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Characteristics of Grain Filling and Starch Accumulation of Brewing Functional *Indica* Rice in Southern Sichuan Eco-Region

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Abstract

In order to study the ecological adaptability of brewing functional *Indica* rice varieties in the ecological area of southern Sichuan, six *Indica* hybrid rice varieties were used as experimental materials to analyze the differences in the rice growth period, yield and yield components, grain filling characteristics, starch and soluble sugar accumulation dynamics under the ecological conditions of southern Sichuan. The results showed that there were significant differences in yield formation, grain filling and starch accumulation among varieties under the same ecological conditions. Affected by the climate in the ecological area of southern Sichuan, the time when CGY6684, GY900 and FY1 reached the maximum grain filling rate moved forward, while GY188, CY6203 and YXY2115 moved backward. At the same time, the high peak value of YXY2115 and GY900 grain filling rate was the highest, while the peak value of CY6203 grain filling rate was the lowest. The peak value of grain starch accumulation of YXY2115 was much higher than that of other varieties, with an increase of 44.07% - 109.71%. At maturity, the order of grain starch content from high to low was YXY2115 > CY6203 > FY1 > GY900 > GY188 > CGY6684. The 1000 grain weight of YXY2115 was significantly higher than that of other varieties, but its grains per panicle and panicles per unit area were lower, resulting in a slightly lower yield than other varieties. On the premise that the number of grains per panicle is genetically determined, in order to improve its yield, we can further improve the number of panicles per unit area through cultivation measures.

Subject Areas

Agricultural Science, Environmental Sciences

Keywords

Brewing Function, *Indica* Rice, Yield, Ecological Adaptability, Grain-Filling, Starch

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops and major food sources in the world [1] [2]. Maximizing the yield potential of rice is the eternal theme of modern agricultural production [3] [4]. The raw materials used in the production of traditional multi-grain Luzhou-flavor liquor are 36% sorghum, 22% rice, 18% glutinous rice, 16% wheat, and 8% corn [5]. The liquor is brewed through a specific process. The liquor yield and excellent grade rate of suitable special grain for brewing are increased by 1.5% and 6% respectively compared with ordinary grain, which has an obvious effect on improving the quality of original Liquor [6]. During the 13th Five-Year-Plan period, "three hundred-billion-yuan industrial clusters" had been formed in Yibin City, Luzhou City, Sichuan Province and Renhuai City, Guizhou Province, and the grain demand had reached tens of millions of tons. "Thirteenth Five-Year" strategic plan of Wuliangye Group Limited Company clearly put forward the goal of building a 100-billion group and building another 100,000 tons of raw Liquor production base, and the corresponding grain procurement scale would be doubled. On the basis of establishing a special grain base in line with the strategic development requirements of Wuliangye brand, it is of great research value to regulate the rice filling process through agronomic and chemical control measures to improve the yield and quality of special grain for brewing [7].

Agricultural production is highly dependent on climatic resources. Under the same ecological conditions, the growth and development, yield and quality formation, grain filling process, and starch formation process of different varieties will be significantly affected by meteorological resources [8] [9] [10]. Some researchers regulated the utilization of light and temperature resources through the sowing date to affect the growth process, yield and quality of *Indica*-japonica hybrid rice [11] [12]. The ecological region of southern Sichuan, represented by Yibin, belongs to the humid monsoon climate in the middle subtropical zone. The climate is mild; the rainfall is abundant; the rain and heat are in the same season; the annual sunshine hours are 1073.7 h; the frost-free period is 335 - 355 d. It is beneficial to play the potential for high photosynthesis and high biomass of rice. The unique high-temperature environment from July to August is also conducive to the formation of the unique starch accumulation process of alcoholic rice. However, there are few studies on the variety selection, grain filling, starch formation characteristics and yield formation process of brewing functional *Indica* rice in the Southern Sichuan eco-region. Therefore, this study compared the differences in yield and yield components, grain filling characteristics, starch and soluble sugar accumulation characteristics of different brewing functional *Indica* rice varieties in the southern Sichuan ecological region, in order to provide a basis for the study of ecological adaptability and support cultivation techniques of brewing functional *Indica* rice in southern Sichuan ecological area.

2. Materials and Methods

2.1. Experimental Design and Material Cultivation

This study was conducted in Yibin City, Sichuan Province in 2021. Six *Indica* hybridrice varieties, GY188 (V1), CGY6684 (V2), CY6203 (V3), GY900 (V4), FY1 (V5) and YXY2115 (V6), were selected as test materials.

Three plots were set up for each variety, arranged randomly, and the area of each plot was 30 m^2. The seedling age is 20 - 21 d, and the planting density is 30 cm × 16 cm. During the whole growth period, nitrogen was 270 kg/hm^2, and the ratio of basal fertilizer and ear fertilizer was 7:3. Phosphorus and potassium fertilizers, irrigation and pest management refer to local conventional management methods. Table 1 shows the development stage and growth period of the tested varieties under different sowing dates.

2.2. Determination Parameters and Methods

2.2.1. Yield and Yield Components

In each plot, 50 clumps of rice were investigated at the mature stage, the number of effective panicles was calculated, and 6 clumps were taken according to the average number of stems and tillers, and the number of grains per panicle, 1000-grain weight and seed setting rate were determined.

2.2.2. Plant Grain Filling Dynamics

When heading 20% - 30% in each plot, select the same size of the single stem spike (including the main stem spike and tillering spike, the top of the spike out the sword leaf sheath < 2 cm) to sign, each plot marked 200 spikes. The listed

Table 1. Development stage and growth period of the tested varieties.

Variety	No.	Sowing (month-day)	Transplanting (month-day)	Heading (month-day)	Maturity (month-day)	Duration from heading to maturity (d)
V1	GY188	4-23	5-13	8-3	9-9	37
V2	CGY6684	4-23	5-13	8-3	9-9	37
V3	CY6203	4-23	5-13	7-28	9-9	43
V4	GY900	4-23	5-13	8-2	9-9	38
V5	FY1	4-23	5-13	8-1	9-9	39
V6	YXY2115	4-23	5-13	8-1	9-9	39

single stem was taken from heading stage (50% heading) and sampled every 7 - 10 days until mature harvest. The middle grains were taken from each group of rice panicles and put into envelopes, respectively. They were all killed at 105˚C for 30 min, dried at 80˚C to constant weight and threshed. The dry matter weight of each group of rice panicles was determined and the single grain weight ($mg \cdot grain^{-1}$) was calculated. Five listed rice panicles were randomly selected for each variety.

2.2.3. Dynamics of Starch and Soluble Sugar Accumulation in Plant Grains

The dried grains were grinded into fine powder by high-throughput grinding instrument (Shanghai Jingxin), and the starch and soluble sugar concentrations (%) were determined by anthrone colorimetric method, and repeated five times. The accumulation of starch and soluble sugar in single grain (μg) was calculated according to the dry weight of grain.

2.3. Data Statistics and Analysis

Statistical analysis of data was performed using Microsoft Excel and SPSS25.0 software (IBM SPSS statistics for windows, version 25.0); Origin 2021 software was used for graphing.

Fitting of rice ear filling process by Richards equation [13] [14]:

$$W = A \Big/ \left(1 + B\mathrm{e}^{-Kt}\right)^{1/N} \tag{1}$$

In Formula (1), W is the grain weight, t is the days after heading (DAH), A is the final grain weight of a single grain ($mg \cdot grain^{-1}$), and B, K, and N are the estimated values of the Richards equation parameters, and the fitting accuracy was expressed by the determination coefficient R^2.

According to Richards equation, the secondary parameters such as the initial growth potential (R_0), the maximum grain-filling rate (GR_{max}, $mg \cdot grain^{-1} \cdot d^{-1}$), the average grain-filling rate (GR_{mean}, $mg \cdot grain^{-1} \cdot d^{-1}$), time to reach maximum grain-filling rate ($T_{max \cdot G}$, d), the effective grain filling period (t_{99}) and active grain-filling period (D, d) are derived.

Calculate the first derivative of (1) to obtain the equation of the grain filling rate (G) according to t:

$$G = \left(KW/N\right)\left[1 - \left(W/A\right)^{N}\right] \tag{2}$$

The grain filling rate equation (Formula (2)) has two inflection points, and its second-order derivative to t is calculated and set to be zero, and the values of t_1 and t_2 of the two inflection points on the t coordinate can be obtained. Combined with the above t_{99}, the whole grain filling process can be divided into three stages: the early stage (0 - t_1), the middle stage (t_1 - t_2) and the late stage (t_2 - t_{99}), and the ratio of the grain filling duration in each stage to t_{99} (RGD, %) and the contribution rate of grain accumulation in each stage to the final grain weight (RGC, %) could be calculated.

3. Results and Analysis

3.1. Differences in Yield and Components of Different *Indica* Rice Varieties in Southern Sichuan Ecological Region

It can be seen from Figure 1 that under the same ecological conditions, there are significant indigenous differences in the yield and some yield components of different *Indica* rice varieties, and the difference in seed setting rate among varieties does not reach the significant indigenous level, so it is not listed. In terms of yield, the yield of V5 (FY1) was significantly lower than that of other varieties ($P < 0.05$), and the difference was 39.34% - 53.45%. In terms of yield components, the panicle number per unit area of V4 (GY900) was 8.33% - 30.00% higher than that of other varieties, and the difference between treatments was significant ($P < 0.05$). The seed setting rate of V6 (YXY2115) was significantly higher than that of other treatments ($P < 0.05$), with an increase of 15.15% - 32.01%. The grain number per panicle of V1 (GY188) was the highest, which was 10.90% - 44.25% higher than that of other treatments, and the difference between treatments reached significant indigenous level.

3.2. Fitting Process of Grain Weight Increase of Different *Indica* Rice Varieties in Southern Sichuan Eco-Region

Rice yield formation process is actually grain filling process. The grain weight growth process of different *Indica* rice varieties at grain filling stage was in line with Richards equation. The fitting results of grain weight growth and grain filling rate of different *Indica* rice varieties at grain filling stage (Figure 2) were

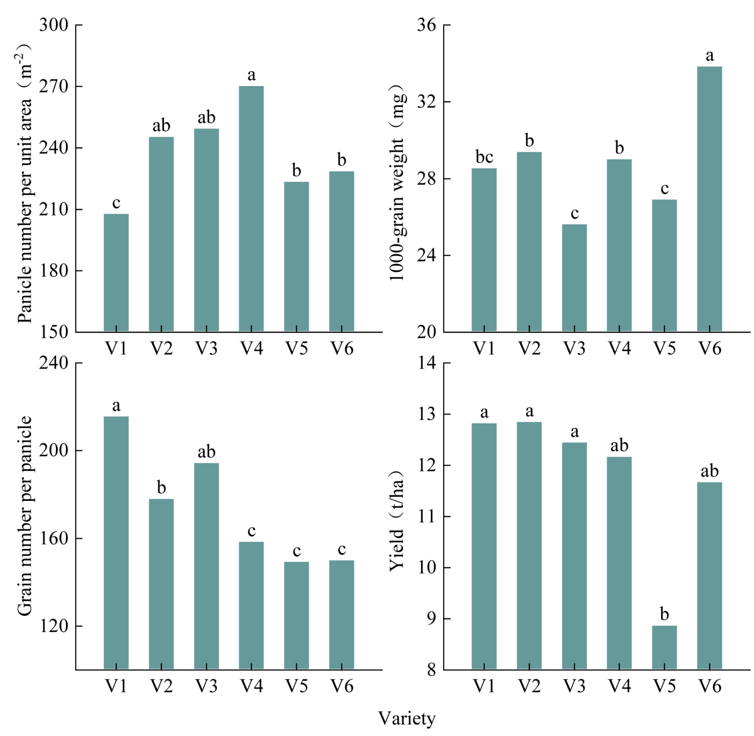

Figure 1. Yield and yield components of different *Indica* rice varieties.

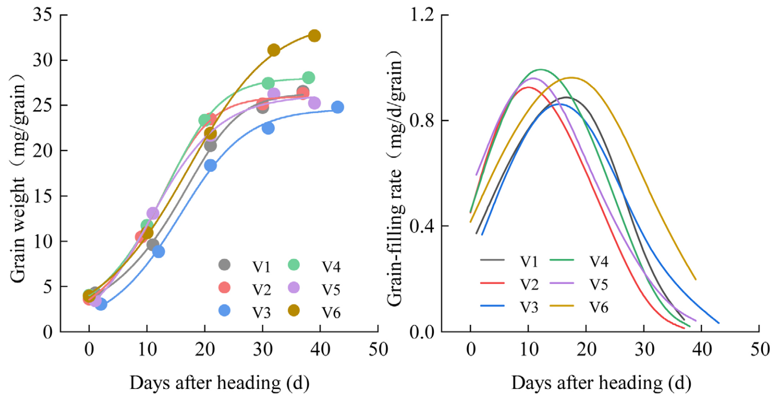

Figure 2. Fitting of grain weight and grain filling rate of different *Indica* rice varieties.

obtained. R^2 was above 0.990, and the fitting results were good. The results can be used for further analysis. Figure 2 shows that the final grain weight of V6 (YXY 2115) was significantly higher than that of other varieties, followed by V4 (GY 900), and the final grain weight of V3 (CY 6203) was the lowest. Comparing the filling rates of various varieties at the filling stage, it can be seen that V2, V4, and V5 reach their maximum filling rates significantly earlier than V1, V3, and V6. At the same time, V4 has the highest grain filling rate, followed by V6 and V5, while V3 was the lowest.

3.3. Dynamics of Starch and Soluble Sugar Accumulation in Grains of Different *Indica* Rice Varieties during Grain Filling Stage

During rice grain filling, photosynthetic carbohydrates and pre-anthesis storage substances are continuously transported to grains in the form of sucrose, and transformed into starch storage to enrich the grain. Figure 3 shows that during the grain filling process, the starch content in rice grains shows a trend of slow increase in the early stage (about 0 - 20 d), rapid increase in the middle stage (about 20 - 31 d) and decrease in the later stage (after 31 d), and the turning point of rapid increase and decrease was about 20 d and 31 d after heading. The comparison among varieties showed that the peak starch content in grains of V6 was much higher than that of other varieties, with an increase of 44.07% - 109.71%. At the mature stage, the starch content in grains of each variety ranked from high to low as V6 > V3 > V5 > V4 > V1 > V2. The soluble sugar content in grains showed an overall gradual increase trend, and the soluble sugar content increased rapidly at the late filling stage, which occurred synchronously with the decrease of starch content.

3.4. Grain Filling Characteristic Parameters of Different *Indica* Rice Varieties

The grain-filling initiation potential (Ro) reflects the growth potential of the seed room, and the larger the value is, the shorter the endosperm cell division cycle is, the faster the division is, and the earlier the grain filling starts. It can be seen from Figure 4 that the Ro difference between the varieties is significant,

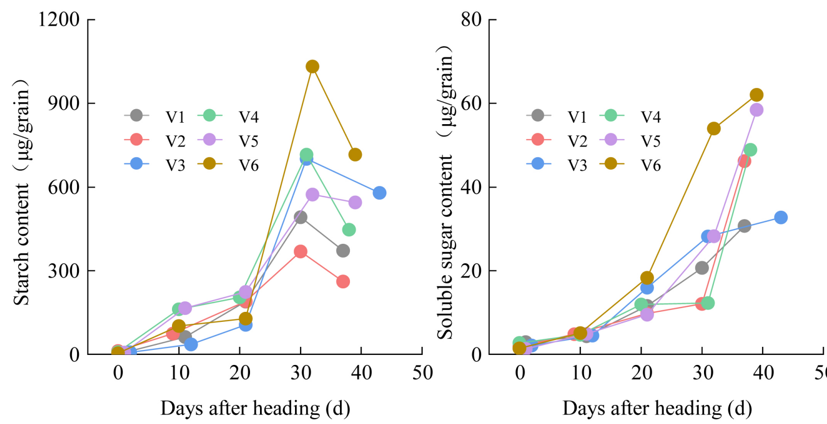

Figure 3. Accumulation dynamics of starch and soluble sugar in grains during grain filling.

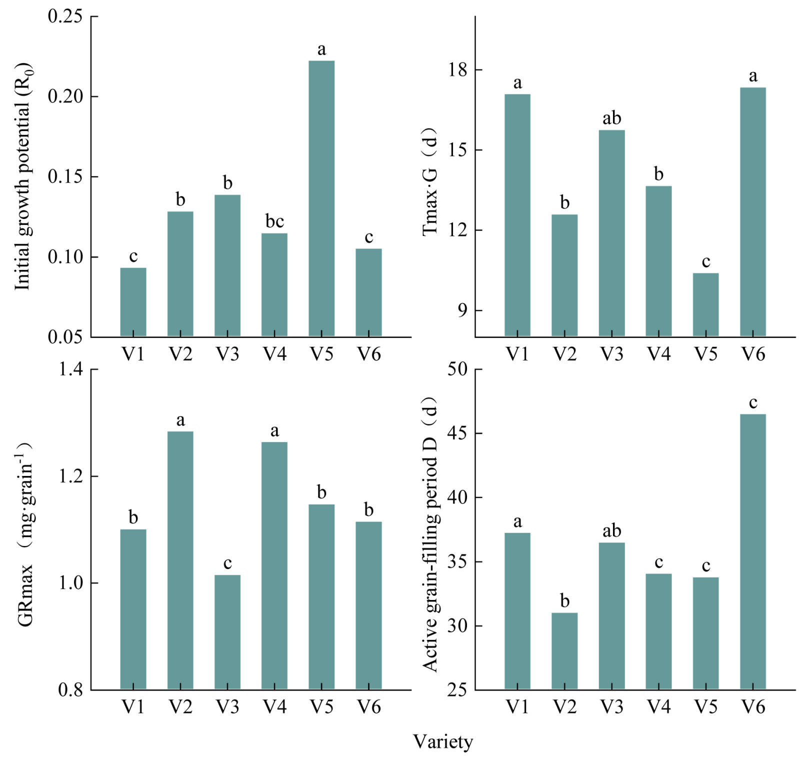

Figure 4. Grain filling characteristic parameters of different *Indica* rice varieties.

and the V5 can reach 1.60 to 2.37 times that of other varieties. It indicated that its grain filling initiation potential and rate were much higher than other varieties. The maximal filling rate (GR_{max}) of each variety showed that V2 and V4 were significantly higher than other varieties, while V3 was the lowest, which was basically consistent with the performance of the grain filling rate in Figure 2. Active grain-filling period (D represents the number of days of grain-filling, which to a certain extent reflects the grain-filling accumulation ability of rice.) It can be seen from Figure 4 that the active filling days of V6 are significantly higher than other varieties, which means that there is more sufficient time to

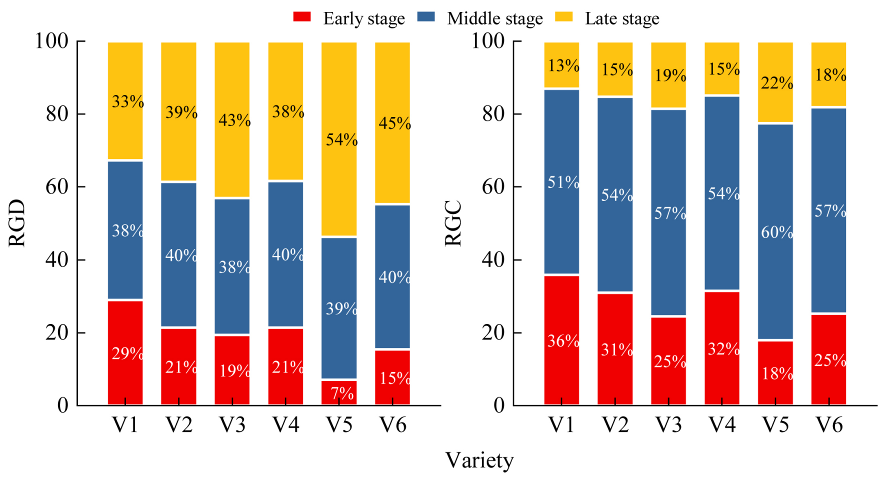

Figure 5. Comparison of RGD and RGC at different filling stages of different *Indica* rice varieties. RGD is the ratio of grain filling days at each stage to effective grain filling stage (%); RGC is the contribution rate of grain filling accumulation at each stage to final grain weight (%).

complete the filling. The earlier the time for grain filling to reach the maximum filling rate ($T_{\text{max}\cdot\text{G}}$), it means that the peak grain filling rate can be reached earlier, which promotes the accumulation of grain material. By comparison, the days of reaching the maximum grain filling rate of the V2 and V5 varieties are the shortest, which is basically consistent with the performance in **Figure 2**.

3.5. Comparison of Grain Filling Stages of Different *Indica* Rice Varieties

From **Figure 2**, it can be seen that the grain filling rate of each stage of the tested varieties conforms to the filling law of gradual increase (early stage), rapid increase (middle stage) and slow increase (late stage). After further analyzing the grain filling characteristics of each stage, it could be found that (**Figure 5**, RGD), there were significant differences in the proportion of filling duration days in each stage to their effective filling period (RGD). In general, among the effective grain-filling days, the V5 pre-filling days accounted for the lowest, accounting for only 7%, while the latter accounted for the highest, reaching 54%. It means that 39% of the effective grain filling days of this variety were in the stage of rapid increase of grain weight. At the same time, in the contribution rate (RGC) of grain filling amount to final grain weight at each stage of grain filling (**Figure 5**, RGC), the proportion of RGC of V5 stage of grain filling of V5 variety reached 60%, It means that the variety had the shortest rapid filling days, the highest proportion of dry matter accumulation and the lowest yield potential compared with other varieties.

4. Discussion and Conclusions

The yield of different varieties not only depends on their own genetic differences but also is affected by environmental factors, which is the result of the interaction between the varieties and the environment [15]. The southern Sichuan ecological area represented by Yibin City has a humid monsoon climate in the middle subtropical zone in terms of geographical location and has three-dimensional climate characteristics from the southern subtropical zone to the warm temperate zone. It is very suitable for the growth of sorghum, glutinous rice, rice, corn, wheat and other brewing functional crops. It is the most suitable area for a high-quality raw materials base [16]. And the raw materials produced have high starch content, moderate tannin content, high quality and high liquor yield, and can fully brew Luzhou-flavor, Maotai-flavor and concurrent-flavor liquor. While establishing a special grain base for brewing in the original area of Wuliangye, by exploring the mechanism of grain filling and starch formation of special rice for liquor making, and then improving the yield and quality of special grain for liquor making through cultivation control measures, it can effectively supply raw materials for liquor making and ensure the quality and characteristics of Wuliangye, and it is also conducive to improving the efficiency of grain cultivation, reducing the cost of grain production, promoting grain transformation, and driving local economic development [7].

The grain filling process is affected by genotype, environmental conditions and cultivation measures [17] [18] [19] [20] [21]. It is a feasible way to improve the brewing quality and liquor yield of rice by exploring the differences in grain filling accumulation and starch formation of different brewing functional rice varieties, based on which cultivation regulation is carried out. Through comparison, this study has found that under the conditions of the southern Sichuan ecological region, there are significant differences in the grain filling process, starch accumulation and yield formation process of different varieties of brewing functional rice. All varieties have basically the same growth period and filling days, but their filling characteristics are significantly different. Among them, the peak period of grain filling rate of V1, V3 and V6 varieties is obviously shifted back; the active grain filling days are significantly prolonged; the grain starch accumulation rate and accumulation amount of V3 and V6 are significantly higher than other varieties. From the comparison of the yield formation process, it is found that the 1000-grain weight of the V6 variety is significantly higher than that of other varieties, but its grain number per panicle and panicle number per unit area are lower, resulting in a slightly lower yield than other varieties. Further analysis shows that the number of grains per panicle of V6 cultivar Yixiangyou 2115 is mainly determined by genetic characteristics (the seed setting rate could reach 93.2%). Therefore, in order to increase its yield, cultivation measures can be used to further increase the number of panicles per unit area. At the same time, in order to give full play to the yield potential of each variety and prolong the filling period, Chuanguyou 6684, Gangyou 900 and Fuyou 1 should be sown early (earlier than April 23).

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Conflicts of Interest

The authors declare no conflicts of interest.

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