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Genetic Analysis of Early Generation Stability in Rice

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Abstract: The mechanism of early generation stability (EGS) in rice was studied via genetic analysis. Three types of crosses were made, namely between EGS varieties, EGS and conventional rice variety, and conventional rice varieties. The genetic analysis was based on the stable lines in F_2 population. The stable lines may appear from some combinations of EGS rice crossing with each other and EGS rice crossing with conventional varieties at different frequencies, but stable lines didn't appear in conventional varieties crossing with conventional varieties. Genetic analysis results indicated that the EGS phenomena should just exist in special rice materials, and the frequency of stable lines was closely related to the EGS traits of parents. The EGS traits were neither qualitative nor quantitative traits, and they were controlled by neither dominant genes nor recessive genes. The EGS traits might be inherited by F_1 single plant, and the traits of F_3 and F_4 were corresponded to those of F_2 population, i.e. F_3 and F_4 lines derived from non-segregating F_2 showed uniform agronomic traits, and those from segregating F_2 did not. The agronomic traits of EGS lines were consistent with those of F_1 single plant. On the other hand, when EGS lines occurred, the segregating lines in Mendelian manner were also observed in all F_2 population of the same combination. It was suggested that the reason why the stable strains occurred might be a special factor to control (open/close) gene at the beginning of cell division in zygote, resulting in closing mitosis and opening somatic reduction. The somatic reduction of zygote resulted in recombination and homozygosity forming in F_1 single plant, and some lines with uniform agronomic traits were observed in some lines of F_2 population.

Key words: rice (*Oryza sativa*); early generation stability; stable line; genetic analysis

As demonstrated by rice breeding practice, it generally requires 7-10 generations of self-pollination to obtain pure lines (almost complete homozygosity) from initial cross [1, 2]. However, some plant individuals with uniform performances, showing no character segregation in later generations, might occur in F_2 or F_3 rice breeding population [3–9]. Such occurrence was referred to as early generation stability (EGS) by Wu et al [7]. EGS was attributed to apomixis in earlier studies [5, 6]; however, investigation on the reproductive property disapproved the presence of apomixis in EGS lines [10]. The stable lines were obtained in F_2 or F_3 progenies by SAR-4 and SAR-3 crossing with conventional varieties and the stable lines crossing with conventional varieties at Rice Research Institute of Sichuan Agricultural University. It showed that the stable speciality could be passed on in its progenies [7–9]. In recent ten years, the studies on the phenomenon of EGS were mainly focused on identifying its true or false hybrids and ensuring its homozygosity by molecular marker, but no linkage-gene about EGS had been reported. Genetic analysis of EGS had not been conducted because genetic population was not easily to be constructed by crossing SAR with conventional variety.

In the present study, 130 combinations obtained from nine early generation stability rice (E) crossing with seven conventional varieties (C) were used to study the early generation stability and its genetic mechanism.

MATERIALS AND METHODS

Materials

The origin and type of parents were listed in Table 1.

Methods

The 130 combinations were obtained from E×E, E×C, C×C at the Rice Research Institute of Sichuan Agricultural University, Wenjiang, Sichuan Province

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Table 1. Origin and type of parents.

Name	Type	Origin	Name	Type	Origin
950	EI	SAR-3×Nanjing 11	429	EI	SAR-4×84-15
422	EI	SAR-3×Sheng 47	992	EI	SAR-3×638480
407	EI	SAR-3×Kang 391	84-15	EJ	*Oryza longistaminate*×80-6195 (CAC)
412	EI	SAR-3×N625	84-23	EJ	Sisters of 84-15 (CAC)
421	EI	407×638480	718	indica	RRI, SAU
Nipponbare	japonica	Japan	93-11	indica	Jiangsu
Zhonghua 15	japonica	CAC	Shuhui 527	indica	RRI, SAU
IR60159	japonica	IRRI	Minghui 63	indica	Fujian

EI, Early generation stability rice (indica); EJ, Early generation stability rice (japonica).

in July 2002(Table 1).

F_1 population

F_1 progenies were grown in Lingshui, Hainan Province. Each combination was planted in 3-row plot, 12 plants per row, at 26.6 cm×16.6 cm row spacing. Female parents were planted beside for favoring the identification of pseudo hybrids.

F_2 population

Ten plants were selected randomly from each F_1 population and grown at Rice Research Institute of Sichuan Agricultural University, Wenjiang, Sichuan Province in 2003. Each combination was planted in 5-row plot, 12 plants per row, at 26.6 cm×16.6 cm row spacing. The populations with uniform agronomic traits were considered as stable lines and the others as segregating lines.

F_3 population

Ten plants selected randomly from F_2 stable lines and 30 plants selected randomly from F_2 segregating lines were planted in Lingshui, Hainan Province.

Statistical analysis of agronomic traits

The agronomic traits, including heading days, length of panicle, plant height, 1000-grain weight, length of grain and effective panicle number were recorded according to the method of Ying[11].

The results were statistically analyzed by using DPS 3.01 and SPSS 10.0 software.

Statistical method of frequency of stable lines

The frequency of stable lines was counted, based on the amount of stable lines in total lines of F_2 population. The frequency of stable lines of direct crossing was counted in combination E×C, and reciprocal crossing frequency was counted in C×E.

RESULTS

Genetic analysis of early generation stability rice

Genetic behave of E×E in F_2 population

The crossing of EI×EI, EJ×EI, EI×EJ and EJ×EJ were performed. The frequencies of stable lines in each crossing type were showed in Table 2.

In 21 combinations of E×E, 42 stable lines in F_2 populations were found in 9 combinations(4 EI×EI, 2 EJ×EJ and 3 EI×EJ), and segregating lines were observed in the other combinations (Table 2). Supposing that early generation stability genes were dominant genes or recessive genes, stable lines would be observed in all combinations derived from the same origin material of SAR-3. However, the results showed stable lines only appeared in the combinations of 992×950, 407×412, 421×412 and 407×421, no stable lines appeared in the combinations of 950×992, 412×407, 412×421 and 421×407, implying that the early generation stability was not controlled by single dominate genes or recessive genes, and could not be explained by the theory of classical genetics.

Genetic behave of E×C in F_2 population

As listed in Table 3, 36 stable lines were observed in 19 combinations (totalled 736 lines) of F_2 population in total of 90 combinations. The frequency was 4.77%. The stable lines were obtained in each crossing type of EI×I, EI×J, EJ×I and EJ×J

Table 2. Stable lines in F_2 population of early generation stability crossing with early generation stability rice.

Cross	Crossing type	Total lines in F_2	Stable lines	Stable：Segregating
950×84-15	EI×EJ	8	0	0：8
950×84-23	EI×EJ	4	0	0：4
950×429	EI×EI	8	0	0：8
950×992	EI×EI	6	0	0：6
950×84-23	EI×EJ	4	0	0：4
992×950	EI×EI	8	1	1：7
992×429	EI×EI	8	0	0：8
84-15×950	EJ×EI	8	0	0：8
84-15×992	EJ×EI	8	3	3：5
84-15×84-23	EJ×EJ	9	9	9：0
84-23×84-15	EJ×EJ	4	3	3：1
84-23×429	EJ×EI	8	3	3：5
84-23×950	EJ×EI	8	1	7：1
84-23×992	EJ×EI	7	0	0：7
412×421	EI×EI	8	0	0：8
412×407	EI×EI	8	0	0：8
421×429	EI×EI	7	0	0：7
421×412	EI×EI	7	7	7：0
421×407	EI×EI	7	0	0：7
407×412	EI×EI	7	7	7：0
407×421	EI×EI	8	8	8：0
Total		146	42	42：104

EI, Early generation stability rice (indica); EJ, Early generation stability rice (japonica); I, indica rice; J, japonica rice.

combination. Seventeen stable lines appeared in crossing E×C in total of 343 lines, and 19 stable lines in reciprocal crossing C×E in total of 413 lines of F_2 population. The frequency of crossing was equal to that of reciprocal crossing. It was suggested that cytoplasm genetic effect to early generation stability in rice was excluded.

Genetic behave of C×C in F_2 population

No stable line appeared in crossing type of C×C in 61 lines of F_2 population. It showed that there was not special factor in varieties to produce stable lines.

Frequency of stable line

Table 4 showed that the frequency of stable line in crossing type of E×E and E×C was 28.77% and 4.76%, respectively. In crossing type of E×E, the frequency of stable lines of EI×EI, EI×EJ and EJ×EJ was 12.73%, 28.49%, and 92.31%, respectively. In direct crossing type of E×C and reciprocal cross of C×E, the frequency was 4.96%, and 4.6%, respectively (Table 3, Table 4). No stable line appeared in crossing type of C×C. The frequency of early generation stable line was 8.96% in F_2 population.

Comparative analysis of stable lines

Comparison among stable lines obtained from crossing type 429×9311 in different years

One stable line was obtained in crossing type 429×9311 by Xie[13] in 2002. It was testified to be true hybrid by SSR marker, and 30 single plants were identified to be homozygote by agronomic traits analysis and SSR marker. The other stable line was obtained by Li in 2003 [14], and it was identified by the same methods as Xie [6]. But the two stable lines greatly differed in agronomic traits (Table 5).

It is difficult to explain the occurrence of the two stable lines with different agronomic traits in the same crossing combination in different years. Even though gamete mutated, if there was no chromosome recombination in the development of embryo, this phenomena should not happen. So it should be attributed to gene recombination in zygote development caused by gamete mutation.

Comparison on the two stable lines with different agronomic traits in the same combination

There were two kinds of stable lines with different agronomic traits in the four combinations

Table 3. Statistics of stable lines in F_2 population of early generation stability rice crossing with conventional rice varieties.

Cross	Crossing type	Total lines in F_2 population	Stable lines	Stable：Segregating
950×718	EI×I	7	1	1：6
422×IR60159	EI×J	5	1	1：4
992×9311	EI×I	8	1	1：7
992×Shuhui 527	EI×I	7	1	1：6
992×718	EI×I	8	1	1：7
84-15×IR60159	EJ×J	3	2	2：1
84-15×718	EJ×I	7	1	1：6
84-23×Nipponbare	EJ×J	4	2	2：2
84-15×Zhonghua 15	EJ×J	8	2	2：6
718×950	I×EI	8	3	3：5
718×84-15	I×EJ	5	2	2：3
412×9311	EI×I	8	3	3：5
421×IR60159	EI×J	8	1	1：7
407×Shuhui 527	EI×I	9	1	1：8
Nipponbare×992	J×EI	8	3	3：5
Nipponbare×84-15	J×EJ	8	5	5：3
Zhonghua 15×84-15	J×EJ	8	3	3：5
Zhonghua 15×84-23	J×EJ	8	2	2：6
Shuhui 527×407	I×EI	8	1	1：7
Other crosses		621	0	0：621
Total		756	36	36：720

EI, Early generation stability rice (indica); EJ, Early generation stability rice (japonica); I, indica rice; J, japonica rice.

Table 4. Frequencies of early stable lines appeared in different crossing types.

Frequency	E×E				E×C			C×C
	EI×EJ	EI×EI	EJ×EJ	Total	E×C	C×E	Total	
Total of F_2 lines	55	78	13	146	343	413	756	61
No. of early stable lines	7	23	12	42	17	19	36	0
Stable lines rate (%)	12.727	29.487	92.308	28.767	4.956	4.600	4.762	0

E, Early generation stability rice; C, Conventional variety; EI, Early generation stability rice (indica); EJ, Early generation stability rice (japonica); I, indica rice; J, japonica rice.

Table 5. Statistics based on agronomic traits of stable lines in different years by crossing 429×9311.

Origin of line	Year	Days to heading (d)	Plant height (cm)	Panicle length (cm)
Xie et al[14]	2002	118.39±1.88	112.50±3.27	25.94±0.75
Li et al[15]	2003	107.96±4.02	128.19±7.58	26.37±2.27

(Table 6), which were testified to be true hybrid of the two parents. For several characters, including plant height, days to heading, panicle length and no. of panicles, the two kinds of stable lines within the descendants of the same combination were rather different. This result showed that stable lines were produced not only by gamete mutation but also by other complicated factors.

Comparison of stable lines obtained from direct and reciprocal crossing

Two stable lines were found in F_2 population of 718×84-15, and one stable line in 84-15×718. The three stable lines were different in agronomic traits (Table 7), indicating the complexity of the inheritance of early-generation stability further.

Agrinomic traits in F_3 population of segregating line

The seeds from 30 single plants in F_2 segregating lines were sown in Lingshui, Hainan in 2003, and no stable lines were observed.

The seeds from 70 single plants in F_2 segregating lines were planted into F_3 population in Wenjiang, Sichuan in 2004. The results showed that segregating lines were the same as those in Hainan, but no stable line appeared. This results showed that the differences existed in ployploid (SAR-4, SAR-3)×diploid [7, 8] and ployploid×ployploid.

Table 6. Comparison of two stable lines with different agronomic traits in the same cross.

Cross	No. of stable lines	Days to heading (d)	No. of effective panicles per plant	Plant height (cm)	Panicle length (cm)
84-15×IR60159	553	107.0±1.6(0.503)	16.6±3.8(20.579)	123.8±2.7(2.176)	22.9±1.1(4.893)
	558	107.2±0.8(0.790)	14.7±3.1(20.483)	111.7±3.9(3.493)	21.4±0.7(3.320)
84-15×992	628	108.5±1.2(0.154)	10.2±3.8(20.355)	116.9±9.9(2.689)	22.9±1.9(1.460)
	638	107.6±0.9(0.801)	8.6±1.8(20.019)	123.0±3.8(3.044)	21.5±0.9(3.970)
84-23×Zhonghua 15	713	106.7±1.2(1.100)	9.0±2.6(17.627)	126.3±3.6(2.818)	22.0±1.7(4.588)
	723	108.9±0.8(0.728)	10.1±2.1(20.592)	120.0±3.0(2.499)	21.9±1.0(4.535)
718×84-15	1058	107.2±0.9(0.872)	9.1±2.4(25.391)	117.8±3.2(2.757)	20.8±1.0(4.955)
	1064	113.9±1.6(0.398)	6.4±2.6(17.671)	62.1±4.7(1.503)	15.5±1.3(1.556)

Data in parentheses are variance coefficient.

Table 7. Agronomic traits of stable lines in reciprocal crosses.

Cross	No. of stable lines	Days to heading (d)	No. of effective panicles per plant	Plant height (cm)	Panicle length (cm)
718×84-15	1058	107.2±0.9(0.872)	9.1±2.4(25.391)	117.8±3.2 (2.757)	20.8±1.0(4.955)
	1064	113.9±1.6(0.398)	6.4±2.6(17.671)	62.1±4. 7(1.503)	15.5±1.3(1.556)
84-15×718	684	106.4±1.9(0.754)	8.6±2.7(29.464)	118. 6±3.6 (3.080)	21.4±0.8(3.515)

Data in parentheses are variance coefficient.

DISCUSSION

This study approved that early generation stability phenomena surely existed in hybrid progenies of rice. Stable lines were obtained from the combination of early stable parents crossing with other conventional varieties, whereas no stable lines appeared in the combination of conventional varieties crossing with each other. The frequency of stable line by direct crosses was almost equal to that by reciprocal crosses. No stable lines were obtained in combinations 718×84-15, 84-15×718, 407×Shuhui 527, Shuhui 527×407, 950×718 and 718×950. We suggested that cytoplasmic inheritance should be excluded.

The stable line(s) of F_2 population was just deriving from the F_1 single plant, which could be testified by comparing the field records about agronomic traits. The F_2 stable line always had similar agronomic performance with the F_1 single plant, which revealed that the F_1 single plant was homozygote. The results had been identified by using SSR marker [7-9, 13] and also indicated that the character segregation in F_1 population might be caused by mutated gamete.

Within the F_2 descendants of the same combination, two types of lines existed, one with uniform agronomic traits and its progenies were not segregating, and the other was segregating line. And the character was quite uniform within the lines generated from the same panicle. The result was different from Wang et al [3], and consistent with Chen et al [5].

Genetic analysis implied that the early generation stable lines had been homozygosity in their F_1 single plant and its previous period, and gene recombination had happened in development of early generation stable line. So studies on early generation stability in rice should be focused on cytological study.

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ERRATUM

At Page 57 (Vol. 12, No. 1, 2005), institution of the authors, and corresponding author should be amended. The following is correct.

Canopy Spectral Reflectance Characteristics of Rice with Different Cultural Practices and Their Fuzzy Cluster Analysis

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Abstract: The influence of major cultural practices including different nitrogen application rates, population densities, transplanting leaf ages of seedling, and water regimes on rice canopy spectral reflectance was investigated. Results showed that increased nitrogen rates, water regimes and population densities and decreased seedling ages could enhance reflectance at NIR (near infrared) bands and reduce reflectance at visible bands. Using reflectance of green, red and NIR band and ratio index of 810–560 nm could distinguish the different type of rice by fuzzy cluster analysis.

Key words: rice; cultural practices; canopy reflectance spectrum; fuzzy cluster analysis

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