Genetic Diversity among Parents of Hybrid Rice Based on Cluster Analysis of Morphological Traits and Simple Sequence Repeat Markers

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Abstract: The genetic diversity of 41 parental lines popularized in commercial hybrid rice production in China was studied by using cluster analysis of morphological traits and simple sequence repeat (SSR) markers. Forty-one entries were assigned into two clusters (i.e. early or medium-maturing cluster; medium or late-maturing cluster) and further assigned into six sub-clusters based on morphological trait cluster analysis. The early or medium-maturing cluster was composed of 15 maintainer lines, four early-maturing restorer lines and two thermo-sensitive genic male sterile lines, and the medium or late-maturing cluster included 16 restorer lines and 4 medium or late-maturing maintainer lines. Moreover, the SSR cluster analysis classified 41 entries into two groups (i.e. maintainer line group and restorer line group) and seven sub-groups. The maintainer line group consisted of all 19 maintainer lines, two thermo-sensitive genic male sterile lines, while the restorer line group was composed of all 20 restorer lines. The SSR analysis fitted better with the pedigree information. From the views on hybrid rice breeding, the results suggested that SSR analysis might be a better method to study the diversity of parental lines in indica hybrid rice.

Key words: parental lines; hybrid rice; morphological trait; simple sequence repeats; clustering analysis; genetic diversity; pedigree

Heterosis has been successfully exploited on a large scale in rice (*Oryza sativa* L.), which is a self-pollinated crop. The selection of parental lines plays a vital role in developing ideal combinations. Therefore, it is essential to study the relationship and genetic diversity among parental lines in hybrid rice. In fact, plant breeders often select parental lines in combinations with morphological trait and pedigree information. However, this breeding method is less effective and accurate due to environmental effect. Molecular markers have been widely used to study the genetic variation and diversity of breeding materials, which were less influenced by temporal, spatial and environmental conditions [1-2]. Previously, Chen et al [3] studied the genetic diversity of 20 rice varieties using AFLP and RFLP markers. Liu et al [4] classified 31 restorer lines of hybrid rice into four groups based on RAPD analysis. Moreover, Ji et al [5] quantified the genetic diversity among 53 rice varieties resistant to bacterial leaf blight by RAPD technology. Compared

with above molecular markers, simple sequence repeat (SSR) marker has several advantages and is popular in studying genetic diversity among rice varieties. Duan et al [6] studied the genetic diversity of 35 restorer lines of hybrid rice using 25 SSR markers, and found that the restorer lines had abundant resource with smaller genetic diversity and vulnerable genetic background. Qiu et al [7] inspected the genetic variation in main parents of japonica hybrid rice by SSR. The molecular phylogenetic tree using UPMGA method showed that the main parents were divided into five groups, and the parents between groups had great genetic differentiation relatively. Yu et al [8] also studied the genetic variation in rice varieties derived from Aizizhan using morphological traits, allozymes and SSR. Their cluster analysis showed the difference in genetic distance among varieties determined by morphological traits, allozymes and SSR markers.

The main objectives of this research were to assess the genetic diversity exists among 41 parental lines that were widely used in hybrid rice breeding, and to compare the two grouping methods based on morphological traits and SSR cluster analysis in

studying genetic diversity among parents of hybrid rice.

MATERIALS AND METHODS

Plant materials

Forty-one hybrid parental lines were chosen to represent a wide diversity in indica hybrid rice breeding in indica rice, including 19 maintainer lines, 20 restorer lines and 2 thermo-sensitive genic male sterile (TGMS) lines (Table 1). Most of them have been used as parents in hybrid rice breeding.

Field experiment and data analysis

The tested materials were sowed on May 15 and transplanted at 30 cm ×25 cm spacing on June 15, 2004 in Nanjing, Jiangsu Province. The experiment was a randomized block design with two replications. Each plot consisted of 20 plants.

The morphological traits such as, plant height, number of panicles per plant, panicle length, number of spikelets per panicle, number of filled grains per panicle, grain weight per plant, maximum number of

tillers per plant, days from sowing to initial heading, flag leaf length, flag leaf width, color of sheath and leaf, plant type, leaf angle and growth vigor were surveyed by sampling ten plants in the center of each plot. The standardized mean values of every morphological trait were used to perform the Furthest Neighbour cluster analysis using appropriate procedures of the program SPSS 10.0[9].

SSR analysis

Twenty seedlings (10-day-old) for each parental line were collected, frozen and ground into fine powder. The DNA was extracted according to Dellaporta et al [10]. The 66 SSR primers distributed throughout the rice genome were chosen from State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University. Primers, PCR conditions and silver staining procedures were followed as described by Chen et al [11].

The genetic similarity (GS) was calculated for all possible pairs of parental lines according to the equation $GS=M/(M+N)$[12], where M is the number of alleles between two parental lines, N is the number of

Table 1.   The entries and their pedigrees.

No.	Material	Pedigree	No.	Material	Pedigree
1	Xieqingzao B	Junxie / Wenxuanqing // Qiutangzao 5	22	Fuhui 016	Selected from Fuyin 1
2	Jinxie B	Jin 23B / Xieqingzao B	23	Minghui 77	Minghui 63 / Ce 64-7
3	Zhong 9B	You 1B / L301B // Neijiangfeigai B	24	Ce 64-7	Selected from IR9761-19
4	Y33B	Jiazhe B	25	Gui 99	Longye 5-3 /(IR661/IR206)
5	Xinlu B	(Ce 64-7 / Lituo) / (V20B / 26 Zhaizao)	26	R818	Minghui 63 / Nannong 3005 //
6	Zhenshan 97B	Zhenzhu'ai 11 / Shan'aixuan 4			(Liunanzao A / Minghui 63//02428)
7	Gang 46B	Erjiu'ai 7 / V41B // Zhenshan 97 /	27	IR24	IR8 / IR1317
		Ya'aizao	28	Uni 2	Duoxi 1 / Qianhui 481
8	Ⅱ-32B	Zhenshan 97B / IR665	29	Kang 85	Minghui 63 / TD-1 // Minghui 63
9	D62B	Hongtu 31 / D297B	30	R351	C418 / Minghui 63
10	Xinxiehuang B	Xinlu B / Xieqingzao B	31	Shuhui 527	1318 / 88-R3360
11	Feng 7B	Unknown	32	Yu 18	Minghui 63 mutant
12	Jin 23B	Huangjin 3 / (Feigai B / M) F$_5$	33	9308	C57 // 300 / IR26
13	Simiao B	Unknown	34	Kehui 752	Erliuzhaizao / BG910-11
14	Yuefeng B	Xieqingzao B / IR58025B	35	9311	Yangdao 4 / 3021
15	Longtepu B	Longwan 1 / Tetep	36	CDR22	IR50 / Minghui 63
16	Bo B	Gangzhizhan / Zhenshan 97	37	Minghui 86	(IR54 / Minghui 63 // IR60 / Gui 630) /
17	Ⅱ Xie B	Ⅱ-32B / Xieqingzao B			GK148 // Minghui 63
18	Chuan 7B	Unknown	38	Maosan	677/IR36
19	Yuetai B	Congguang 41 / Zaoshutaiyin 1	39	Shuangqizhan	Qiqingzhan / Qihuangzhan
20	Pei'ai 64S	Nongken 58S / (Pei'ai F$_7$/ Ce 64-7)	40	Minghui 63	IR30 / Gui 630
21	6311S	N422S / Aiguangzhan 63 // Yangdao 6	41	Yanhui 559	Teqing / Minghui 75

non-alleles between two parental lines. Un-weighted pair-group method using an arithmetic average (UPGMA) cluster analysis was performed with the GS matrix using appropriate procedures of the program NTSYS-pc[13].

RESULTS

Cluster analysis of morphological traits

Based on average values of morphological traits, 41 varieties were classified into two clusters, early or medium-maturing cluster and medium or late-maturing cluster (Fig. 1 and Table 2).

The early or medium-maturing cluster contained 15 maintainer lines, 4 early-maturing restorer lines and 2 TGMS lines. These 21 lines had shown a shorter growth period and weaker growth vigor with the average plant height of 99.0 cm, and the average grain weight of 43.0 g per plant, respectively (Table 3). Based on the threshold distance value of 13, the

cluster could be sub-divided into three sub-clusters i.e. the first sub-cluster of four early-maturing restorer lines and five maintainer lines, the second sub-cluster of eight maintainer lines and the third sub-cluster of two TGMS lines, Pei'ai 64S and 6311S. The medium or late-maturing cluster contained 16 restorer lines and four medium or late-maturing maintainer lines with a longer growth period and stronger growth vigor, while the average value of the grain weight per plant was 68.0 g. This cluster was further divided into three sub-clusters by the critical distance value of 13, the first sub-cluster consisted of 6 restorer lines and 4 maintainer lines, the second sub-cluster was made up of nine restorer lines, and only one parental line, 9308 was in the third sub-cluster.

SSR cluster analysis

During the experiment, 66 SSR primers produced 301 alleles among the 41 hybrid rice parental lines, while the allele numbers for the SSR loci ranged from
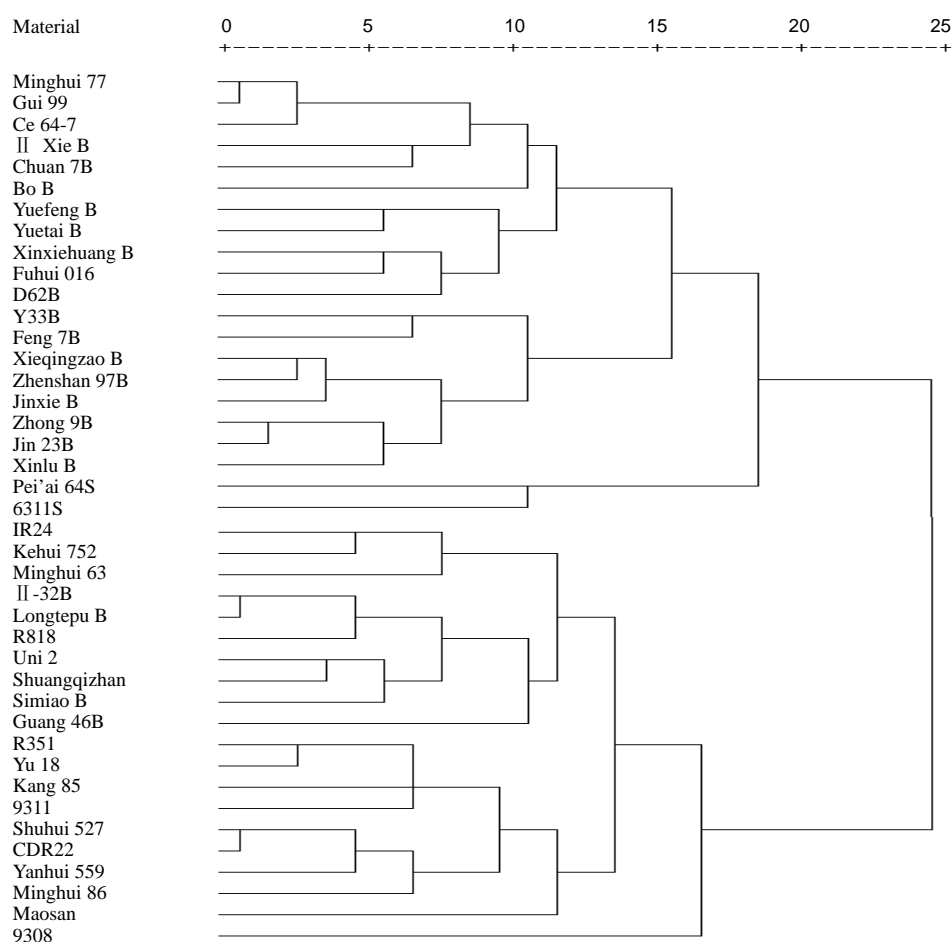

Fig. 1.   Dendrogram for 41 parental lines of hybrid rice by morphological trait data.

Table 2.   The grouping result for 41 parental lines of hybrid rice.

Group	Material
EMG	Zhenshan 97B, Xieqingzao B, Jin 23B, D62B, Zhong 9B, Bo B, Xinlu B, Yuefeng B, Yuetai B, Y33B, Chuan 7B, Feng 7B, Jinxie B, Xinxiehuang B, Ⅱ Xie B, Fuhui 016, Ce 64-7, Minghui 77, Gui 99, 6311S, Pei'ai 64S
MLG	IR24, Minghui 63, Shuhui 527, CDR22, Kehui 752, Yanhui 559, R351, Yu 18, 9311, Kang 85, Maosan, Minghui 86, R818, Shuangqizhan, Uni 2, Longtepu B, Ⅱ-32B, Gang 46B, Simiao B

EMG, Early or medium-maturing group; MLG, Medium or late-maturing group.

Table 3.   Mean values of nine characters within two groups.

Group	Maximum number of tillers per plant	From sowing to initial heading (d)	No. of panicles per plant	Panicle length (cm)	Number of spikelets per panicle	Plant height (cm)	Length of flag leaf (cm)	Width of flag leaf (cm)	Grain weight per plant (g)
EMG	22.9	71	19.99	26.60	184.35	99	34.2	1.5	43.00
MLG	27.7	89	17.37	28.22	269.90	118	40.0	1.9	68.00

EMG, Early or medium-maturing cluster; MLG, Medium or late-maturing cluster.

2 to 13. The average allele number was 5.60, the *GS* values among 41 parental lines was 0.745 and ranged from 0.643 to 0.928.

The cluster analysis divided the 41 parental lines into maintainer line group and restorer line group according to the threshold *GS* value of 0.735 (Fig. 2).

The maintainer line group consisted of 19 maintainer lines and two TGMS lines, which was further divided into three subgroups (the critical *GS* was 0.764). Subgroup A (Xieqingzao subgroup) included three maintainer lines (Xieqingzao B, Jinxie B and Zhong 9B). The result is in agreement with pedigree information, e.g. Jinxie B and Zhong 9B both derived from Xieqingzao B. Subgroup B (Zhenshan 97 subgroup) included 16 main maintainer lines widely utilized in hybrid rice breeding, of which Zhenshan 97B, Gang 46B, Ⅱ-32B, Bo B, D62B, Ⅱ Xie B, Xinlu B and Xinxiehuang B all derived from Zhenshan 97. The Zhenshan 97-derived lines accounted for 50% of the 16 maintainer lines. Subgroup C was composed of two TGMS lines (Pei'ai 64S and 6311S), both are offspring of javanica rice.

The restorer line group contained 20 lines, which could be further divided into four subgroups according the threshold *GS* value of 0.760 (Fig. 2). Subgroup D (Minghui 63 subgroup) included 14 restorer lines (i.e. Minghui 63, Minghui 77, Minghui 86, Yanhui 559, CDR22, R818, Uni 2, Kang 85, Ce 64-7, Yu 18, Kehui 752, Gui 99, Shuhui 527 and Fuhui 016), of which ten are derived from Minghui 63, while the subgroup E had only one restorer line (9308) with 12.5% consanguinity of japonica. Therefore it has a special genetic background. Subgroup E

(two-line restorer line subgroup), included 9311, Maosan and Shuangqizhan, which were exploited as restorer lines of TGMS lines. Subgroup F (IR24 subgroup) was composed of two restorer lines (IR24 and R351), R351 was directly derived from IR24.

DISCUSSION

In this study, most of the maintainer lines were grouped into early or medium-maturing cluster, while the restorer lines were clustered into late or medium-maturing cluster, based on morphological trait cluster analysis. In addition, two TGMS lines were assigned into unique sub-cluster. It can be suggested that morphological trait analysis is a useful tool in studying the difference in ecological type, which is closely related to heterosis. Therefore, morphological trait analysis may be helpful to identify heterotic patterns of combinations between the diverse ecological type variety groups for hybrid rice breeding. However, the cluster result based on morphological trait data could not reveal the genetic relationship among the parental lines adequately. For example, Ⅱ-32B, Gang 46B, D62B, Xinlu B and Xinxiehuang B were all derived from Zhenshan 97B, however, D62B, Xinlu B, Xinxiehuang B and Zhenshan 97B were classified into early or medium-maturing group, while Ⅱ-32B, Gang 46B were assigned into late or medium-maturing group. Therefore, the morphological variation does not always reflect real genetic variation because of genotype×environment interaction and the largely unknown genetic control of polygenic morphological and agronomic traits[14].

Xieqingzao B
Jinxie B
Zhong 9B
Xinlu B
Y33B
Zhenshan 97B
Yuetai B
Longtepu B
Chuan 7B
D62B
Feng 7B
Guang 46B
Ⅱ Xie B
Ⅱ-32B
Jin 23B
Simiao B
Bo B
Yuefeng B
Xinxiehuang B
Pei'ai 64S
6311S
Kang 85
Yanhui 559
R818
Ce 64-7
Kehui 752
Shuhui 527
Minghui 63
CDR22
Yu 18
Uni 2
Minghui 86
Minghui 77
Fuhui 016
Gui 99
9308
9311
Maosan
Shuangqizhan
R351
IR24

0.70   0.77   0.85   0.93   1.00
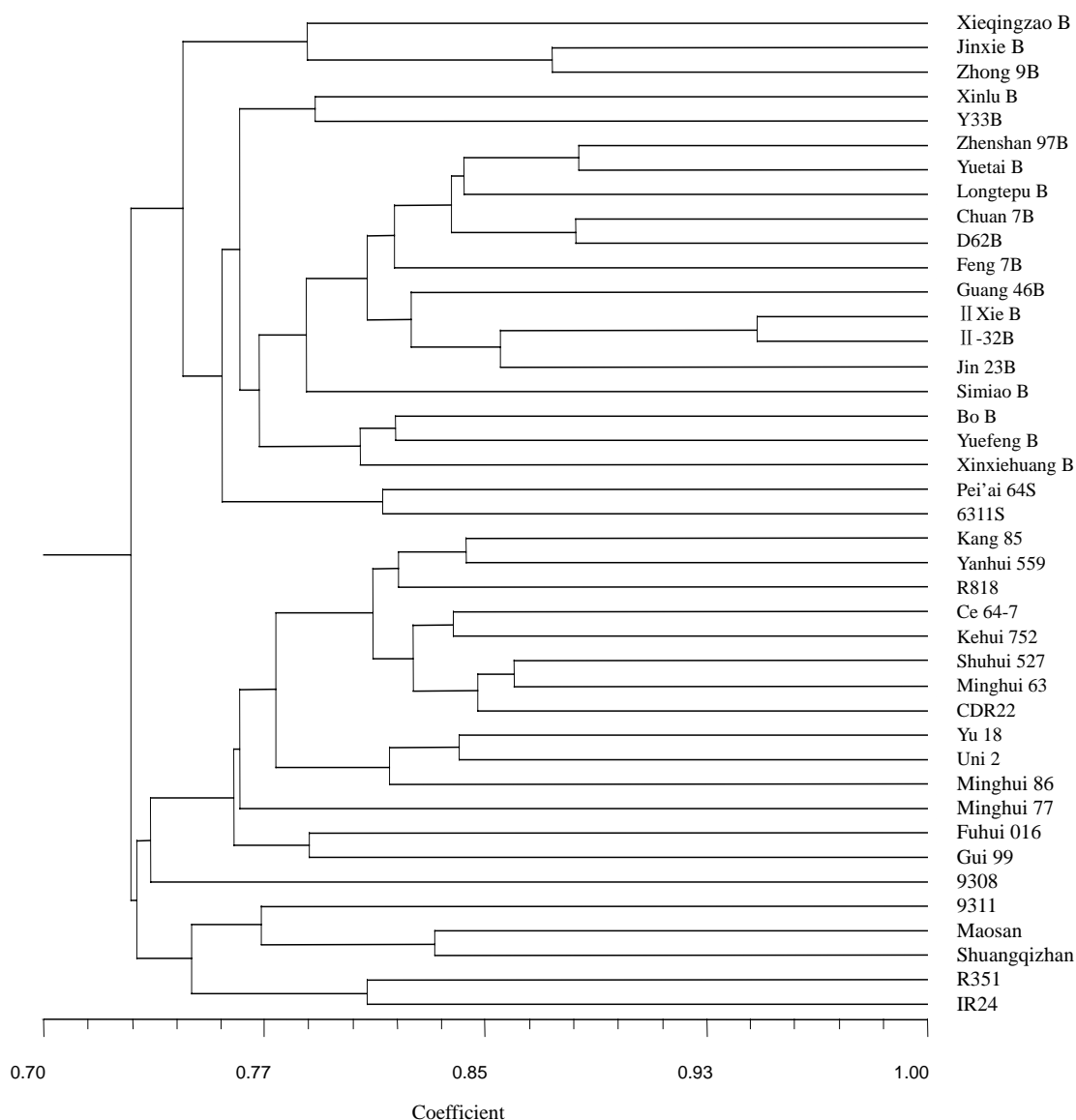
Coefficient

Fig. 2. Dendrogram of 41 parental lines of hybrid rice based on genetic similarity determined by SSR analysis.

In contrast to morphological trait, the molecular markers revealed polymorphism at the DNA level, suggesting a very powerful tool for characterization of genotype and estimation of genetic diversity. Among them the microsatellite or SSR (simple sequence repeats) markers showed a high potential for identification and estimation of genetic diversity [15-16]. The SSR markers played an important role in studying the germplasm diversity in rice [17-18]. During this experiment, the maintainer lines and restorer lines were clearly discriminated on the basis of SSR analysis. Furthermore, with higher polymorphism revealed by SSR markers, some of the parental lines have the similar genetic background from pedigree information. This grouping result is in agreement with pedigree information and hybrid rice breeding. The results and the current status of hybrid rice breeding indicated that SSR analysis could be a better method to study the diversity of parental lines in indica hybrid rice.

The current result indicates that SSR markers are of an indispensable complementation to pedigree analysis in identification of parental groups. In general, the pedigree analysis is considered to have no effect on selection and mutation. Therefore, pedigree analysis can't reveal the relationship between progeny and their parents exactly. On the contrary, SSR markers can detect genetic variation at DNA level. For example, Xieqingzao B and Jin 23B are the parents of Jinxie B, Xieqingzao B and Ⅱ-32B are the parents of Ⅱ Xie B. From pedigree, each parent contributes 50% of its genome on average to its F_2-derived inbred lines [19], so it is difficult to identify which parent is more similar to its progeny. SSR markers analysis

showed that Jinxie B was more similar to Xieqingzao B than to Jin 23B, and ⅡXie B was more similar to Ⅱ-32B than to Xieqingzao B (Fig. 2). Furthermore, the lines without any clear pedigree record can also be classified into their corresponding parental groups by SSR markers. In other words, combination of pedigree analysis and SSR markers will be helpful in more reliable grouping.

Heterotic groups and patterns are important in hybrid breeding. A heterotic group can be defined as a collection of germplasm, which tends to exhibit a higher degree of heterosis (on average) when crossed with germplasm from an external group than those crossed with a member of its own group [20]. In general, a heterotic group is a collection of closely related inbred lines. Generally, the higher co-ancestries found within a heterotic group, while the lower co-ancestries noted between the two heterotic groups [21]. The identification of heterotic groups and patterns among breeding populations and lines provides fundamental information in order to help the plant breeders to gain more information on heterosis [22]. To date, in hybrid rice breeding, a systematic study aimed at classifying these breeding lines into heterotic groups has not been reported. Therefore, the grouping result based on SSR analysis may be helpful to identify heterotic group for hybrid rice breeding.

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