. (2011) with minor modification. The sensory evaluation of rice was made up of 30-35 people of different genders, different ages and with professional ability to identify EQ. Eight samples (including 1 control sample) were assessed each time. A control sample was set for sensory evaluation to better distinguish the differences of food and taste among varieties. According to the hardness, viscosity, elasticity, appearance, taste, palatability and cold rice texture of the sample (rice), the taster will give a comprehensive score after comparing with the control sample, the full score is 100 points. The average value is calculated according to the comprehensive scoring result of each evaluator, which is taken as the sensory comprehensive evaluation result of the EQ of the sample. The calculated result is reserved to two decimal places.

Amylose content and Rapid Visco Analyser (RVA) determination

Determination of apparent amylose content (AAC, %) was performed based on the colorimetric reaction of the amyloseiodine complex developed using the method of ISO 6647 (International Organization for Standardization). The absorbance of the solution was measured at a wavelength of 620 nm against the blank solution using a spectrophotometer (Lambda 365; Perkin Elmer). AAC% was calculated using a standard curve made from four rice samples with known AAC% (AAC% = 1.5%, 9.2%, 17.1% and 26%). Rice pasting properties were measured using a Rapid Visco Analyser (RVA) (TechMaster RVA; Perten) using samples of milled rice flour according to the method reported previously by Umemoto et al. (2004). The peak time, pasting temperature, peak viscosity, trough viscosity, final viscosity and their derivative parameters breakdown and setback were recorded using the Thermocline for Windows software (Version 1.2). According to the standard method of the American Cereal Chemistry Association Operating Regulations (1995-61-02), the water content of rice flour was 12 %, the sample volume was 3 g, and the distilled water was 25 ml.

Measurement of total protein and protein components in grains

Determination of total protein content: determination of protein content by kjeldahl method. Determination of component protein content: referring to the methods of Yang et al. (2006). Continuous extraction method was used to separate and extract different components with distilled water, 5 % NaCl, 70 % ethanol and 0.1 mol·L⁻¹ NaOH in turn. Then the protein content of each component was determined by Coomassie brilliant blue method.

Enzyme activity determination

The activity of GS according to Sun et al. (2014), NADH-GOGAT according to Singh (1986), GDH according to Yamaya et al. (1984), and AS was conducted according to Nakano et al. (2000).

Statistical analysis

The data were statistically analyzed with Excel 2003 (Microsoft Office 2003) and SPSS 24.0 for Windows (IBM Corporation), and means were tested by least significant difference at $P < 0.05$ (LSD 0.05).

Abbreviations

AKI: Akihikari; ALB, albumin; AS: asparagine synthetase; CK: control check; Ep: erect panicle; EQ: eating quality; FGN, filled grain number per panicle; GAGOT: glutamine-2-oxoglutarate aminotransferase; GDH: glutamate dehydrogenase; GLO, globulin; GLU, glutelin; GNP, grain number per panicle; GS: glutamine synthetase; H: high nitrogen; HD: heading date; L: low nitrogen; LG5: Liaogeng 5; N: nitrogen; NA/NADH: nicotinamide adenine dinucleotide; NILs: near-isogenic lines; NUE: nitrogen use efficiency; PBI: protein body I; PBII: protein body II; PBN, primary branches; PGN, primary grain; PH: plant height; PL, panicle length; PNP, panicle number per square meter; PRO, prolamin; RVA: rapid visco analyser

Declarations

Authors' Contributions

Conceived and designed the experiments: LT; Performed the experiments: SC, SC, YJ, QL, ZL, WL, and WS; Analyzed the data: SC; Wrote the paper: SC; Provide amendments to the manuscript: QX, JS, FZ. All authors read and approved the final manuscript.

Funding

This research was supported by the Training Plan for Excellent Talents in Agricultural Scientific Research, National Key Research and Development Program of China (2016YFD0300504).

Availability of Data and Materials

All data supporting the conclusions of this article are provided within the article (and in the Additional files).

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Competing Interests

The authors declare that they have no competing interests.

References

1. Amagliani L, O'Regan J, Kelly AL, O'Mahony JA (2017) The composition, extraction, functionality and applications of rice proteins: A review. *Trends in Food Science & Technology* 64:1–12
2. Balindong JL, Ward RM, Liu L, Rose TJ, Pallas LA, Ovenden BW, Snell PJ, E. Waters DL (2018) Rice grain protein composition influences instrumental measures of rice cooking and eating quality. *Journal of Cereal Science* 79: 35–42
3. Bornhorst GM, Ferrua MJ, Rutherford SM, Heldman DR, Singh RP (2013) Rheological Properties and Textural Attributes of Cooked Brown and White Rice During Gastric Digestion in Vivo. *Food Biophysics* 8:137–150
4. Cai C, Huang J, Zhao L, Liu Q, Zhang C (2014) Heterogeneous structure and spatial distribution in endosperm of high-amylose rice starch granules with different morphologies. *Journal of Agricultural & Food Chemistry* 62:10143–10152
5. Che R, Tong H, Shi B, Liu Y, Fang S, Liu D, Xiao Y, Hu B, Liu L, Wang H, Zhao M, Chu C (2015) Control of grain size and rice yield by GL2-mediated brassinosteroid responses. *Nature Plants* 2:15195
6. Chen S, Liu S, Zheng X, Yin M, Chu G, Xu C, Yan J, Chen L, Wang D, Zhang X (2018) Effect of various crop rotations on rice yield and nitrogen use efficiency in paddy–upland systems in southeastern China. *The Crop Journal* 6:576–588
7. Chen S, Tang L, Sun J, Xu Q, Xu Z, Chen W (2021) Contribution and prospect of erect panicle Type to *japonica* super rice. *Rice Science* 28:431–441
8. Crofts N, Nakamura Y, Fujita N (2017) Critical and speculative review of the roles of multi-protein complexes in starch biosynthesis in cereals. *Plant Science* 262:1–8
9. Derycke V, Veraverbeke WS, Vandeputte GE, Man WD, Hosney RC, Delcour JA (2005) Impact of proteins on pasting and cooking properties of nonparboiled and parboiled rice. *Cereal Chemistry Journal* 82:468–474

10. Fan C, Xing Y, Mao H, Lu T, Han B, Xu C, Li X, Zhang Q (2006) *GS3*, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theor Appl Genet* 112:1164–1171
11. Fei C, Yu J, Xu Z, Xu Q (2019) Erect panicle architecture contributes to increased rice production through the improvement of canopy structure. *Mol Breeding* 39:128.
12. Fitzgerald MA, Martin M, Ward RM, Park WD, Shead HJ (2003) Viscosity of Rice Flour: A Rheological and Biological Study. *Journal of Agricultural and Food Chemistry* 51:2295–2299
13. Hu J, Wang Y, Fang Y, Zeng L, Xu J, Yu H, Shi Z, Pan J, Zhang D, Kang S, Zhu L, Dong G, Guo L, Zeng D, Zhang G, Xie L, Xiong G, Li J, Qian Q (2015) A rare allele of *GS2* enhances grain size and grain yield in rice. *Molecular Plant* 8:1455–1465.
14. Hu X, Yang J, Cheng C, Zhou J, Niu F, Wang X, Zhang M, Cao L, Chu H (2018) Targeted editing of rice *SD1* gene using CRISPR/Cas9 system. *Chin J Rice Sci* 32:219–225 (in Chinese with English abstract)
15. Huang X, Su F, Huang S, Mei F, Niu X, Ma C, Zhang H, Zhu X, Zhu JK, Zhang J (2021) Novel *Wx* alleles generated by base editing for improvement of rice grain quality. *J Integr Plant Biol* 63: 1632–1638
16. Lai S, Motonobu K, Wang Z, Mikami T, Huang D, Li H, Lu D, Zhou D, Zhou S (2011) Cooking and eating quality of indica rice varieties from south china by using rice taste analyzer, *Chin J Rice Sci* 25:435–438 (in Chinese with English abstract)
17. Ma Z, Cheng H, Nitta Y, Aoki N, Chen Y, Chen H, Ohsugi R, Lyu W (2017) Differences in viscosity of superior and inferior spikelets of *Japonica* rice with various percentages of apparent amylose content. *J Agric Food Chem* 65:4237–4246
18. Nakano K, Suzuki T, Hayakawa T, Yamaya T (2000) Organ and cellular localization of asparagine synthetase in rice plants. *Plant Cell Physiol* 41:874–880
19. Palme K, Li X, Teale WD (2014) Towards second green revolution: engineering nitrogen use efficiency. *Journal of Genetics and Genomics* 41:315–316
20. Qi P, Lin YS, Song XJ, Shen JB, Huang W, Shan JX, Zhu MZ, Jiang L, Gao JP, Lin HX (2012) The novel quantitative trait locus *GL3.1* controls rice grain size and yield by regulating Cyclin-T1;3. *Cell Research* 22:1666–1680
21. Saleh MI (2017) Protein-starch matrix microstructure during rice flour pastes formation. *Journal of Cereal Science* 74:183–186
22. Si L, Chen J, Huang X, Gong H, Luo J, Hou Q, Zhou T, Lu T, Zhu J, Shangguan Y, Chen E, Gong C, Zhao Q, Jing Y, Zhao Y, Li Y, Cui L, Fan D, Lu Y, Weng Q, Wang Y, Zhan Q, Liu K, Wei X, An K, An G, Han B (2016) *OsSPL13* controls grain size in cultivated rice. *Nat Genet* 48:447–456
23. Singh RD, Srivastava HS (1986) Increase in glutamate synthase (NADH) activity in maize seedlings in response to nitrate and ammonium nitrogen. *Physiol. Plantarum* 66:413–416
24. Sun H, Qian Q, Wu K, Luo J, Wang S, Zhang C, Ma Y, Liu Q, Huang X, Yuan Q, Han R, Zhao M, Dong G, Guo L, Zhu X, Gou Z, Wang W, Wu Y, Lin H, Fu X (2014) Heterotrimeric G proteins regulate nitrogen-use efficiency in rice. *Nat Genet* 46:652–656
25. Tang L, Gao H, Yoshihiro H, Koki H, Tetsuya N, Liu T, Tatsuhiko S, Xu Z (2017) Erect panicle super rice varieties enhance yield by harvest index advantages in high nitrogen and density conditions. *J Integr Agric* 16:1467–1473
26. Tang L, Zhang F, Liu A, Sun J, Mei S, Wang X, Liu Z, Liu W, Lu Q, Chen S (2019) Genome-Wide Association analysis dissects the genetic basis of the grain carbon and nitrogen contents in milled rice. *Rice* 12:101-116
27. Umemoto T, Aoki N, Lin H, Nakamura Y, Inouchi N, Sato Y, Maruyama S (2004) Natural variation in rice starch synthase IIa affects enzyme and starch properties. *Functional Plant Biology* 31:671–684
28. Vidal V, Pons B, Brunnschweiler J, Handschin S, Rouau X, Mestres C (2007) Cooking behavior of rice in relation to kernel physicochemical and structural properties, *Journal of Agricultural & Food Chemistry* 55:336–346
29. Wang Y, Zhang N, Chen H, Wang F, Huang Y, Jia B, Wang S, Wang Y, Xu Z (2019) Effects of *DEP1* on grain yield and grain quality in the background of two *japonica* rice (*Oryza sativa*) cultivars. *Plant Breeding* 139:608–617
30. Wu J, Xiong R, Xie J, Jiang H, Tan X, Pan X, Zeng Y, Shi Q, Zeng Y (2021) Research advances on rice eating grain quality formation and its response to nitrogen application. *China Rice* 27: 28–37 (in Chinese with English abstract)
31. Xu Z, Chen W, Zhou H, Zhang L, Yang S (1996) Physiological and ecological characteristics of rice with erect panicle and prospects of their utilization. *Chin Sci Bull* 41:1642–1648 (in Chinese)
32. Xu Q, Zhao M, Wu K, Fu X, Liu Q (2016) Emerging insights into heterotrimeric G protein signaling in plants. *J Genet Genomics* 43:495–502
33. Yamaya T, Oaks A, Matsumoto H (1984) Characteristics of glutamate dehydrogenase in mitochondria prepared from corn shoots. *Plant Physiol* 76:1009–1013
34. Yang J, Luo Q, Qian C, Liu H, Jin Z (2006) Effect of nitrogen on the protein fractions content and cooking and eating quality of rice grain. *Journal of Northeast Agricultural University* 37:145–150 (in Chinese with English abstract)
35. Zhang X, Wang J, Huang J, Lan H, Wang C, Yin C, Wu Y, Tang H, Qian Q, Li J, Zhang H (2012) Rare allele of *OsPPKL1* associated with grain length causes extra-large grain and a significant yield increase in rice. *Proceedings of the National Academy of Sciences* 109:21534–21539
36. Zhao M, Zhao M, Gu S, Sun J, Ma Z, Wang L, Zheng W, Xu Z (2019) *DEP1* is involved in regulating the carbon nitrogen metabolic balance to affect grain yield and quality in rice (*Oryza sativa* L.). *Plos One* 14:e0213504
37. Zhu L, Wu G, Cheng L, Zhang H, Wang L, Qian H, Qi X (2020) Investigation on molecular and morphology changes of protein and starch in rice kernel during cooking. *Food Chemistry* 316: 126262

Figures

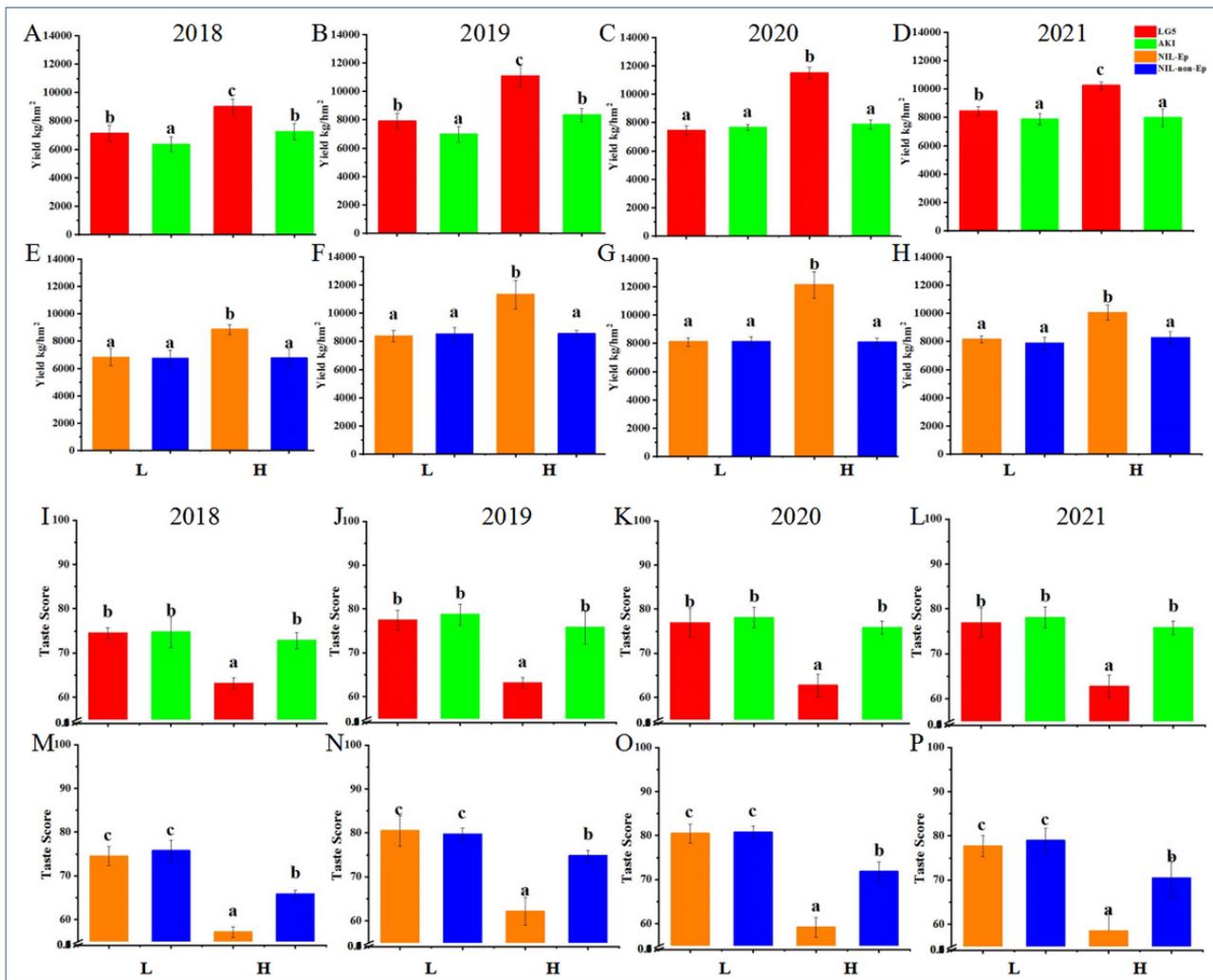


Figure 1
 Phenotypes of yield and taste quality of test materials from 2018 to 2021. A-H Yield for LG5, AKI, NIL-Ep and NIL-non Ep. I-P taste score for LG5, AKI, NIL-Ep and NIL-non Ep. The a,b denote significance at the 0.05 level.

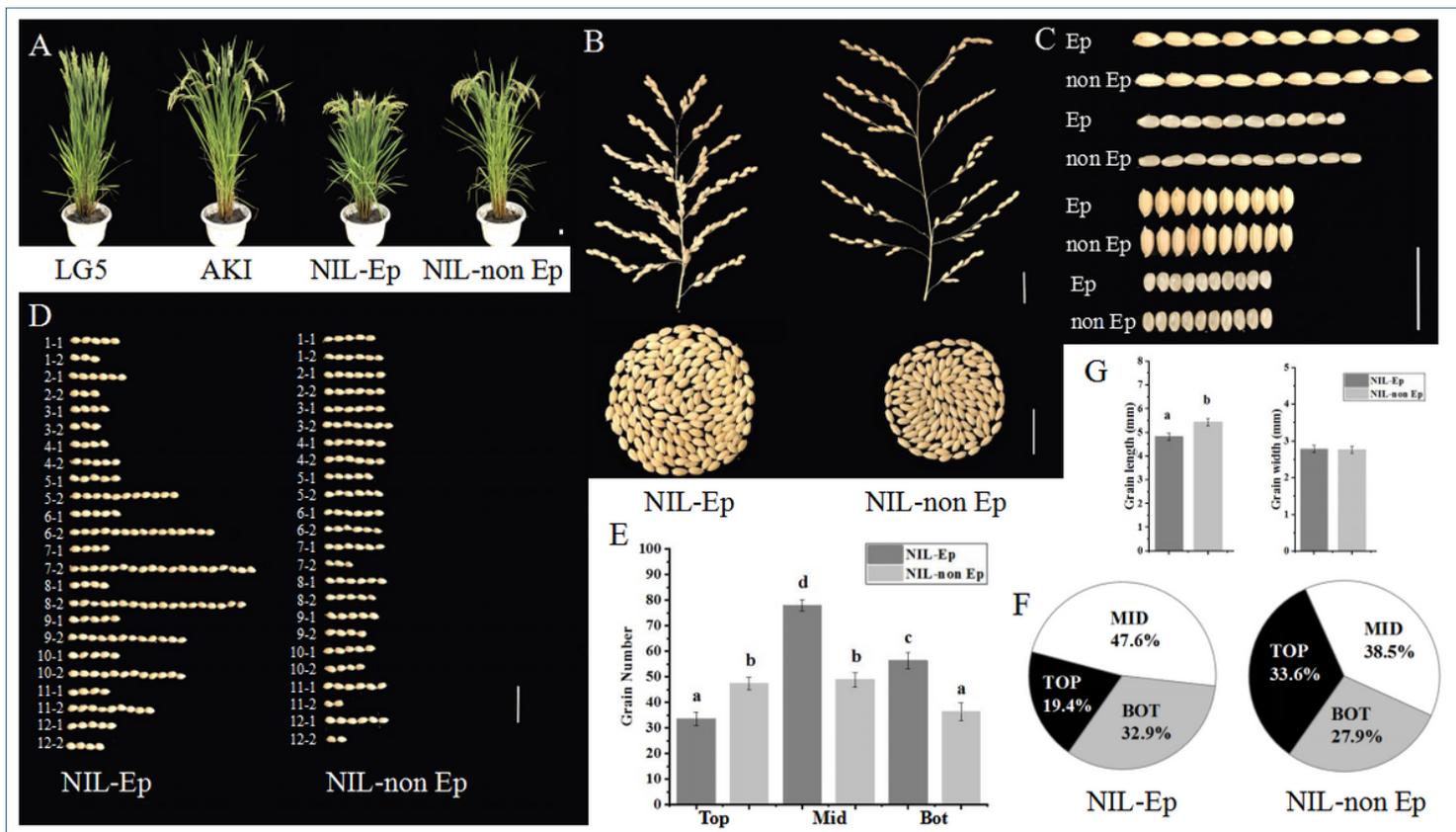


Figure 2

The yield performance and grain shape of NIL-Ep and NIL-non Ep plants. A The four experimental materials plant in pot. B The panicles and grain numbers per panicle of the NIL-Ep and NIL non Ep. C The grain size of the NIL-Ep and NIL non Ep. D The grain numbers of different panicle locations for NIL-Ep and NIL non Ep. E Difference analysis of grain number in different panicle position. F Proportion of grain number in different panicle positions. G Difference analysis of grain shape. The a,b denote significance at the 0.05 level. Scale bar, 2 cm.

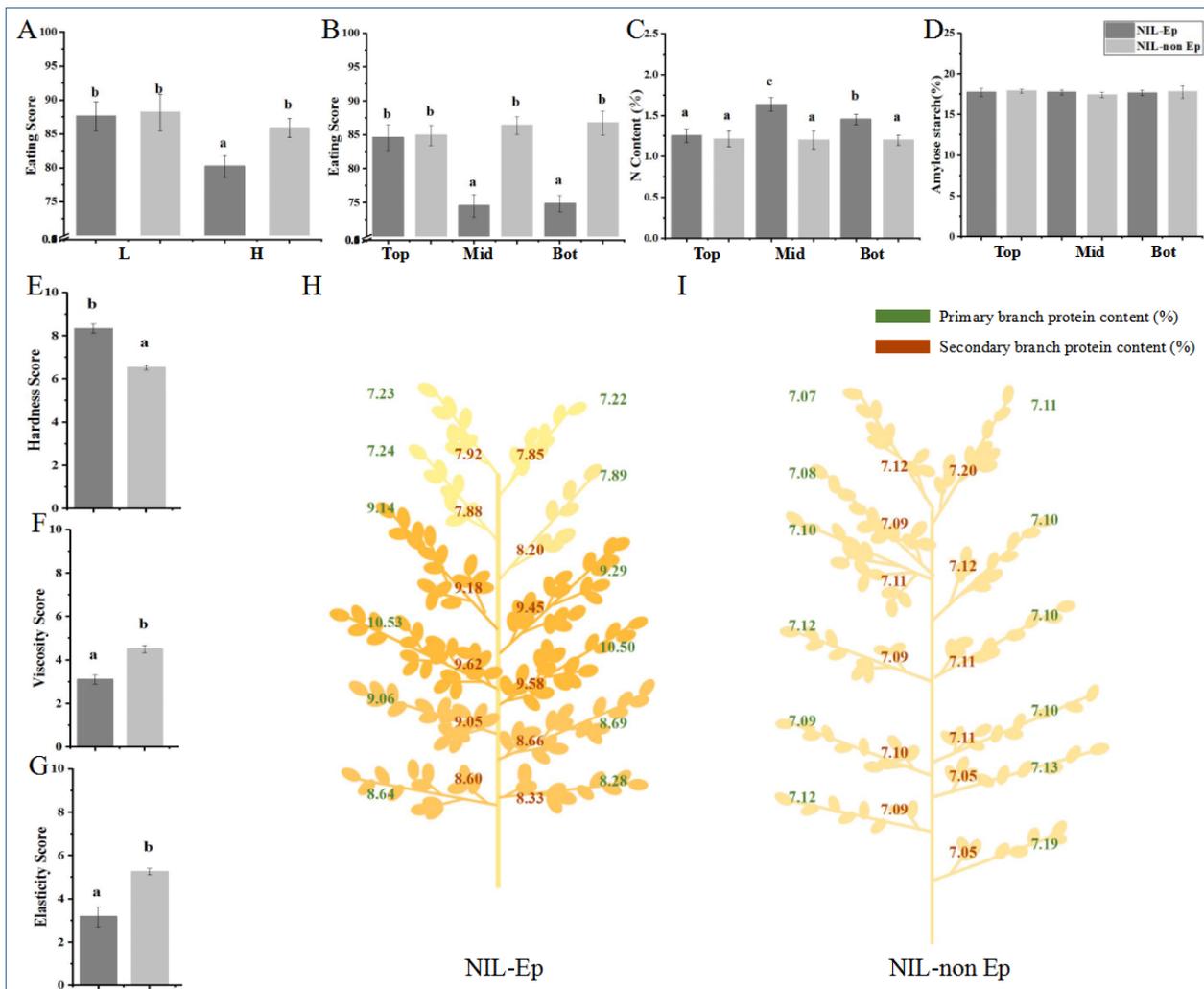


Figure 3
 The eating quality performance and grain protein, starch content of NIL-Ep and NIL-non Ep plants. A Eating quality under two nitrogen fertilizer treatments. B Eating quality of different panicle locations under H treatment. C N content of different panicle locations under H treatment. D Amylose content of different panicle locations under H treatment. E-G Components of eating quality of NIL-Ep and NIL-non Ep under H treatment. H-I Protein content(%) of different panicle locations under H treatment. The a,b denote significance at the 0.05 level.

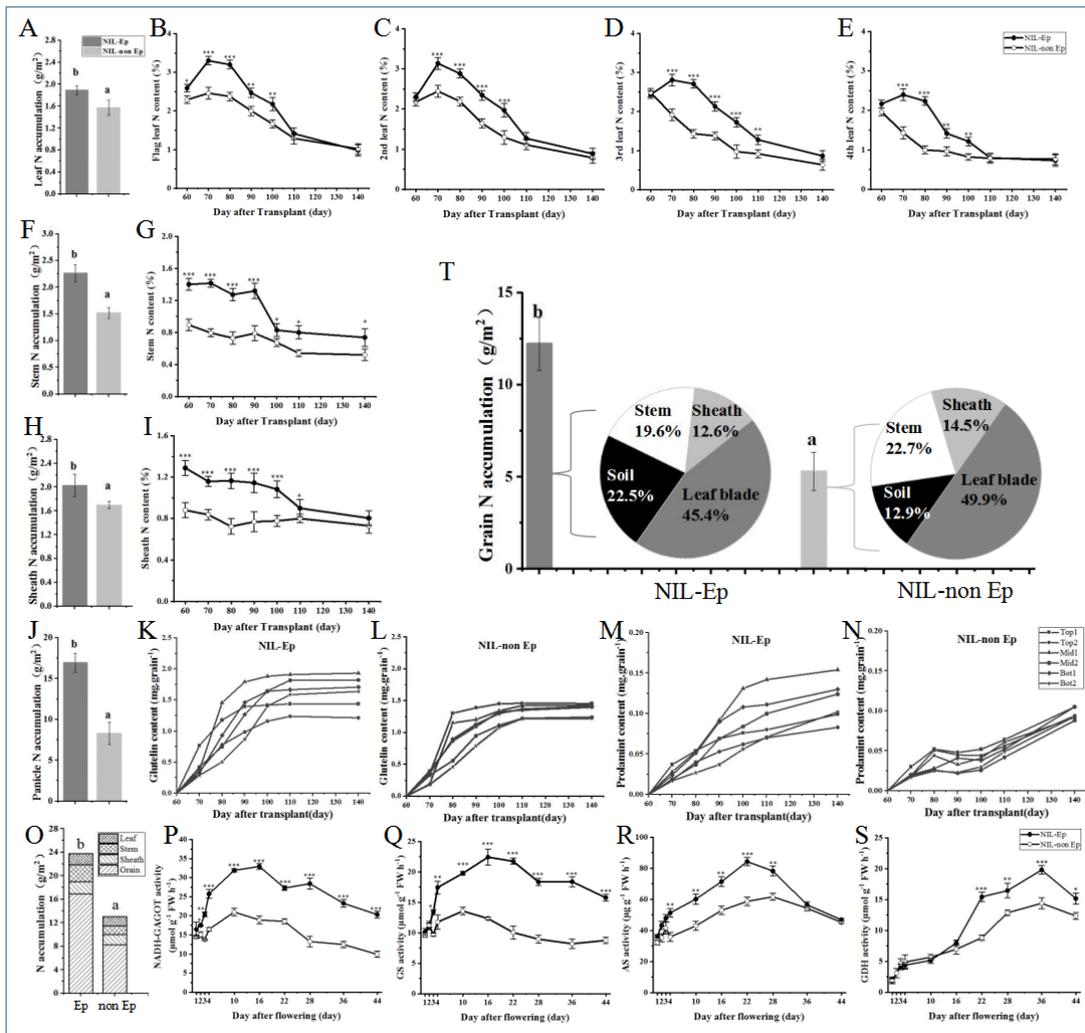


Figure 4 Differences in nitrogen transfer and nitrogen metabolism-related enzyme activities of NIL-Ep and NIL-non Ep plants under H treatment. A Leaf nitrogen accumulation at maturity stage. B-E Dynamics of leaf (from flag leaf to 4th leaf) nitrogen content F Stem nitrogen accumulation at maturity stage. G Dynamics of stem nitrogen content. H Sheath nitrogen accumulation at maturity stage. I Dynamics of leaf sheath nitrogen content. J Grain nitrogen accumulation at maturity stage. K-N Dynamics of glutelin and prolamin content in different panicle positions. O Total nitrogen accumulation at maturity stage. P-S Activities of enzymes related to nitrogen metabolism. T Origin of nitrogen in panicles from various organs and soils in rice from heading to mature period. The a,b denote significance at the 0.05 level. The *, ** and *** denote significance at 0.05, 0.01, and 0.001 level respectively.

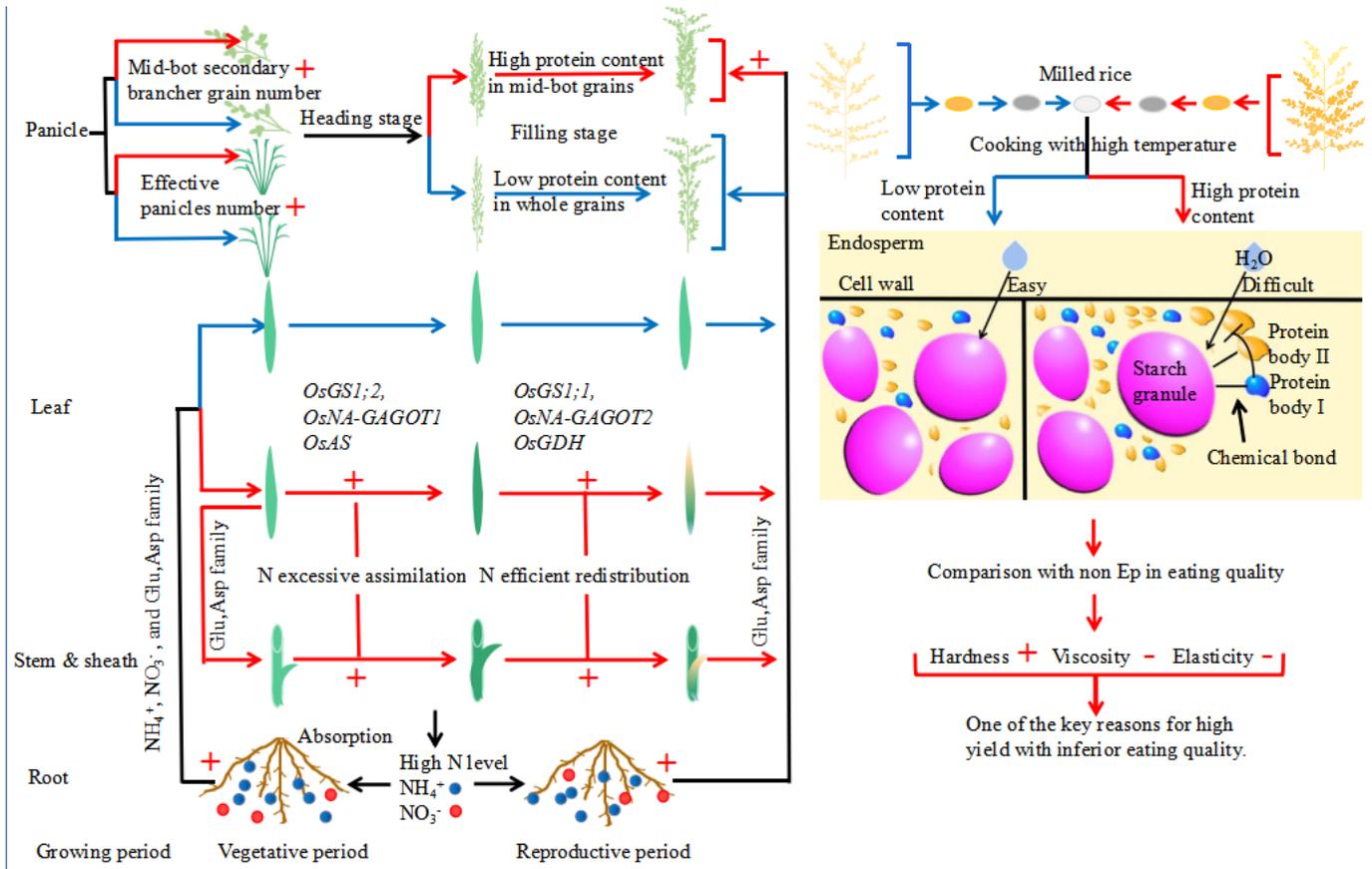


Figure 5

Diagram of Nitrogen utilization of Ep and non Ep panicle type at different growth stages under high nitrogen condition, and interaction between protein and starch granule during cooking. Details are described in the text. Red and blue lines represent the growth and development process of Ep and non Ep type respectively, black lines for common process. Red plus and minus indicate promoting and demoting effect of Ep compared with non Ep type.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xls
- TableS2.xls
- Fig.S1.doc
- Fig.S2.doc