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Characterization and Grouping of All Primary Branches at Various Positions on a Rice Panicle Based on Grain Growth Dynamics

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Abstract: Grain filling can directly influence rice yield. However, there is limited information on the growth relationship among grains at different positions on the entire panicle during grain filling. In this study, field experiments were conducted in 2014–2015 to compare the growth dynamics of grains at various positions for two rice cultivars (Nongle 1 and Guifeng 2). The results showed that a high similarity and a slow–fast–slow trend of dry-matter accumulation occurred in all primary branches. However, the maximum grain growth rates of the top primary branches were 86% and 44% higher than basal primary branches of Nongle 1 and Guifeng 2, respectively. Similarly, the maximum final grain weights were 32% and 18% greater in the top primary branches than in the basal primary branches of Nongle 1 and Guifeng 2, respectively. In contrast, the active grain filling duration was 1.5 and 1.3 times longer in the basal primary branches than the top primary branches of Nongle 1 and Guifeng 2, respectively. The time to reach the maximum rate of grain growth of the basal primary branches for Nongle 1 and Guifeng 2 was 2.2 and 2.5 times longer than those of the top primary branches, respectively. Based on cluster analysis of growth characteristics of all primary branches, Group I (superior primary-branches) was considered to be the fastest for grain filling and greatest for dry matter weight, followed by Group II (medium primary-branches). The poorest growth occurred in Group III (inferior primary-branches). Therefore, the yield of poor-filling grains at the basal panicle could be achieved primarily by improving the growth of Group III.

Keywords: rice; grain growth; grain position; mathematical simulation; primary branch grouping

1. Introduction

Rice (*Oryza sativa* L.) is the most important food crop worldwide. It is a staple food for about 3 billion people [1], and it provides 76% of the calories for Southeast Asian people [2]. Nearly 90% of the world's rice is obtained from Asia (almost 640 million tons), and China and India are the major contributors [3]. The increase in the population and the improvement of living standards has resulted in the demand for further enhancing rice yield and quality.

The formation process of grain weight and rice yield is essentially dependent on grain growth and grain filling. The flowerings of spikelets and grain growth on the rice panicle have shown an obvious sequence in time [4]. It is commonly known that the weight of superior grains is heavier than that of inferior grains. Generally, the superior spikelets are located on the primary branches of the top rice panicle or top rachides, they flower earlier and have the advantages of fast grain filling rate, maximum assimilate enrichment, and high grain weight [5,6]. In contrast, the inferior spikelets are located on the secondary branches of the basal panicle or bottom rachides [5,6]. Inferior spikelets flower later, have poor grain plumpness, minimum weight, and poor cooking quality [7,8].

There are significant differences (about 25%) between superior and inferior grains in seed setting rate and grain plumpness. In addition, inferior grains can account for about one-third of rice panicle grains, and result in a decrease in both the 1000-grain weight and the seed setting rate, which further diminishes the high-yield potential of rice [6]. This is mainly caused by the asynchronous grain filling of superior and inferior grains in different positions [9–11]. Ishimaru et al. [10] found via morphological analysis that inferior grains located on the secondary branches of the bottom panicle experienced a lag time to begin filling, whereas superior grains have the highest grain filling rate and heaviest dry-matter accumulation in the rice panicle over the same period. This growth advantage of rice often leads to a higher seed setting percentage and a higher grain weight in the grains from the top of the rice panicle than the basal grains on the panicle [12–15]. A phenomenon similar to grain-grouping at different positions of the panicle during the grain filling stage is also common in other cereal crops; for example, inferior grains on the upper ear flower later and accumulate dry matter slower than superior grains on the middle and basal ear in wheat and maize [16–20]. They are characterized by both slower and later grain filling in inferior grains compared with superior grains, which ultimately results in low grain weight.

However, previous studies have used different definitions of superior and inferior grains. Nagato [21] first reported that superior grains are located in the middle and upper parts of a panicle which start flowering earlier and accumulate assimilates quickly, whereas inferior grains are located in the lower part of a panicle, flowering late and accumulation rate was slower. Wang et al. [22] then proposed that superior grains are defined as those located on the three topmost primary branches, but those on the secondary branches are excluded, and inferior grains are located on the secondary branches of the three basal primary branches. Peng et al. [23] confirmed that superior grains are often localized in the middle of the primary branches, whereas inferior grains are mostly located on the last two secondary branches of the lower half of the panicle, according to the order of the grain weight. Nevertheless, Fu et al. [15] and Jiang et al. [24] suggested that superior spikelets flower on the first two days after flowering, and are localized at the primary branches of the top panicle, and inferior spikelets flower on the last two days before the end of flowering, and are located on the secondary branches of the basal panicle. Despite many efforts made by researchers, there is still no clear standard for the classification of superior and inferior grains on the whole panicle. It has been concluded from previous studies that superior and inferior grains are determined by the flowering time, grain weight, and the position of the grains on the panicle; that is, superior grains, which are those located on the primary branch at the upper panicle, have an earlier flowering time and heavier weight [22,25–28], whereas inferior grains are usually formed on secondary branches at the basal panicle, and are characterized by later flowering and lower grain weight [4,22]. The differences between superior and inferior grains in grain quality and weight are primarily caused by varying grain fillings at different positions [28]. A lot of research has mainly focused on characteristics of grain filling, physiological and biochemical mechanisms [4–6] for limited parts of the panicle, such as superior and inferior grains, instead of the whole rice panicle. Furthermore, they classified superior grains and inferior grains according to their own experience and subjective judgment. However, (i) where is the actual position of superior grains and inferior grains on the rice panicle for different rice cultivars? (ii) On what basis was it grouped? (iii) Is it reasonable to divide the whole rice panicle into two groups of superior and inferior grains? Therefore, it is of great significance to sort out these ambiguities by evaluating the characteristics of all

the grains at different positions on the whole panicle and to establish the classification for superior and inferior grains.

Moreover, the increasing trend of dry-matter accumulation of cereal crops from flowering to maturity is generally an “S” curve and is usually fitted by the logistic equation, Gompertz equation, Mitscherlich equation, polynomial regression equation, and Richards equation for the growth process of plants [29,30]. However, the Richards model has more equation parameters than other growth models and includes three famous models (logistic, Gompertz, Mitscherlich) for plant growth according to the value of N in Richards equation [30]. It means that this model has a wider and more adaptive application for truly simulating the growth process of various plants with more equation parameters [30]. Furthermore, the parameters of the Richards model provide a reasonable and practical meaning to explain and predict plant growth [30]. In a study on the grain filling characteristics of superior and inferior grains, Zhu et al. [31] found that the Richards model well fitted the increasing weight of superior and inferior grains, and the initial growth power and maximum grain growth rate in superior grains were higher than in inferior grains. Likewise, Zhang et al. [32] studied that the grain-filling process of inferior grains was well fitted by the Richards equation under different treatments of controlling water application during grain filling, and dry-matter accumulation of inferior grain in the moderate soil drying treatment markedly increased more than in other treatments. Many previous studies of superior and inferior grains were limited to two extreme parts of a panicle, and few considered the actual grain filling of the whole panicle. There is limited information available on the relationship and characteristics of grain growth for all primary branches at different positions on the whole rice panicle, and a lack of methods to classify grains on the panicle by objective judgment. The key purpose of this study was to determine the grain-filling characteristics of all primary branches at different positions and to establish the method to classify grains according to the dry-matter accumulation of all primary-branches at different positions.

2. Materials and Methods

2.1. Experimental Site and Cultivar Details

The field experiment was conducted at the experimental farm of Guangxi University (22°51' N, 108°17' E, 78 m a.s.l.), Guangxi Province, China in the late season of 2014 and in the early season of 2015. The soil of the experimental field was a Ultisol (U.S. taxonomy) with a pH of 6.36, organic matter (35.7 g kg^{-1}), NaOH hydrolysable N ($129.52 \text{ mg kg}^{-1}$), Olsen P (25.61 mg kg^{-1}), and NH_4OAc extractable K (95.5 mg kg^{-1}). Monthly average air temperatures and rainfall from March to November over two years are presented in detail in Figure 1. Based on the monthly meteorological data, the rainfall was 793.00 mm in the late season and 772.10 mm in the early season. The average temperature was 26.10 °C in the late season and 25.81 °C in the early season.

The test crops were indica cultivars Nongle 1 and Guifeng 2, which have extensive adaptability, a suitable growth period, and are widely grown in many areas of Guangxi province. They are also stable in inheritance and could be reserved for subsequent experiments.

Figure 1. Summary of the average air temperature and rainfall in 2014 and 2015 growing seasons.

2.2. Experimental Design and Crop Management

The experiment was conducted in a completely randomized block design with three replications for each cultivar; there were six experimental plots for the two cultivars. Each experimental plot was 8 m long and 7 m wide, with a total plot size of 56 m². Pre-germinated seeds were sown in a seedbed on 19 July for the late season, and on 13 March for the early season. After 20 days, seedlings were transplanted at a hill spacing of 0.3 m × 0.1 m with four seedlings per hill. Urea was used for N fertilizer at the rate of 150 kg N ha⁻¹. It was applied in a split with 50% at basal (1 day before transplanting), 30% at early tillering (6 days after transplanting), and 20% at panicle initiation. Potassium chloride was used as the K fertilizer at a rate of 120 kg K₂O ha⁻¹. It was applied in a split with 50% at basal and 50% at early-tillering. For phosphorus fertilizer, superphosphate was applied as a basal with a rate of 55 kg P₂O₅ ha⁻¹. The experimental field was kept flooded from the day of seedlings being transplanted until 6 days before maturity. Insects and diseases were intensively controlled to avoid yield loss.

2.3. Sampling and Measurements

Five hundred panicles that headed on the same day were chosen and tagged in each plot. Most of the rice panicles for Nongle 1 and Guifeng 2 included 13 and 14 primary branches in the field, respectively. The panicles of Nongle 1 including 13 primary branches and the panicles of Guifeng 2 including 14 primary branches were regarded as target samples. One hundred target panicles selected from tagged panicles in each plot were sampled at 7-day intervals from the anthesis (0 days) to the maturity stage (28 days after heading). The grains from the primary branches at the top to the base of the panicle were labeled from B1 to B13 for Nongle 1, and from B1 to B14 for Guifeng 2 (Figure 2). Therefore, B1 represented the first primary-branch grains from the topmost panicle, and B13 or B14 represented the last primary-branch grains from the lowest panicle. Grains from the same primary branch were combined as one sample. These were dried in an oven at 105 °C for 30 min, and then at 70 °C to a constant weight to measure the grain weight.

Figure 2. Thirteen primary branch grains of Nongle 1 panicle and fourteen primary branch grains of Guifeng 2 panicle. B1 to B14 represent the grains from the top 1st primary branch to the basal 14th primary branch on rice panicles for Nongle 1 and Guifeng 2.

2.4. Mathematical Simulation of Grain Growth

The Richards equation [29,31] was used to fit the grain filling process, and the basic parameters of the equation in the tested rice cultivars were calculated as follows:

$$Y = \frac{A}{(1 + Be^{-kt})^{1/N}} \quad (1)$$

where Y is the weight (g per 100 grains), A is the final grain weight (g per 100 grains), t is the number of days after anthesis, and B , k , and N are the parameters determined by the regression equation. k is the parameter of growth speed, B and N do not have obvious biological meaning, but they can decide flexibility and position of growth curve; R^2 is the coefficient regarding the fitfulness of the equation.

The grain growth rate (V) was calculated from the derivation of Equation (1):

$$V = (kY/N) \left[1 - (Y/A)^N \right] \quad (2)$$

Based on the above equations, the secondary parameters were calculated by the methods used by Zhu et al. [31] to describe the characteristics of grain growth as follows:

$$T_{max} = \frac{(\ln B - \ln N)}{k} \quad (3)$$

$$Y_{max} = \frac{A}{(N + 1)^{1/N}} \quad (4)$$

$$V_{max} = (kY_{max}/N) \left[1 - \left(\frac{Y_{max}}{A} \right)^N \right] \quad (5)$$

$$R_0 = \frac{k}{N} \quad (6)$$

$$D = \frac{2(N + 2)}{k} \quad (7)$$

$$I = \frac{Y_{max}}{A} \quad (8)$$

where T_{max} is the time of the maximum grain growth rate (days after anthesis), Y_{max} is the grain weight of the maximum grain growth rate (g per 100 grains), V_{max} is the maximum grain growth rate

(g per 100 grains d^{-1}), R_0 is the initial growth power that refers to the growth potential of the fertilized ovary, D is the active grain filling duration (days after anthesis), and I is the ratio of Y_{max} to A .

There are two inflection points t_1 and t_2 (days after anthesis) that are calculated with the grain growth rate Equation (2) to divide the grain filling process into beginning ($0-t_1$), middle (t_1-t_2), and end (t_2-t_3) stages. Additionally, t_3 (days after anthesis) is the time point at which the grain weight is close to 99% of A , and the grain filling is assumed to end. The formulas are as follows:

$$t_1 = -\frac{\ln\left(\frac{N^2+3N+N\sqrt{N^2+6N+5}}{2B}\right)}{K} \quad (9)$$

$$t_2 = -\frac{\ln\left(\frac{N^2+3N-N\sqrt{N^2+6N+5}}{2B}\right)}{K} \quad (10)$$

$$t_3 = \frac{-\ln\left[\left(\frac{100}{99}\right)^{\frac{N}{B}}-1\right]}{K} \quad (11)$$

2.5. Statistical Analysis

The software SPSS 19.0 was used to fit the grain filling process equation, the correlation analysis, and the cluster [33] analysis of the data. Figures were plotted using Sigma Pot 12.5 software. Means of cultivars were compared based on the least significant difference test at the 0.05 probability level.

3. Results

3.1. Dynamics of Dry-Matter Accumulation for Primary-Branch Grains

The dynamics of dry matter accumulation of the grains of all the primary branches were plotted (Figure 3A–D) using the Richards growth equation, and the grain growth rates of all the primary branches were calculated according to the dry-matter accumulation and grain growth time (Figure 3E–H). The dry matter of grains of all the primary branches in the tested cultivars accumulated slowly in the early stage, quickly in the middle stage, and again slowly in the latter stage. The dry-matter accumulations of all the primary-branch grains gradually decreased from the top to the base of the panicle in the same period. The difference in dry-matter accumulation between primary-branch grains at different positions was small at the early stage of grain growth, the greatest at the middle stage and then smallest at the latter stage (Figure 3A–D). What is more, the maximum grain growth rates of B1–B6 in 2014 and B1–B12 in 2015 for Nongle 1, and those of B1–B7 in 2014 and B1–B9 in 2015 for Guifeng 2, were achieved between 7 and 14 days after anthesis, while the maximum grain growth rates of B7–B13 in 2014 and B13 in 2015 for Nongle 1, and those of B8–B14 in 2014 and B10–B14 in 2015 for Guifeng 2, were later achieved between 14 and 21 days after anthesis (Figure 3E–H). It was found that the main differences in the primary-branch grains at different positions were the starting time of grain growth, the maximum grain growth rate, and the time taken to reach the maximum grain growth rate. The grain growth rates of the primary branches at different positions decreased gradually, and the number of days taken to reach the maximum grain growth rate increased gradually from the top to the base of the panicle.

Figure 3. Dynamics of dry-matter accumulation and grain growth rate of primary-branch grains at different positions for two rice cultivars in 2014 and 2015 at different growth stages. (A,B) dry-matter accumulation for Nongle 1, (C,D) dry-matter accumulation for Guifeng 2, (E,F) grain growth rate for Nongle 1, (G,H) grain growth rate for Guifeng 2. Data are the mean across three replications. 0, 7, 14, 21 and 28 indicate 0 days, 7 days, 14 days, 21 days and 28 days after anthesis. B1 to B14 represent the grains from the top 1st primary branch to the basal 14th primary branch on rice panicles for Nongle 1 and Guifeng 2.

3.2. Mathematical Simulation of Grain Growth

The Richards equation was used to fit the growth process of all the primary-branch grains and the whole-panicle grains of Nongle 1 and Guifeng 2. The determinant coefficients R^2 were both greater than 0.990 (Table 1), which indicates that the growth processes of grains of all the primary branches in both Nongle 1 and Guifeng 2 were very similar, and they were suitable for Richards equation fitting. The values of the final grain weight (A) at the maturity of the primary branch grains decreased gradually from the top to the base of the panicles. It was concluded that the maximum values of A at the upper primary branches of Nongle 1 and Guifeng 2 were 32% and 18% higher than the minimum values of A at the lower primary branches, respectively (Table 1).

Table 1. Parameters of Richards equation for fitting grain growth of primary branches and the whole panicle of Nongle 1 and Guifeng 2 in 2014 and 2015. B1 to B14 represent the 1st primary-branch grains to the 14th primary-branch grains from the top to the base of the panicle for Nongle 1 and Guifeng 2. Panicle indicates the whole panicle of Nongle 1 and Guifeng 2. *A* is the final grain weight (g per 100 grains). *B*, *k* and *N* are the parameters set by the regression equation. *k* is the parameter of growth speed, *B* and *N* do not have the obvious biological meaning but they can decide flexibility and position of the growth curve. R^2 is the determinant coefficient.

Cultivars	Branches	2014 (Late Rice)					Branches	2015 (Early Rice)				
		<i>A</i>	<i>B</i>	<i>k</i>	<i>N</i>	R^2		<i>A</i>	<i>B</i>	<i>k</i>	<i>N</i>	R^2
Nongle 1	B1	2.011	45.84	0.289	1.750	0.996	B1	1.935	57.56	0.402	2.133	0.995
	B2	2.040	52.75	0.269	1.856	0.994	B2	1.951	24.91	0.322	1.661	0.999
	B3	1.895	56.62	0.259	1.948	0.997	B3	1.873	49.37	0.325	2.043	0.999
	B4	1.865	60.82	0.255	2.011	0.997	B4	1.845	12.17	0.229	1.311	0.997
	B5	1.835	131.13	0.279	2.411	0.998	B5	1.762	20.67	0.228	1.580	0.998
	B6	1.742	311.13	0.299	2.791	0.998	B6	1.708	27.32	0.231	1.651	0.998
	B7	1.727	597.14	0.321	3.211	0.995	B7	1.681	305.80	0.322	3.100	0.995
	B8	1.739	4521.61	0.392	4.334	0.999	B8	1.659	393.61	0.325	3.180	0.998
	B9	1.634	11139.48	0.407	4.867	0.997	B9	1.620	665.46	0.331	3.411	0.996
	B10	1.611	140115.85	0.498	6.071	0.998	B10	1.519	1239.35	0.350	3.750	0.993
	B11	1.645	756627.73	0.526	6.812	0.996	B11	1.544	1614.66	0.352	3.790	0.989
	B12	1.587	1186041.88	0.537	7.410	0.992	B12	1.611	2507.23	0.353	4.064	0.994
	B13	1.417	8710836.16	0.594	8.519	0.993	B13	1.602	13194.86	0.401	4.990	0.990
	panicle	1.855	69.38	0.22	2.122	0.999	panicle	1.765	14.45	0.199	1.394	0.998
Guifeng 2	B1	1.572	21.11	0.283	1.622	0.999	B1	1.651	23.31	0.311	1.687	0.998
	B2	1.531	30.47	0.283	1.792	0.999	B2	1.621	20.56	0.281	1.551	0.975
	B3	1.560	27.06	0.265	1.674	0.998	B3	1.620	13.09	0.261	1.279	0.993
	B4	1.529	33.10	0.266	1.787	0.998	B4	1.629	14.82	0.252	1.361	0.995
	B5	1.560	45.09	0.260	1.895	0.997	B5	1.680	20.31	0.250	1.457	0.997
	B6	1.530	53.49	0.256	1.992	0.997	B6	1.654	25.67	0.244	1.568	0.997
	B7	1.497	89.62	0.261	2.100	0.996	B7	1.610	36.38	0.234	1.701	0.995
	B8	1.480	180.82	0.281	2.311	0.996	B8	1.601	75.35	0.252	2.011	0.997
	B9	1.465	268.17	0.289	2.544	0.996	B9	1.603	197.98	0.286	2.488	0.995
	B10	1.370	3598.77	0.377	3.844	0.996	B10	1.530	1516.54	0.345	3.477	0.995
	B11	1.301	23999.85	0.432	4.751	0.995	B11	1.484	4021.75	0.369	3.885	0.994
	B12	1.311	138239.93	0.485	5.751	0.993	B12	1.471	42367.89	0.451	5.102	0.994
	B13	1.306	354717.29	0.512	6.250	0.993	B13	1.474	118240.28	0.491	5.801	0.990
	B14	1.271	1603900.56	0.544	6.890	0.990	B14	1.479	328969.32	0.511	6.119	0.984
	panicle	1.527	25.84	0.207	1.629	0.998	panicle	1.659	12.31	0.189	1.252	0.998

3.3. Characteristics of Growth Parameters

The values of the initial growth power (R_0) and the maximum grain growth rate (V_{max}) of the primary-branch grains showed a decreased trend from the top to the base of the panicles of both cultivars during both years (Figure 4B,E). In our study, the values of V_{max} of the upper primary branch grains of Nongle 1 and Guifeng 2 were 86% and 44% higher than those of the basal primary branch grains, respectively. On the contrary, the values of the active grain filling duration (D), the time of the maximum grain growth rate (T_{max}), and the ratio of Y_{max} to A (I) of the primary-branch grains exhibited an obvious increasing trend from the top to the base of the panicles (Figure 4A,C,D). The maximum values of D of the grains at the lowest primary branches of Nongle 1 and Guifeng 2 were 1.5 times and 1.3 times higher, respectively than the minimum values of D of the grains at the uppermost primary branches. The T_{max} of the basal primary branch grains of Nongle 1 and Guifeng 2 were 2.2 times and 2.5 times later, respectively than those of the upper primary branch grains. However, the values of the grain weight of the maximum grain growth rate (Y_{max}) of the primary-branch grains gradually reduced from the base to the top of the panicle (Figure 4F).

Figure 4. Variation of grain growth parameters of primary-branch grains at different positions for Nongle 1 and Guifeng 2 in 2014 and 2015. (A) active grain-filling period, (B) maximal grain growth rate, (C) time of the maximum grain growth rate, (D) the ratio of Y_{max} to A , (E) initial growth power,

(F) grain weight of the maximum grain growth rate. B1 to B14 represent the 1st primary-branch grains to the 14th primary-branch grains from the top to the base of the panicle for Nongle 1 and Guifeng 2.

3.4. Division of Growth Stages

The beginning ($0-t_1$), middle (t_1-t_2), and end (t_2-t_3) of grain-filling stages represented gradual, fast, and slow accumulation of dry matter, respectively (Figure 5); $0-t_1$, t_1-t_2 , and t_2-t_3 of all the primary-branch grains of Nongle 1 were, respectively, 6.1 to 19.2 days, 8.2 to 12.3 days, and 3.6 to 11.9 days in 2014, and 3.6 to 14.5 days, 7.9 to 12.9 days, and 6.3 to 13.9 days in 2015 (Figure 5A,B). Those of Guifeng 2 were, respectively, 3.8 to 18.5 days, 8.4 to 12.2 days, and 4.2 to 11.8 days in 2014, and 3.6 to 17.0 days, 8.6 to 12.8 days, and 4.6 to 13.2 days in 2015 (Figure 5C,D).

Figure 5. The period for the beginning ($0-t_1$), middle (t_1-t_2) and end (t_2-t_3) of grain filling of primary-branch grains at different positions for Nongle 1 (A,B) and Guifeng 2 (C,D) in 2014 and 2015. B1 to B14 represent the 1st primary-branch grains to the 14th primary-branch grains from the top to the base of the panicle for Nongle 1 and Guifeng 2. $0-t_1$, t_1-t_2 and t_2-t_3 represent the period with gradual, fast, slow dry matter accumulation, respectively.

The days of $0-t_1$ had the largest changing range than the other two stages from the top primary branch to the basal primary branch on the panicle. The days of $0-t_1$ increased obviously from top to base on the panicle. The $0-t_1$ was the longest for the basal primary branch. The days of t_2-t_3 had a larger changing range than t_1-t_2 from the top primary branch to the basal primary branch on the panicle. However, the changing trends of t_1-t_2 and t_2-t_3 was the same from top to base on the panicle, and the days of t_1-t_2 and t_2-t_3 was the highest for the middle primary branches.

3.5. Correlation of Dry-Matter Accumulation between Primary-Branch Grains

There was a significant positive correlation of grain dry-matter accumulation between the primary branches, and the values of the correlation coefficients were related to the distance between the primary branches (Tables 2 and 3). The values of the correlation coefficients decreased with the increase of the

distance between primary branches. Therefore, the dry-matter accumulation of the first primary-branch grains was most closely related to that of the second primary-branch grains, but least closely related to that of the lowest primary-branch grains.

There was a significant positive correlation of the dry-matter accumulation between the whole panicle grains and primary-branch grains. The value of the correlation coefficient was largest in dry-matter accumulation between the whole-panicle grains and the middle primary-branch grains (B6 and B7) in 2014 and (B5 and B6) in 2015 for Nongle 1, and that was most closely related to the middle primary-branch grains (B6 and B7) in 2014 and in 2015 for Guifeng 2 (Tables 2 and 3).

[illegible]

[illegible]

3.6. Grouping of Primary Branches

Cluster analysis can partition grains of all the primary branches into several groups according to the similarities and differences of specific traits. All grains on the panicle regarding an individual primary branch as a unit underwent cluster analysis according to the grain-growth parameters of the primary branches by using the methods of class average and Euclidean distance. Grains of all primary branches on the panicle of Nongle 1 (Figure 6A,B) and Guifeng 2 (Figure 6C,D) were clustered into three groups. Details of the three groups were provided in Table 4.

Figure 6. Cluster analysis of all primary-branch grains at different positions for Nongle 1 (A,B) and Guifeng 2 (C,D) in 2014 and 2015. B1 to B14 represent the 1st primary-branch grains to the 14th primary-branch grains from the top to the base of the panicle for Nongle 1 and Guifeng 2.

The growth of the three groups of grains was simulated mathematically by the Richards equation, and the grain filling parameters of each group were obtained. There were significant differences in grain growth among the three groups. The values of A , V_{max} , and R_0 in Group I were the highest, followed by Group II, and the lowest values occurred in Group III. The values of A in Group I for Nongle 1 and Guifeng 2 were 10.4% and 2.2% greater than in Group II, respectively; those in Group II were 12.8% and 10.5% greater than in Group III, respectively. The differences of V_{max} and R_0 between groups were particularly significant. For Nongle 1 and Guifeng 2, the values of V_{max} of Group I were 31.4% and 17.8% higher than those of Group II, respectively; the values of V_{max} of Group II were 6.4% and 3.4% higher than those of Group III, respectively. The values of R_0 in Group I for Nongle 1 and Guifeng 2 were 42.6% and 48.6% greater than in Group II, respectively; those in Group II were 62.9% and 52.8% greater than in Group III, respectively (Table 4). However, the values of D , T_{max} , and $0-t_1$

exhibited the opposite trend. Those parameters in Group III were the highest, followed by Group II, and the lowest values occurred in Group I. The differences between T_{max} and $0-t_1$ among groups were particularly significant. For Nongle 1 and Guifeng 2, respectively, the days of T_{max} in Group II were 2.9 days and 4.8 days longer than in Group I; those in Group III were 6.7 days and 7.1 days longer than in Group II, respectively. The days of $0-t_1$ in Group II for Nongle 1 and Guifeng 2 were 2.1 days and 4.3 days longer than in Group I, respectively; the days of $0-t_1$ of Group III were 8.7 days and 9.2 days longer than those of Group II, respectively. The days of t_1-t_2 between Group I and Group II were not significantly different (except Nongle in 2015), whereas those in Group III for Nongle 1 and Guifeng 2 were 2.4 days and 3.3 days shorter in Group I, respectively. The days of t_2-t_3 between Group I and Group II were not significantly different, whereas those in Group III for Nongle 1 and Guifeng 2 were 8.0 days and 8.9 days shorter than in Group I, respectively (Table 4).

Table 4. Comparison of grain growth parameters at different primary-branch groups of Nongle 1 and Guifeng 2 in 2014 and 2015. Means followed by the same letters within the column are not significantly different at $p < 0.05$.

Cultivars	Season	Branch Groups	Branches	A	V_{max}	D	T_{max}	R_0	$0-t_1$	t_1-t_2	t_2-t_3
Nongle 1	late rice (2014)	I	B1-B2	2.081a	0.101a	31.4b	12.0b	0.143a	5.6b	12.8a	13.5a
		II	B3-B7	1.857b	0.082b	34.8a	14.5b	0.112ab	7.7b	13.5a	12.8a
		III	B8-B13	1.619c	0.079b	34.1a	21.3a	0.076b	16.9a	8.7b	4.3b
	early rice (2015)	I	B1-B3	1.941a	0.120a	24.3b	7.7c	0.238a	2.4b	10.6b	13.0a
		II	B4-B6	1.785b	0.086b	31.6a	11.0b	0.151b	4.4b	13.2a	14.5a
		III	B7-B13	1.610c	0.079b	33.3a	17.6a	0.085c	12.6a	10.0b	6.3b
Guifeng 2	late rice (2014)	I	B1-B4	1.563a	0.085a	28.0b	10.0c	0.165a	4.2c	11.5a	12.4a
		II	B5-B9	1.524a	0.072b	32.7a	14.3b	0.118b	8.0b	12.5a	11.7a
		III	B10-B14	1.339b	0.068b	33.2a	22.2a	0.075c	18.2a	8.0b	3.6b
	early rice (2015)	I	B1-B6	1.658a	0.091a	27.6b	9.4c	0.190a	3.5c	11.8a	13.7a
		II	B7-B9	1.629a	0.077a	32.5a	14.6b	0.121b	8.2b	12.7a	12.0a
		III	B10-B14	1.519b	0.077a	32.8a	20.9a	0.081c	16.4a	8.8b	4.7b

4. Discussion

Previous studies showed that grain filling at different positions of the rice panicle has shown obvious sequence in time [4]. The spikelets on the upper panicle are early flowering, filling fast and producing heavier grains, whereas the spikelets on the lower panicle are late flowering, filling slowly and poorly [34–36]. However, previous studies on grain filling at different positions of the rice panicle were limited to examining superior grains on the topmost panicle and inferior grains on the bottom panicle, not all grains on the whole panicle were studied. Additionally, they took the superior and inferior grains as samples by their own judgment of experience and subjectivity before the study. This resulted in a lack of information on the relationship of grain filling between all grains at different positions of the panicle and grouping for all the grains on the whole panicle so far. Thus, the objective of the present research was to determine the grain-filling characteristics of all primary branches at different positions and to establish the method to classify grains according to the dry-matter accumulation of all primary-branches at different positions.

In this study, the grain-growth characteristics for all branches on the panicle of two rice cultivars were compared via field testing in 2014 and 2015. Our results showed that grains of all primary branches of both cultivars exhibited a high similarity in growth. The slow–fast–slow trend was recorded for dry-matter accumulation during the whole grain-filling stage because all primary branches on the panicle showed an increasing and decreasing trend for the grain-filling rate (Figure 3). Similar work reported that the dry-matter accumulation of the whole panicle, superior grains and inferior grains all exhibit a slow–fast–slow trend at the grain filling stage, and their growth processes can be simulated by the Richards equation [36,37]. In the present study, our results indicated that it was most closely related

between the two adjacent primary branches in dry-matter accumulation, whereas it was least closely related between two primary branches far apart (Tables 2 and 3). In addition, dry-matter accumulation of the whole-panicle grains was most closely related to that of the middle primary-branch grains.

However, the grain growth of all primary branches exhibited significant differences due to the different positions and flowering times. Firstly, the final grain weight (A) of the grains decreased in turn from the top to the base of the primary branches, and there was a significant difference in A between the primary branches. A similar finding was made by Liang et al. [38], who reported that a great difference in the final grain weight at maturity was observed between superior and inferior grains. A possible explanation for this is a higher number of cells in superior spikelets than inferior spikelets because the cell-division activity is vital in attracting assimilation to sink organs in the early stages of development [39,40]. Secondly, our results showed that the values of V_{max} and R_0 on the upper primary-branch grains were significantly greater than those on the basal primary-branch grains (Figure 4B,E). This was because the upper primary branch grains could compete for more nutrients at the beginning stage through higher V_{max} and R_0 , whereas the basal primary-branch grains might experience a long lag period before starting to accumulate dry matter at this stage [36]. Our present study indicated that T_{max} of the basal primary-branch grains was significantly later than those of the upper primary-branch grains (Figure 4C). Thirdly, D increased significantly from the top to the base of the primary branches (Figure 4A). Furthermore, the period of grain filling can be divided into three stages: the beginning stage, middle stage, and the end stage, as analyzed by the Richards equation [24]. The days of the three stages of the primary branches varied significantly depending on the position of the primary-branch (Figure 5). The most notable feature was that the days of the beginning stage at the lowest primary branch was four times longer than that at the uppermost primary branch. A possible explanation for this is that the lowest spikelets might have a long lag period at the early grain-filling stage [36] caused by low V_{max} and R_0 . Then, the long lag period may further extend D and $0-t_1$ in the lowest spikelets. Last, the grains of the whole panicle [37,41], the primary-branch grains [42], and the single grains [20,43], all exhibited a slow-fast-slow trend for growth rate. This means that there must be a “peak” period caused by V_{max} and T_{max} in rice grain growth for all grains.

The possible reasons for the significant difference in grain growth between all the primary branches in rice are related to the flowering habits and the location of spikelets. On the one hand, the spikelets of the top branch usually blossom one to two days after heading, whereas those of the basal branch blossom later after heading [22,27]. Huang et al. [6] reported that the spikelets of the top branch blossom on the day of heading, where those of the basal branch bloomed seven days after heading. The upper spikelets of rice spikes exhibited high physiological activity and a strong ability to accumulate substances transported from the source [44–46], whereas poor grain filling of the inferior spikelets from the base panicle was caused by the low sink strength [38]. The low activity of starch synthesis-related enzymes in endosperm could obviously reduce grain weight by the decline of starch synthesis in inferior spikelets [38]. In addition, Panigrahi et al. [47] stated that poor quality and low grain weight in inferior spikelets were caused by high ethylene production in inferior spikelets which suppresses expression of genes encoding endosperm starch synthesizing enzymes. However, the poor physiological and biochemical activity in inferior spikelets can be improved by alternate wetting and moderate soil drying (AMD) which could enhance grain weight, grain filling rate, protein expression, and transcript level of the gene encoding these proteins in the inferior spikelets [48]. Furthermore, AMD could increase the remobilization of non-structural carbohydrates by 19.6–36.7% [49] and remobilize the nitrogen assimilated from source to the grain through coordinately expressed proteins involved in photosynthesis, systematic senescence, and other metabolisms [50]. Similarly, the moderate increase of nitrogen fertilizer application in the later growth stage [24] and spraying exogenous ABA on grains at the active grain filling stage [51–53] can effectively improve physiological activity in inferior spikelets. These findings indicate that the flowering time and physiological activity of the spikelets are the most important factors that affect the grain growth at varied positions of the panicle. On the other hand, the varied locations of the grains also affect grain growth. The vascular bundles of the primary branch at the upper panicle of rice were rapidly matured

and their ducts and sieve tubes were very developed [6]. However, the ducts area and phloem area in the vascular bundles of the basal secondary branch of the lower panicle are smaller than those of the primary branch at the upper panicle [6]. Additionally, the differentiation of catheters, sieve tubes, and companion cells of the basal secondary branch of the lower panicle is insufficient and results in the poor transportation of materials [6]. This is one of the most important reasons for the differences in grain growth at different positions. However, the growth of rice grains also has an obvious apical advantage [12,54], that is, the growth of superior grains from the top panicle obviously inhibits that of inferior grains from the lower panicle. After the superior spikelets were thinned or removed, the seed setting rate, grain weight and sucrose content, hormone levels, activities of key enzymes, and the expression levels of genes involved in sucrose-to-starch metabolism in inferior spikelets were all enhanced [55,56]. Moreover, the growth of inferior grains can be significantly promoted by eliminating or reducing the inhibition from the superior grains of the top panicle [55,56]. This is another important reason for the differences in grain growth between the upper and lower branches.

All grains that regard an individual primary branch as a unit on the panicle of the two rice cultivars were clustered into three different groups according to the grain growth parameters of the primary branches. In the current work, our results showed that grain growth was significantly different between groups. Because the values of V_{max} and R_0 in Group I for both cultivars were highest, whereas those in Group III were lowest (Table 4). The days of T_{max} and $0-t_1$ of Group III for both cultivars were longest, whereas those in Group I were shortest (Table 4). This indicated that the grains in Group I had the strongest initial growth power, and first arrived at the fastest grain-filling rate during the shortest early grain-filling stage. On the contrary, the grains in Group III were the last to start their growth and had the slowest grain-filling rate during the longest early grain-filling stage, which might be related to the lowest initial growth power.

The growth parameters (A , V_{max} , T_{max} , D , $0-t_1$, t_1-t_2 , t_2-t_3 , and grain weight) of the primary-branch grains changed gradually from the top to the base of the panicle. However, it was found that the changes between adjacent primary branches for these parameters were various—that is, the increase or reduction was different and non-uniform between all adjacent primary branches for the same parameter. Figure 7 shows the distribution of the primary branches on the spike axis. It was found that the distances between adjacent primary branches were also non-uniform. Furthermore, the spikelet numbers of primary branches on the rice panicle were different (Table 5). Therefore, further study is required to determine whether the non-uniform changes of parameters between adjacent primary branches are related to the non-uniform distribution of the primary branches on the spike axis and different spikelet numbers of primary branches.

Figure 7. The distribution of all primary-branch grains at different positions for Nongle 1 (A) and Guifeng 2 (B) on the panicle axis. B1 to B14 represent the 1st primary-branch grains to the 14th primary-branch grains from the top to the base of the panicle for Nongle 1 and Guifeng 2.

Table 5. The numbers of spikelets of each primary-branch at different positions for Nongle 1 and Guifeng 2 in 2014 and 2015. B1 to B14 represent the 1st primary-branch grains to the 14th primary-branch grains from the top to the base of the panicle for Nongle 1 and Guifeng 2.

	Nongle 1		Guifeng 2	
	2014	2015	2014	2015
B1	16.6	16.1	15.6	15.6
B2	18.1	18.5	16.96	17.2
B3	18.7	20.7	16.50	17.3
B4	18.7	23.2	16.5	16.7
B5	18.4	23.3	16.5	17.2
B6	17.1	22.3	16.30	16.5
B7	17.9	23.3	14.6	14.7
B8	17.5	22.5	13.50	14.1
B9	15.6	20.9	13.50	13.9
B10	16.3	21.8	11.7	12.0
B11	14.3	19.0	10.4	10.7
B12	14.6	18.7	9.9	10.2
B13	15.2	18.5	9.6	10.3
B14			9.4	9.2

5. Conclusions

Our results showed a high correlation in dry-matter accumulation between adjacent primary branches, and a lower correlation between distanced primary branches. Moreover, there was a significant difference between the primary branches situated far apart in final grain weight, active grain filling duration, maximum grain growth rate, and the time of maximum grain growth rate. For both cultivars, the rice grains at different positions on a panicle could be clustered into three different groups based on the changes in these parameters of grain growth. Group I (superior primary-branches) is considered to be the highest in dry-matter accumulation, and the highest in maximum grain growth rate, followed by Group II (medium primary-branches), which has less growing ability than Group I. Group III (inferior primary-branches) was found to be the poorest for grain filling in the three groups. Therefore, the increase of grain yield can be achieved by improving the growth of Group III. This study could provide a new method of classification for all primary branches on a panicle based on growth parameters of every primary branch, and lays an important foundation for the directional cultivation and regulation of grain filling at middle and basal positions for large panicle cultivars.

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