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Preliminary Exploration of Physiology and Genetic Basis Underlying High Yield in Indica–Japonica Hybrid Rice

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Abstract: The utilization of heterosis is of great significance in improving rice yield. To explore the physiological and genetic basis for high yield in indica–japonica hybrid rice, Zheyou18 (z18) and Yongyou12 (y12) were used as materials and compared with indica hybrid rice, Zheyou12 (z12); japonica hybrid rice, Liangyoupeijiu (LYPJ); and the conventional lines zhe04B (04B) and zhehui818 (h818) under seedling growth vigor, functional leaf morphology, chlorophyll content, yield component, panicle trait, and InDel heterosis analysis. Z18 and y12 showed the largest increase in plant height 6 d and 9 d after germination; the root dry weight of z18 was 31.2% and 42.0% higher than its parents on the 12th d. The length of functional leaves ranked in the middle, while the width was the largest, resulting in z18 and y12 having the largest leaf area. Yield components showed that z18 and y12 had the highest number of primary branches, spikelets, and grains, and grain yield, which was 58.1 g in z18, increased by 29.8% and 8.7%, respectively, in comparison with h818 and LYPJ. The InDel genetic distance was significantly positively correlated with single spike weight, with r reaching 0.771, making it the only consistent and most correlated among the seven traits. Therefore, we speculated that as the InDel genetic distance expands, heterosis mainly manifests in the increase in single spike weight. This study comprehensively explored the physiological mechanism of yield improvement in indica–japonica-hybrid rice and used InDel genetic distances to study the genetic basis of heterosis, which will be helpful for future rice yield improvement.

Keywords: breeding; indica–japonica hybrid; marker; PCA analysis; rice; yield

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1. Introduction

Rice (*Oryza. Sativa* L.) is the best-known cultivated crop, providing staple food for more than half of the world's population. The domestication of cultivated rice began about 10,000 years ago. During domestication, the cultivated rice differentiated into two varietal groups, Xian and Geng [1]. The two subspecies of cultivated rice are two different ecological types; indica rice is suitable to be grown in tropical and subtropical areas at low latitudes and low altitudes, while japonica rice is suitable to be grown in temperate areas at high latitudes or high altitudes [2].

Rice yield is mainly determined by several important quantitative traits, including the effective panicle number, the grain number per panicle, and grain weight. The grain number is influenced by leaves, which significantly correlate with rice yield. Leaves are the main photosynthetic organs with high developmental plasticity [3]. As an important part of plant architecture, the flag leaf of rice is the main material basis of rice panicle size, which has an impact on the filling speed and the degree of grain fullness [4]. More than 50% of the carbohydrates in rice grains come from the photosynthesis of the rice flag leaves, and the photosynthetic products synthesized by the first two leaves and the flag leaves account for more than 80% of the grain yield. For the agronomic traits of hybrid rice, flag leaf-related heterosis is an indispensable component of productivity and a major element for plant breeding [5]. The main reasons for the higher yield of super rice LYPJ than the control variety were its longer leaf area before spike-flushing, higher number of grains per spike, and longer root length. It has been shown that the canopy of rice populations with ultra-high yields is characterized by a larger leaf area in the upper three leaves with a smaller lance and a higher leaf curl index, and that this plant structure favors the light exposure of the lower and middle leaves in the canopy and increases the photosynthetic production capacity of the lower leaves in the canopy, which improves the thousand-matter production of the population as well as its yield.

Heterosis plays a key role in the enhancement of crop yield and, thus, is regarded as a cause of the far superior phenotypic performance of hybrids in comparison with their parents [6]. The much-enhanced yield of heterosis has been utilized in numerous crops and vegetables [7,8]. The magnitude of heterosis depends on the genetic diversity between the two parents of the hybrids. The greater the genetic difference between the parents, the higher the heterosis [8,9]. Yield heterosis in rice can be arranged in descending order for subspecific combinations as indica–temperate japonica > indica–tropical japonica > temperate japonica–tropical japonica > indica–indica > japonica--japonica [10]. Hybrids of indica and japonica varieties have a yield advantage of approximately 25% [11]. Numerous inter-subspecific lines have been developed for hybrid varieties with stronger heterosis and normal seed sets. In recent years, the breeding of 5G inter-subspecific indica–japonica hybrid rice has progressed rapidly, and some cultivars with high yield have been successfully released [2].

China has achieved great success in utilizing the heterosis of indica–japonica, releasing many super hybrid combinations such as Zheyou 18, Yongyou 6, Yongyou 9, Yongyou 12, Chunyou 658, Chunyou 84, and Chunyou 927 by introgressing japonica composition and the wide-compatibility gene (S5n) into the indica restorer line background. These indica–japonica hybrid rice varieties, with high yield potential, are widely cultivated in southern China. In spite of the extensive research, the genetic basis of the heterosis of indica–japonica is not yet completely understood. In Zhejiang Province, China, the mean grain yields of Zheyou 18, Yongyou 6, Yongyou 9, Yongyou 12, Chunyou658, Chunyou84, and Chunyou 927 were all over 12.0 t ha^{-1}, which was significantly higher than conventional rice [12].

Considering that heterosis is determined by the divergence of the parents, we hypothesized the existence of a critical point at which the conflict between the greater degree of heterosis and the increased reproductive isolation is balanced. At this point, the heterosis of inter-subspecific hybridization can be acquired. In search of this point, six rice varieties, one indica, one japonica, one indica–indica, one japonica–japonica, and two indica–japonica with different proportions of indica–japonica content, were selected. The physiological mechanisms were compared under the seedling growth vigor, functional leaf morphology, chlorophyll content, yield component, panicle traits. At the same time, the genetic divergence index (GDI) of the indica–japonica parents and grain number per plant and yield-related trait data were collected. Regression analyses were performed to depict the relationship between the yield and GDI of the parents. After integrating the regression results of the raw phenotypic data, the PCA analysis was carried out. This result will help to facilitate inter-subspecific hybrid rice breeding programs and contribute to yield improvement.

2. Materials and Methods

2.1. Plant Materials

The indica–japonica hybrid rice varieties Zheyou18 and Yongyou12 were used as materials for the study of high-yield physiological mechanisms of indica–japonica hybrid rice, and one japonica–japonica hybrid rice variety (Zheyou12), one indica–indica hybrid rice variety (Liangyoupeijiu, LYPJ), and the parents of Zheyou18 (female parent Zhe04B and restoring line Zhehui818) were selected as representative comparative varieties. Twelve super-high-yield Zheyou series combinations and their parents were selected for the genetic study of heterosis, which were Zhe04B, Zhehui818, Zheyou929, Zheyou1017, Zheyou18, 9311, Nanjing11, IR36, Shanyou63, Nipponbare, Youmangzaojing, and Balilla. All plants used in the study were naturally grown in paddy fields in either Zhejiang or Hainan province, China, from 2019 to 2023.

2.2. Determination of Growth in Seedling Stage, Leaf Morphology, and Chlorophyll Contents

The plant height, aboveground dry weight, root length, and root dry weight were measured 6 d, 9 d, and 12 d after germination to determine the growth potential of the 6 varieties in the seedling stage. The plant height was the distance of the highest point to the base of the stem, and the root lengths were measured in the natural stage. Seedlings were sampled and divided into aboveground parts and roots, and the dry weights were measured.

Function leaves including the flag leaf, the top second leaf, and the top third leaf were selected for the determination of leaf morphology and leaf area. Three representative plants were selected at maturity, and their aboveground parts were cut. The length and width of the flag leaf, the top second leaf, and the top third leaf at the widest point were quickly measured from the main spike. Length width correction methods were adopted for the determination of leaf area, and the calculation formula was leaf area (cm^2) = length × width × 0.75 [13].

Fresh leaves were sampled and cut into small pieces with the midribs removed; ethanol extraction methods were used for the measurement of chlorophyll contents according to Hao et al. [14]. Here, 95% ethanol was used as the blank, and the following absorbance values were measured at wavelengths of 663 nm and 645 nm:

Chl a (mg $L^{-1)}$ = 12.72A663−2.59A645;

Chl b (mg L^{-1}) = 22.88A645−4.67A663;

Chl a + b (mg L^{-1}) = 8.02A663 + 20.21A645;

Chl contents (mg g^{-1}) = (Chlorophyll concentration × Extraction volume × Dilution factor)/Fresh weight of the sample.

2.3. Determination of Main Agronomy Characters and Yield Traits

Three representative plants during the mature stage were sampled for the determination of agronomic and yield traits. And the plant height, panicle length, effective panicles per plant, total grains per panicle, actual grains per panicle, and the seed setting rates (total grains divided by actual grains) were measured. Afterwards, all the grains were removed and placed in an electric constant-temperature blast-drying oven at 125 °C for drying, and the dry weight of the grains was recorded. The remaining parts of the straw and stem were air dried in a natural room and then placed in an electric constant-temperature blast-drying oven at 125 °C for drying. Then, 2 × 500 normal-sized seeds were selected from the dried seeds, and the weight of 1000 seeds was recorded using a 0.01 g electronic balance. The sum of straw weight and grain yield is the biological yield. The weight of straw divided by biological yield is the harvest index. The weight of straw divided by grain yield is the ratio of grain weight to straw weight.

2.4. DNA Isolation and InDel Determination for Molecular Distance and Heterosis

During the 3–4 leaf stage, fresh leaves were collected, and total DNA was extracted using the CTAB method; this was performed according to Hao et al. [15]. The InDel markers used were based on the results of Jin et al. [16], and 19 pairs of InDel primers which were relatively uniformly distributed on 12 chromosomes were selected for indica–japonica rice. These primers were confirmed to have higher accuracy and credibility in the identification of indica, japonica, and genetic differentiation in previous studies [16].

The total PCR reaction system was 20 μL, including 2.0 μL of 10 × buffer, 0.6 μL of dNTPs (10 mmol·L^{-1}), 1.0 μL of forward primer (10 mmol·L^{-1}), 1.0 μL of reverse primer (10 mmol·L^{-1}), 0.5 μL of Taq polymerase (2 U·$μL^{-1}$), 13.7 μL of dd H_2O, and 1.2 μL of template DNA (10 ng·$μL^{-1}$). The PCR reaction program consisted of 35 cycles, including 94 °C for 4 min, 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; 72 °C for 7 min and being stored at 16 °C. The amplified products were separated by 3% agarose gel, the DNA samples without electrophoresis bands were re-PCR amplified, and the ones that did not appear after three repeats were treated as null alleles.

In order to detect the phylogenetic relationships and genetic differentiation revealed by InDel molecular markers in various experimental materials, a database was established using "0, 1" statistics. Each pair of InDel primers detected one locus, and each polymorphic band was identified as an allele. At the same migration location, value "1" was assigned when a band was shown, no band was assigned as "0", while "9" was assigned if there were a band missing. The genetic similarity (GS) and genetic distance (GD) between every pair of two materials were calculated according to Nei [17] with the formula GS = 2Mxy/(Mx + My), GD = −ln GS, where Mx and My represent the total number of fragments in X and Y materials, respectively, while Mxy represents the number of common fragments in both materials. Based on the obtained genetic similarity coefficients, the UPGMA (unweighted pair group method with arithmetic mean) was used for genetic similarity clustering. The analysis was carried out using NTSYS-pc2.1 software. At the same time, SPSS 17.0 statistical analysis software was used to perform principal component analysis (PCA) on the database of the experimental materials shown above using the assignment of an InDel band as a variable, and the eigenvector values of the InDel data of each principal component in all samples were obtained. SigmaPlot v 10.0 was used to create a planar scatter plot based on the average component values of the eigenvectors of the first and second principal components.

2.5. Statistical Analysis

SPSS v.22.0 software (IBM, Chicago, IL, USA) was used for the analysis of data, and significant treatment effects evaluation was determined using Duncan's multiple range test (DMRT). Origin v.2023b was used for paper drawing. The data were shown as means ± SD. $p < 0.05$ was used as the significance limit for all comparisons.

3. Results

3.1. Indica-Japonica Hybrid Rice Varieties Showed Better Growth Vigor in Seedling Stage

The seedling height, shoot dry weight, root length, and root dry weight were tested 6 d, 9 d, and 12 d after germination to compare the growth potential of different rice varieties in the seedling stage. The results showed that the increase in the seedling heights of indica–japonica hybrid rice, Z18 and Y12, was the largest in the first two stages, which reached 15.5 cm and 15.0 cm, respectively, by the 12th day (Figure 1A). Meanwhile, Z18 was significantly higher than its parents, 04B and h818, which were only 13.0 cm and 14.4 cm, respectively. Z12 and LYPJ were the lowest, which were only 11.7 cm and 12.0 cm, respectively (Figure 1A). In terms of shoot dry weight, the trend of each variety in the three stages was consistent, with Z12 and LYPJ ranking the highest, followed by Z18 and Y12 (Figure 1C).

The main root length varied greatly among different varieties, with Z18 and Y12 ranking at a moderate level, which reached 8.08 cm and 8.10 cm, respectively, by the 12th day, while 04B and Z12 were the longest, reaching 8.70 cm and 9.92 cm, respectively, but both were significantly longer than h818 and LYPJ (Figure 1B). For root dry weight, h818 and LYPJ still reached the lowest weight, while Z18 and Y12 were ranked first and third, respectively, with weights of 69 mg and 56 mg. Compared with its parents, 04B and h818, Z18 showed a significant advantage over its parents by 31.2% and 42.0%, respectively (Figure 1D).

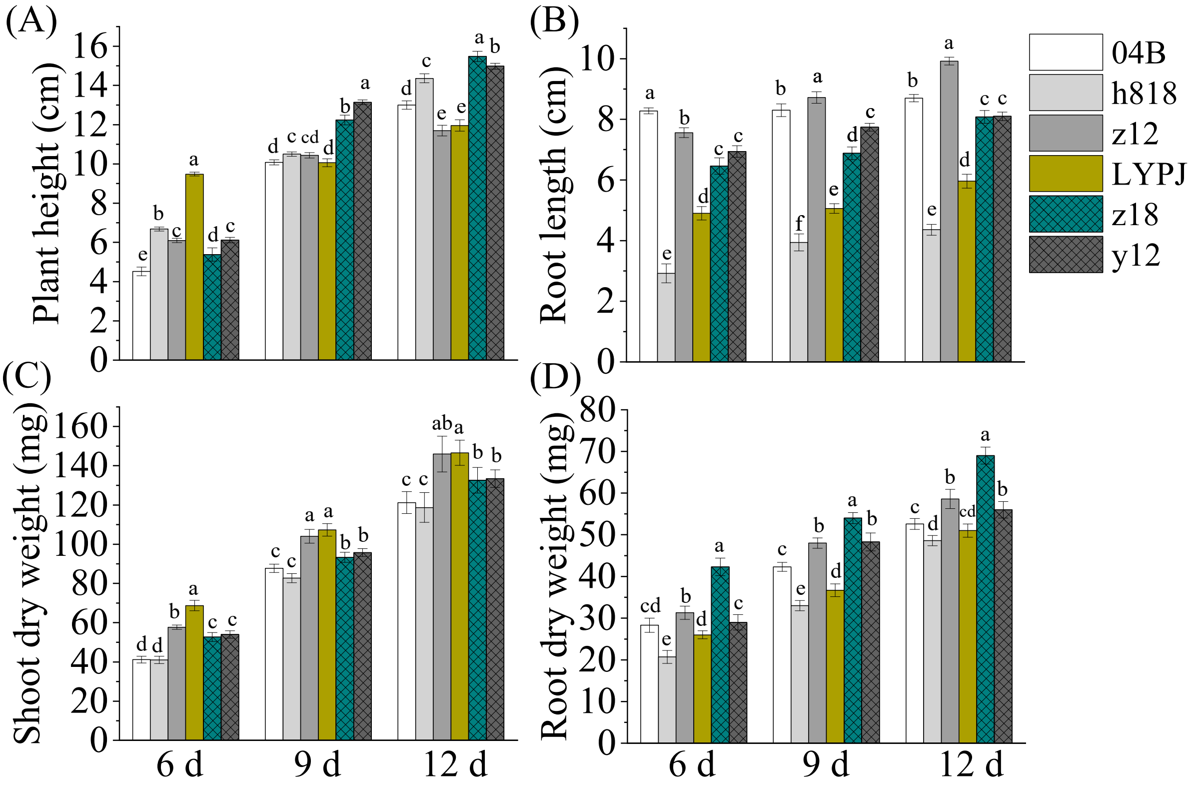

Figure 1. Comparison of growth vigor in seedling stage among different varieties. (**A**) Seedling height; (**B**) root length; (**C**) shoot dry weight; (**D**) root dry weight. Different lowercase letters represent significant differences at $p \leq 0.05$.

3.2. The Indica–Japonica Hybrid Rice Had Higher Leaf Area and Showed Advantages in Chlorophyll Contents in Seedling and Filling Stages

H818 showed the longest flag leaf length, reaching 45.20 cm, while Z18, Y12, and LYPJ were at the same level, which were 36.5 cm, 36.3 cm and 37.2 cm, respectively (Figure 2A). The flag leaf width was the widest in Z18 and Y12, reaching 2.47 cm and 2.57 cm, respectively, and Z18 had increased by 30.0%, 19.3%, 10.8%, and 23.5%, respectively, in comparison with 04B, h818, and z12 (Figure 2B), resulting in the largest area of flag leaf of z18, y12, and h818; the values were 67.5 cm^2, 70.0 cm^2, and 69.9 cm^2, respectively. Meanwhile, 04B and z12 showed the smallest flag areas of 37.06 cm^2 and 44.81 cm^2 (Figure 2C).

The length of the top second and third leaves was consistent, with h818 and LYPJ showing the longest leaf length, followed by z18 and y12, while 04B were the shortest. The width of the top second and third leaves was still the widest in z18 and y12. Taking the top third leaf as an example, the leaf widths of z18 and y12 were 1.83 cm and 1.97 cm, respectively, while the other four varieties were at the same level, which were significantly lower than the indica–japonica hybrid rice (Figure 2B).

The area of the top three leaves is the main indicator affecting the output of photosynthetic products and is closely related to the formation of the final yield. H818 had the largest flag and the top second leaf areas, followed by z18 and y12, while 04B had the smallest. As for the top third leaf, the leaf areas of indica–japonica hybrid rice z18 and y12 ranked as the top two, which were 73.3 cm^2 and 77.3 cm^2, respectively. The top third leaf area in z18 increased by 93.9%, 9.8%, 53.5%, and 31.6%, respectively, in comparison with 04B, h818, z12, and LYPJ (Figure 2C). In terms of the total leaf areas of these three functional leaves, h818, z18, and y12 had the highest. The heterosis was 16.46% and 38.59%, respectively in z18 compared with indica–indica and japonica–japonica hybrid rice, respectively; meanwhile, the super parental advantage was 22.88% compared with the average value of its parents (Figure 2D).

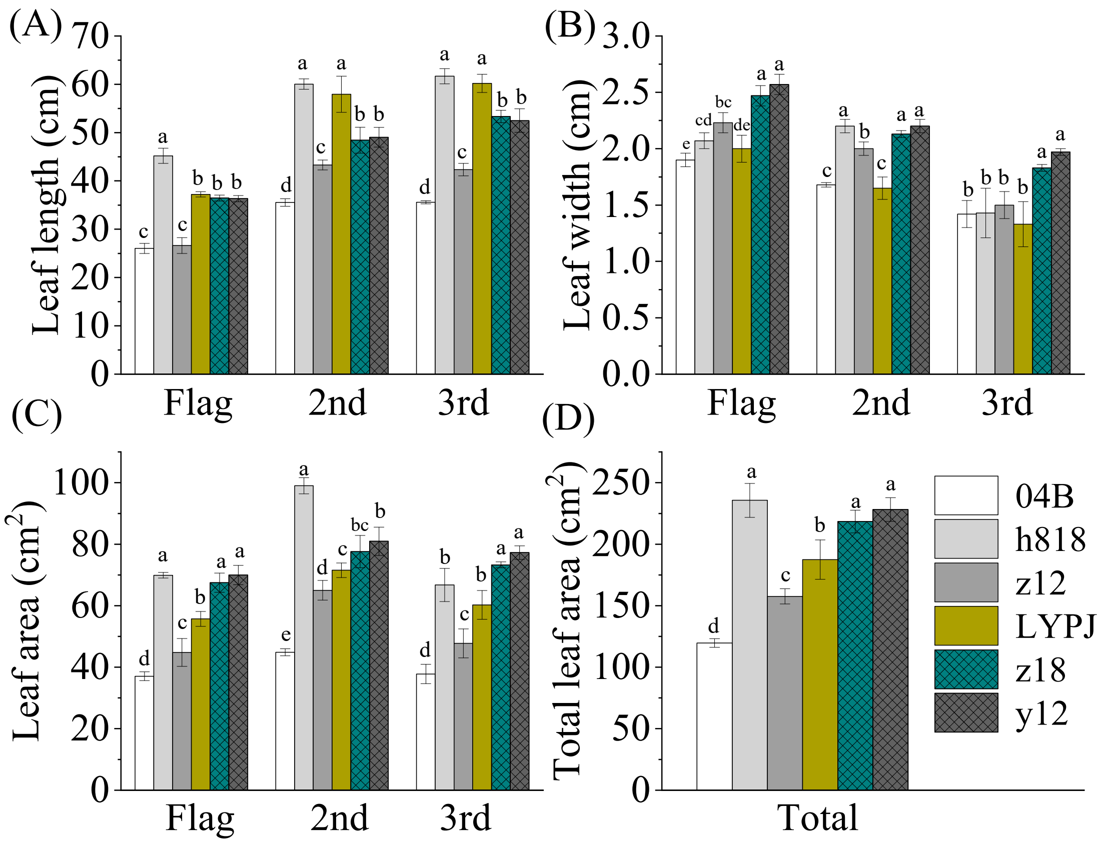

Figure 2. Morphology of functional leaves among different varieties. (**A**) Leaf length; (**B**) leaf width; (**C**) leaf area; (**D**) total leaf area. Flag, flag leaf; 2nd, the top second leaf; 3rd, the top third leaf. Different lowercase letters represent significant differences at $p \leq 0.05$.

The chlorophyll contents were compared in the seedling, heading, and filing stages among different varieties. The contents of chlorophyll a and chlorophyll b in z18 ranked the second highest in the seedling stage, while these values were decreased in the heading and filling stage. Meanwhile, the chlorophyll contents of y12 showed no obvious advantage compared to other varieties. However, comparing z18 with its two parents, the total chlorophyll contents of z18 were increased by 6.1%, 0.8% and 8.4%, respectively, in the seedling, heading, and filling stage, indicating the advantage of the heterosis of indica–japonica hybrid rice in the seedling and filling stages (Figure S1).

3.3. Determination of Agronomy Traits and Yield Components That Contribute to the High Yield of Indica–Japonica Hybrid Rice

The plant heights of z18 and y12 were 127.3 cm and 129.1 cm, respectively, similar to h818 and LYPJ, but significantly higher than the 88.2 cm and 98.4 cm heights of 04B and z12 (Figure 3A). The effective numbers of panicles per plant for z18 and y12 were 12.3 and 10.0, respectively, indicating a moderate tillering variety (Figure 3B). However, the numbers of grains per panicle were significantly increased, reaching 291.0 and 352.5, respectively, indicating a super large panicle variety, ranking in the top two. In terms of 04B and LYPJ, although they had the highest number of effective panicles, reaching 15.33 and 16.67, respectively, their grains numbers per panicle were only 162.4 and 178.5, respectively, reaching the lowest value (Figure 3C).

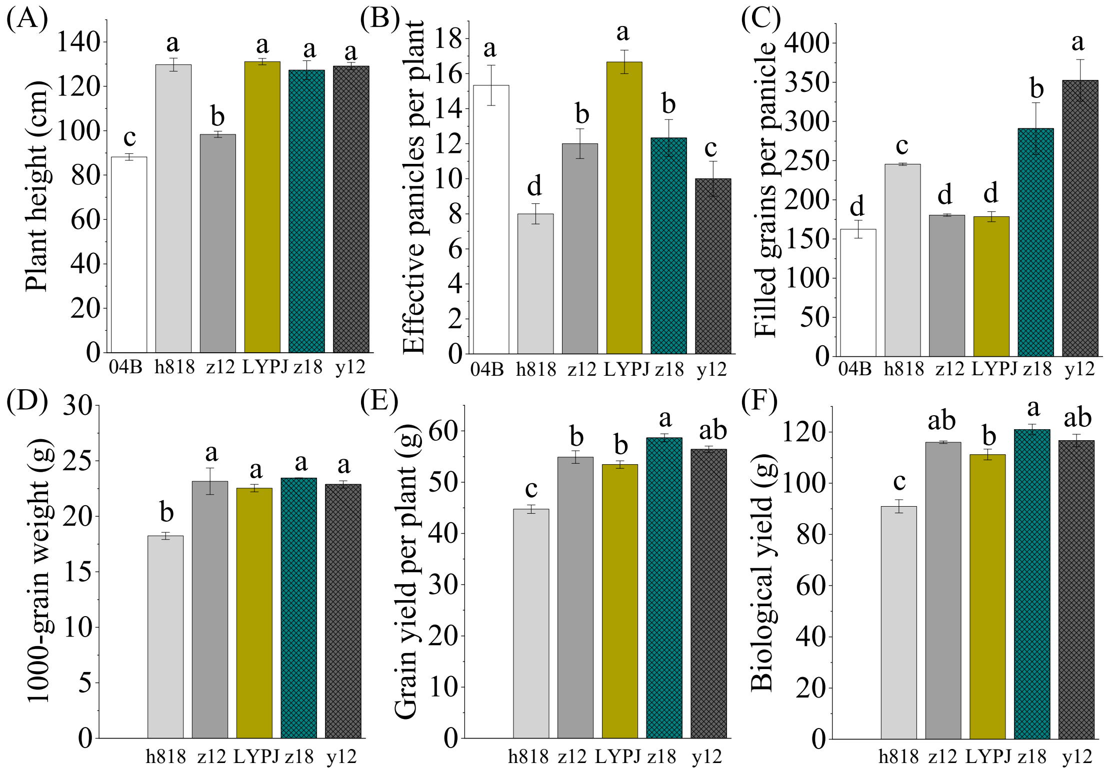

Figure 3. Agronomy characters and yield traits among different rice varieties. (**A**) Plant height; (**B**) effective panicle number per plant; (**C**) full grain numbers per panicle; (**D**) 1000-grain weight; (**E**) grain yield per plant; (**F**) biological yield. Different lowercase letters represent significant differences at $p \leq 0.05$.

The 1000-grain weights of z18 and y12 were 23.4 g and 22.9 g, respectively, which were similar to z12 and LYPJ, with values of 23.2 g and 22.5 g, respectively, and both were significantly higher than the 18.2 g of h818 (Figure 3D). The grain weight per plant was also highest in z18 and y12, with values of 58.1 g and 56.0 g, respectively. Z18 increased by 29.8%, 4.3%, and 8.7%, respectively, compared with the parents, h818, z12, and LYPJ, demonstrating a higher yield-increasing ability. Y12 ranked second in yield, which increased by 25.2% and 4.8% compared to z12 and LYPJ (Figure 3E).

From the perspective of biological yield, z18 and y12 had the highest yield, with 121.0 g and 116.7 g, respectively, while z12 had the lowest yield of 90.97 g (Figure 3). There was no difference in the harvest index and grain grass ratio among the six varieties. The harvest index was between 0.4801 and 0.4920, and the grain grass ratio was between 0.9237 and 0.9688 (Table S1).

To analyze the reasons for the increase in the grain yield per plant of indica–japonica hybrid rice, the yield components such as effective panicles, total grains per panicle, full grains per panicle, seed setting rate, and 1000-grain weight were statistically analyzed, as well as the correlation between shoot weight, biological yield, harvest index, and grain grass ratio (Table S2). The results showed that the grain weights per plant of z18 and y12 were all related to full grains per panicle, thousand-grain weight, and biological yield at a significant level (Table S2).

3.4. The Increases in the Number of Primary Branches, Spikelets, and Grains in the Primary and Intermediate Panicles Ultimately Contribute to the Increasing of Yield in Indica–Japonica Hybrid Rice

We further selected the primary panicle with the longest single panicle length and the intermediate panicle with average panicle length from six varieties and measured the total grains per panicle, full grains per panicle, number of branches per panicle, and seed setting rate of the main panicle and intermediate panicle (Table 1). The data show that the trend of the main panicle and intermediate panicle traits was consistent. Taking the main panicle as an example, z18 and y12 had the highest number of branches per panicle, with each being 26, while the average number of the parents of z18 was 18.5, with an increase of 40.5% in z18 in compared to the average number of its parents (Table 1). However, 04B and LYPJ had the lowest number; each were 16 and 14, respectively. The trend of the number of spikelets and grains was the same, with z18 and y12 having the highest number; each had 323 and 326 grains in the main panicle, respectively. Taking z18 as an example, the average increase compared to both parents was 56.4, indicating a significant super parental advantage. Compared to z12 and LYPJ, the increase was 80.4% and 87.8%, respectively. However, due to hybridization reasons, their seed setting rates were only 81.2% and 80.3%, lower than the conventional varieties, which were 90.9% and 87.1%, respectively, while they were slightly higher than the indica–indica hybrid variety, z12, and japonica–japonica hybrid variety, LYPJ, which had rates of 74.6% and 73.5% (Table 1).

Table 1. Comparison of panicle characteristics of primary and intermediate panicles among six lines.

Line	Primary Panicle						Intermediate Panicle					
	SNP	FNP	PB	SNPB	FNPB	SS (%)	SNP	FNP	PB	SNPB	FNPB	SS (%)
Zhe04B	186 d	169 c	16 de	11.63 c	10.56 bc	90.86 a	177 c	148 d	13 c	13.62 a	11.38 a	83.62 b
Zhehui 818	280 b	244 b	21 b	13.33 b	11.62 ab	87.14 a	247 c	230 c	18 b	13.72 a	12.78 a	93.12 a
Zheyou12	240 c	179 c	18 cd	13.33 b	9.94 c	74.58 c	182 d	152 d	16 b	11.38 b	9.50 a	83.52 b
LYPJ	234 c	172 c	14 e	16.71 a	12.29 a	73.50 c	185 d	146 d	13 c	14.23 a	11.23 a	78.92 b
Zheyou18	398 a	323 a	26 a	15.31 a	12.42 a	81.16 b	324 b	264 b	23 a	14.09 a	11.48 a	81.48 b
Yongyou12	406 a	326 a	26 a	15.62 a	12.54 a	80.30 b	379 a	310 a	26 a	14.58 a	11.92 a	81.79 b

Note: Primary panicle refers to the longest panicle; intermediate panicle refers to the panicle with proximal average length. SNP, spikelet numbers per panicle; FNP, filled grain numbers per panicle; PB, primary branches; SNPB, spikelet numbers per branches; FNPB, filled grain numbers per panicle; SS seed setting rate. Different lowercase letters represent significant differences at $p \leq 0.05$.

The above panicle analysis indicates that the increase in the number of grains per panicle in indica–japonica hybrid rice is mainly due to a significant increase in the number of primary branches, spikelets, and grains per panicle in the main and middle panicles, which ultimately lead to a significant increase in yield.

3.5. PCA Heterosis Correlation Analysis of InDel Molecular Distance

To analyze the genetic relationship between the Zheyou series combination F1 and its parents, principal component analysis (PCA) was performed on 35 materials, the details of which are shown in Table S3. The contribution rate of the first principal component to genetic variation was 51.84%, the contribution rate of the second principal component was 12.96%, and the cumulative contribution rate was 64.8%, which represents the main genetic variation. Each icon represents a material, and their genetic relationships are inferred based on their spatial positions in the scatter plot, with the main variations reflected on the horizontal axis (Figure 4). It can be seen that the PCA scatter plot results are highly consistent with the clustering analysis results, and all 35 research materials are clustered into three different regions, which were ○,the indica/preferred indica district, in which eight restoring lines of indica–japonica hybrid combinations, three indica control varieties, and the indica hybrid regional trial control variety Shanyou63 were gathered; ●, the japonica/preferred japonica district, in which sterile line 04B, four japonica hybrid restoration lines and hybrid F1, three japonica control varieties, and the japonica hybrid regional trial control variety Xiushui09 were gathered; ▼,a mediation to the preferred japonica district, in which eight hybrid F1 combinations of indica and japonica were gathered, and this region was closer to the ● region in space. From this, it can be seen that there were significant genetic differences in the sterile line, restoring line, and hybrid F1 of the Zheyou series of indica–japonica hybrid combinations. These combinations are typical combinations of indica–japonica hybrids, and the large genetic distance between parents is the basis for the strong hybrid advantage of hybrid F1.

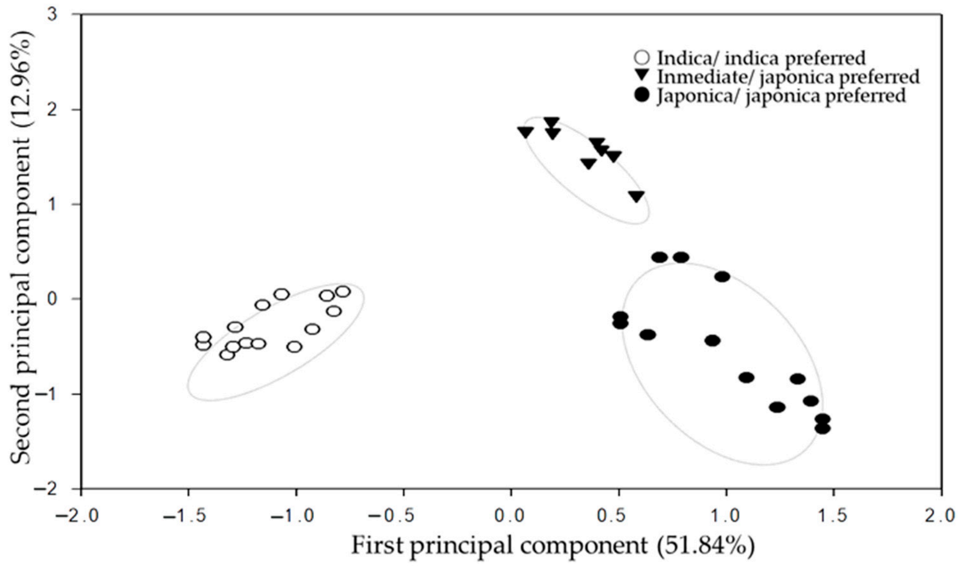

Figure 4. Scatter plotting showing the association among 35 materials based on PCA.

The correlation analysis between InDel genetic distance and heterosis showed that when 12 combinations were analyzed as a whole, InDel genetic distance was highly significantly positively correlated with economic yield, total grain number, solid grain number, and single panicle weight and significantly negatively correlated with seed setting rate, while it was not significantly correlated with effective panicle and thousand-grain weight (Table 2). This indicates that as the genetic distance of InDel increased, although the seed setting rate significantly decreased, the heterosis was significantly positively reflected in the single spike weight, total grain number, and actual grain number, which ultimately contributed to the increase in economic yield. When only eight indica–japonica hybrid combinations were used as research objects, InDel genetic distance was significantly positively correlated with single spike weight and thousand-grain weight, with correlation coefficients r of 0.771 * and 0.710 *, respectively (Table 2).

Table 2. Heterosis of seven traits and the correlation coefficient between genetic distance and traits.

Yield Related Traits	Control Heterosis				Correlation Coefficient between Genetic Distance and Traits	
	Range/%	Mean	No. of Combinations with Positive (Negative) Heterosis	No. of Combinations with Significance at 0.01 Level	The 12 Combinations Analyzed as a Whole	The 8 Indica–Japonica Combinations Analyzed as a Whole
Grain yield	1.89~29.97	20.09	12 (0)	8 (−)	0.819 **	0.474
Panicles per plant	−0.97~−39.12	−29.36	0 (12)	1 (−)	−0.522	−0.685
Total grains per plant	36.31~173.75	108.78	12 (0)	1 (+)	0.845 **	0.599
Filled grains per plant	24.95~126.40	79.08	12 (0)	9 (+)	0.834 **	0.633
Seed setting rates	−19.24~−3.19	−13.32	0 (12)	9 (−)	−0.702 *	−0.010
1000-grain weight	−9.20~10.80	−3.20	2 (10)	3 (−)	−0.349	0.710 *
Panicle weight	20.45~124.59	72.30	12 (0)	11 (+)	0.863 *	0.771 *

** and * mean significant at 0.01 and 0.05 level, respectively.

It can be seen that InDel genetic distance had good predictive ability for heterosis among different types of varieties, but its predictive ability decreased among varieties of the same type. Through comparison between two populations, it was found that InDel genetic distance was significantly positively correlated with single spike weight, which was the only consistent trait among the seven traits. The correlation coefficient r was also the highest among the seven traits. Hence, it can be concluded that InDel genetic distance can be fully used for predicting the heterosis of single spike weight. That is, as the InDel genetic distance expands, heterosis is mainly reflected in the increase in single spike weight.

4. Discussion

Inter-subspecific indica–japonica hybrid rice can take full advantage of its inter-subspecific nature. Many indica–japonica hybrid rice combinations have been extensively tested in different rice-growing areas in China [2]. Rice yield is mainly determined by several important quantitative traits, including effective panicle number, grain number per panicle, and grain weight. The grain number is influenced by leaves, which significantly correlate with rice yield. Leaves are the main photosynthetic organs with high developmental plasticity. For the agronomic traits of hybrid rice, flag leaf-related heterosis is an indispensable component of productivity and a major element for plant breeding [5]. As we measured above, we found that based on the big leaf style of h818, z18 showed a large advantage in leaf area, which was 16.5% and 38.6% higher than the indica–indica hybrid rice variety, z12, and the japonica–japonica hybrid rice variety, LYPJ, in terms of total leaf area. And though z18 showed no significant difference when compared to its parent, h818, z18 still showed a single parent advantage and middle parent advantage (Figure 3). Furthermore, according to our study, we found that the indica–japonica hybrid rice also showed quick growth in terms of plant height, which will contribute to the development and expansion of leaves and, hence, increase the rates of photosynthesis. In addition, a reasonable constitution of panicle style, panicle numbers, grain number, and seed setting rates contributes a lot to the final yield. As has been reported, the number of spikelets and thousand-grain weight per unit area of indica–japonica hybrid rice were lower, the number of grains per spikelet was significantly higher than that of conventional japonica [18], the proportion of secondary pedicel grains to the total number of grains was as high as about 60%, the total number of glumes was very significantly higher than that of conventional japonica, the fertility was 85.83%, and the high yield of indica–japonica hybrid rice was mainly obtained by large spikelets [19,20]. Similar to a previous study, the results of this study showed that indica–japonica hybrid rice significantly increased the number of primary spikelets, the total number of main spikelet solid grains, and the average number of main spikelet solid grains. This resulted in clear superiority and control advantages in single-plant grain weight, single-plant stem weight, and single-plant biological yield, and the yield advantage was significantly greater than that of other types of hybrid rice, but the harvest index decreased instead. This result may reflect the cumulative effects of heterosis on yield-related traits [21,22]. The inter-subspecific hybridization of rice will make a major contribution to solving the problems of a growing world population and decreasing arable land, as well as meeting the demands for environmentally friendly, low-carbon production.

Knowing that reproductive isolation is caused by the genetic divergence between parents, many studies have estimated the degree of differentiation between the indica–japonica rice varieties, and efforts have also been made to determine the relationship between genetic divergence and heterosis [23,24]. Most reports correctly concluded that the larger the genetic distance between parents, the stronger the heterosis of the hybrids [2,23]. However, in regard to the relationships between the yield and heterosis, people found it incomprehensible and obtained different and even contradictory outcomes. The reason for these discrepancies may be that the DNA markers chosen to classify the genetic distance of the parents were not suitable. Because there are so many different loci between the sequences of indica and japonica, these differences may not show the true genetic distance. Therefore, selecting an appropriate estimating tool is quite important. Therefore, the correlations between the genetic divergence and the seed setting rate must be verified as being true. Many studies have been carried out using InDel molecular markers to identify indica and japonica characteristics [1]. In this study, the InDel markers are feasible for and effective at estimating the genetic divergence. It was found that InDel genetic distance was significantly or highly significantly positively correlated with spikelet weight, which was the only consistent trait among the seven traits, and the correlation coefficient, r, was the largest among the seven traits. Considering that economic yield, the total number of grains, the number of grains in a kernel and spikelet weight were consistent, it can be concluded that InDel genetic distance can be fully used for predicting dominance in spikelet weight, i.e., with increasing InDel genetic distance, the heterosis dominance was mainly manifested in the increase in single spikelet weight.

5. Conclusions

In this study, the physiological mechanisms of total grain yield improvement in indica–japonica hybrid rice were comprehensively explored, and InDel genetic distance was used to study the genetic basis of heterosis. Our results showed that indica–japonica hybrid rice varieties showed the advantages of better growth vigor in the seedling stage; higher leaf area; and more primary branches, spikelets, and grains in the primary and intermediate panicles, which contributed to the increase in grain yield. Taken together with our findings of the relationship between InDel genetic distance and spikelet weight, this research will provide support for future rice indica–japonica hybrid breeding and application in practice.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/agriculture14040607/s1, Figure S1: Chlorophyll contents among different rice varieties during seedling, heading, and filling stages; Table S1: Difference in harvest index and ratio of grain to straw among 6 rice lines; Table S2: Correlation between grain yield per plant and other yield composition factors. Table S3: Thirty-five rice materials used to identify *indica* or *japonica* by InDel markers and their results.

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