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Unwanted self-pollination in crossing programs interferes with trait improvement and variety breeding

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ABSTRACT

Clear-cut identification of elite crop varieties and hybrids is essential for guarantying purity of hybrid seeds. Unwanted self-pollination in field or during crossing programs is one of the major sources of impurity of hybrid seeds that interferes with trait improvement via conventional breeding programs or variety improvement via backcross scheme. Conventional characterization of hybrid seeds based on specific morphological and agronomic data is time-consuming, restricted to a few characteristics, and is influenced by environment. In contrast, DNA-based markers are highly heritable, available in high numbers, and exhibit enough polymorphism; hence they can be used to trace the alleles came from a given parent. To estimate the interference rate of self-pollination with the variety improvement, hybrid seeds in several backcross generations were studied using SSR markers. Results showed that in the case of hybrid seeds produced under uncontrolled low-stringent conditions frequency of off-types seeds ranged from ~17% (for BC_1 of cross Sadri x Neda) to as high as 50% (for BC_3 of cross Sadri x Neda) and averaged up to 40%, that may be seem an unexpected value. However, in the case of hybrid seeds produced under completely controlled conditions any off-type seeds were not found. Thus, we suggest that make crosses under completely controlled conditions to guarantee the production of true hybrid seeds.

Key words: Rice, self-pollination, crossing, improvement.

INTRODUCTION

Rice is a major staple food crop and serves as a carbohydrate source for more than one third of the world's population. More than 90% of the world's rice production is produced and consumed in Asia. With respect to doubling of population of rice eaters in this area by 2025, the demand for rice is expected to outstrip its production. Two technological options for overcoming the problem are (1) exploitation of heterosis and (2) improvement of new high-yielding varieties with desirable traits. For exploitation of heterosis rice breeders utilize three-line hybrid system (CMS line, maintainer line and restorer line) [9]. In the case of former program, it is obvious that any impurities in the hybrids would reduce the expected yield. It has been estimated that every 1% mixture of female line seed in the hybrid seed results in yield reduction of 100 kg per hectare [5].

The Indian seed act prescribes that for hybrid rice the purity should be 98% [8], while in China it is mandated that the purity of hybrid rice should be at least 96% [12]. The fingerprinting of rice hybrids is very important for plant improvement, variety registration, distinctness, uniformity and stability testing (DUS), and seed purity testing. Therefore, clear-cut identification of elite crop varieties and hybrids is essential for protection and prevention of unauthorized commercial use [6]. On the other hand, purity of hybrid seeds supplied to farmers must surpass 96% [4]. Conventional characterization of hybrids based on specific morphological and agronomic data is time-consuming, restricted to a few characteristics, and is influenced by environmental condition. In contrast, DNA-based markers are highly heritable, available in high numbers, and exhibit enough polymorphism; hence they can be used to discriminate closely related genotypes of a plant [11, 13]. For these purposes, several types of molecular markers including allozymes [3], RAPDs [1, 4, 10], SSRs [6, 13] and STS [13] have been used.

For improvement of a high-yielding variety with a desirable trait usually rice breeders use the backcross scheme in which a commercial high-yielding variety is crossed to a variety with the desirable trait (such as early maturity, diseases resistance etc.) and then the F_1 hybrid is repeatedly backcrossed to commercial variety as recurrent parent. In the case of this program, unwanted self-pollination during backcross scheme undoubtedly reduces the efficacy of breeding program and makes longer the needed time for recovery of the genome of recurrent parent and even it may not be resulted to a given purpose. In this field, there are not any case studies to estimate the interference rate of self-pollination with the trait improvement via backcross scheme. Therefore, here we firstly report the estimation of interference rate of self-pollination with the trait improvement in several backcross schema utilizing molecular SSR markers.

MATERIAL AND METHODS

Plant material

In this study were analyzed different generations of three separate crosses of rice including Sadri (P_1) x Neda (P_2) (generations BC_1, BC_2 and BC_3), BC_1 generation of NedaA (P_1) x IR36 (P_2), and IR68897 (P_1) x Usen (P_2) (generations BC_5 and BC_6). In first cross, 15, 30 and 25 plants were produced, respectively, for BC_1, BC_2 and BC_3 generations. BC populations for this cross were produced under low-stringent conditions (that is, without removing emerging or emerged panicles in a hill). Six, 10 and 8 plants respectively were randomly selected for molecular evaluations by SSR markers. In second cross, BC1 generation consisted of 25 plants, 11 out of them were evaluated at molecular level by SSR markers. In third cross, 55 and 20 plants were produced, respectively, for BC_5 and BC_6 generations. BC_6 population was produced under completely controlled (high-stringent) conditions in a crossing cabinet (that is, in an isolated environment and removing all the emerging or emerged panicles, except those panicles to be crossed with recurrent parent). Thirty and 8 plants in each BC population, respectively were selected for molecular evaluations by SSR markers.

DNA extraction and PCR conditions

Young leaves were collected from the parental lines and BC plants. Total genomic DNA was isolated from the leaves according to CTAB method [7] with some modifications [2]. DNA was quantified on 1% agarose gel, diluted and used in PCR. Polymerase chain reaction (PCR) was performed in 15 µl volumes containing 0.75 µM/l of each primer, 7.5 µl master mix (200 µM/l dNTPs, 50 mM/l KCl, 10 mM/l Tris HCl, 1.5 mM/l MgCl2, and 1 unit of Taq DNA polymerase (Cinnagen, Iran)) 5 µl H2O and 1 µl DNA (50 ng/µl). The PCR profile was 94 oC for 5 min (denaturation), followed by 35 cycles of 94 ^{o}C for 35 s., 55 ^{o}C for 1 min, 72 ^{o}C for 2 min, and

finally 72 ^{o}C for 7 min in the final extension. The PCR products were resolved by electrophoresis in 3% agarose gel containing 0.5 µg/ml ethidum bromide.

SSR analysis

Ninety SSR primer pairs were used for detection of polymorphism between parents of each cross. Polymorphic markers were used for producing banding pattern in BC progenies. Donor (P_1) allele was scored as BB, recurrent (P_2) allele as AA and a heterozygote as AB. As in all crosses second parent (P_2) was recurrent parent, one can expect that all BC plants should show AB or AA genotype. Thus, observing the BB genotype must be considered as the result of self-pollination (selfing).

RESULTS AND DISCUSSION

Cross Sadri x Neda

Thirty out of 90 SSR primer pairs could detect polymorphism between two parents. These 30 markers were used for tracing the alleles of parents in each progeny within each BC population. In BC_1 generation, six out of 20 progeny were randomly selected and amplified by 30 polymorphic markers. One can expect that all BC_1 plants should show AB or AA genotype with 1:1 ratio. At twenty four marker loci none of 6 BC_1 progenies showed BB genotype. However, at rest 6 loci one BC_1 plant showed BB genotype (Table 1). These results indicated that this BC_1 plant received its two alleles due to segregation after selfing (AB x AB gives AA, AB or BB genotypes with 0.25: 0.50: 0.25 ratio), while its maternal F_1 parent (with AB genotype) was backcrossed to Neda (with AA genotype) as recurrent parent.

In BC_2 generation, 10 out of 25 progenies were selected and amplified by 30 polymorphic markers. At twenty eight marker loci none of 10 BC_1 progenies showed BB genotype. However, at rest 2 loci two BC_2 plants showed BB genotype (Table 1). These results indicated that these BC_2 plants received their two alleles due to segregation after selfing.

In BC_3 generation, 8 out of 30 progenies were selected and amplified by 30 polymorphic markers. At twenty one marker loci none of 8 BC_1 progenies showed BB genotype. However, at rest 9 loci four BC_3 plants showed BB genotype (Table 1). These results indicated that these 4 BC_3 plants received their two alleles due to segregation after selfing. Altogether, results indicated that selfing rate in this cross (sadri x Neda) ranged from 16.7% to 50%, with average of ~29%.

Cross NedaA x IR36

Twenty out of 90 SSR primer pairs could detect a visible polymorphism between NedaA and IR36. At 17 loci no BB genotype was detected. However, at rest three SSR loci 5 out of 11 BC_1 plants showed BB genotype (Table 2). These results indicated that these 5 BC_1 plants received their two alleles due to segregation after selfing, either by pollens from same spikelet due to non-careful emasculation or by pollens from emerged panicles in the same plant.

Cross IR68897 x Usen

Thirty two out of 90 SSR primer pairs could detect polymorphism between two parents (IR68897 and Usen). These 32 markers were used for tracing the alleles of parents in each progeny within each BC population. In BC_5 generation, 30 out of 55 progenies were randomly selected and amplified by 32 polymorphic markers. At twenty eight marker loci none of 30 BC_5 progenies showed BB genotype. However, at rest 4 loci fourteen BC_5 plants showed BB genotype (Table 2). These results indicated that these 14 BC_5 plants received their two alleles due to segregation

Table 1. Allele tracing in randomly selected BC progenies using polymorphic SSR markers in the case of Sadri x Neda cross. BC seeds were produced under low-stringent conditions

Population	Pedigree (donor/recurrent)	N. plants	N. analyzed plants	SSR marker	1	2	3	4	5	6					N. Selfed plants
BC_1	Sadri x Neda	20	6	RM4835	AA	AA	AB	AA	AB	**BB**					
				RM207	AA	AB	AB	AB	AB	**BB**					
				RM219	AB	AB	AA	AB	AA	**BB**					
				RM228	AB	AB	AB	AB	AB	**BB**					
				RM485	AA	AB	AB	AB	AB	**BB**					
				RM1335	AA	AB	AB	AB	AB	**BB**					
				Result	NS	NS	NS	NS	NS	**S**					1 (16.7%)
					1	2	3	4	5	6	7	8	9	10	
BC_2	Sadri x Neda	25	10	RM302	**BB**	AB	AA	AB	AB	AB	AA	AB	AA	AB	
				RM228	**BB**	AA	**BB**	AB	AA	AB	AB	AB	AB	AB	
				Result	**S**	NS	**S**	NS	NS	NS	NS	NS	NS	NS	2 (20%)
					1	2	3	4	5	6	7	8			
BC_3	Sadri x Neda	30	8	RM302	AA	AB	AB	AB	AB	AB	**BB**	AA			
				RM206	**BB**	AB	AB	AB	AB	AB	AB	AB			
				RM505	**BB**	AA	AA	AB	AB	AB	AB	AB			
				RM279	AA	AB	AA	AB	**BB**	AA	AA	AB			
				RM334	AA	AB	AA	AA	**BB**	AA	AB	AA			
				RM485	**BB**	AB	AB	AA	AA	AA	AB	AA			
				RM228	AB	AA	AB	AB	**BB**	AB	AB	AB			
				RM273	**BB**	AB	AA	**BB**	AB	AB	AB	AB			
				RM207	AA	AB	AA	AB	BB	AA	AA	AA			
				Result	**S**	NS	NS	**S**	**S**	NS	**S**	NS			4 (50%)
Total		75	24												7 (29.2%)

S: selfed; NS: non-selfed. A allele came from recurrent parent (P_2) and B allele came from donor parent (P_1).

Table 2. Allele tracing in randomly selected BC progenies using polymorphic SSR markers in the case of NedaA x IR36 and IR68897 x Usen crosses. BC seeds were produced under low-stringent conditions

Population	Pedigree (donor/recurrent)	N. plants	N. analyzed plants	SSR marker	1	2	3	4	5	6	7	8	9	10	11	N. Selfed plants
BC_1	NedaA x IR36	25	11	RM3510	**BB**	AA	**BB**	**BB**	AA	**BB**	**BB**	AB	AA	AA	AA	
				RM1146	**BB**	AA	AB	**BB**	AA	**BB**	AB	AB	AA	AA	AA	
				Result	**Y**	N	**Y**	**Y**	N	**Y**	**Y**	N	N	N	N	5 (45.4%)
					1	2	3	4	5	6	**7**	8	9	10		
				RM566	AA	**BB**	AA	AB	**BB**	AB	**BB**	AA	AA	AB		
				RM334	AA	**BB**	**BB**	AB	AB	AA	**BB**	AA	AA	AB		
				RM505	AA	**BB**	**BB**	AA	**BB**	AB	**BB**	AB	AB	**BB**		
				RM5841	AA	**BB**	**BB**	AB	AB	AB	AA	AB	AB	AB		
				Result	N	**Y**	**Y**	N	**Y**	N	**Y**	N	N	**Y**		5
					11	12	13	14	15	16	17	18	19	20		
				RM566	AA	AB	AA	AB	**BB**	AB	AA	**BB**	**BB**	AB		
				RM334	AB	AA	AB	AB	AB	AA	AA	AA	AA	AB		
BC_5	IR68897 x Usen	55	30	RM505	AA	AA	**BB**	AB	AB	**BB**	AA	AA	AA	**BB**		
				RM5841	AA	AB	AB	AB	AA	AB	AB	**BB**	AB	AB		
				Result	N	N	Y	N	Y	Y	N	Y	Y	Y		6
					21	22	23	24	25	26	27	28	29	30		
				RM566	AA	**BB**	AB	AA	AA	AA	**BB**	AA	AB	AB		
				RM334	AB	AA	AB	AB	AB	AA	AB	AB	AA	AB		
				RM505	AB	AB	AA	**BB**	AA	AA	**BB**	AB	AA	AB		
				RM5841	AB	AB	AA	AB	AB	AB	AB	AB	AB	AA		
				Result	NS	**S**	NS	**S**	NS	NS	**S**	NS	NS	NS		3
Total		75	41													14 (46.7%)

S: selfed; NS: non-selfed. A allele came from recurrent parent (P_2) and B allele came from donor parent (P_1).

Table 3. Allele tracing in randomly selected BC_6 of IR68897 x Usen cross using polymorphic SSR markers. BC_6 seeds were produced under high-stringent conditions

Population	Pedigree (donor/recurrent)	N. plants	N. analyzed plants	SSR marker	1	2	3	4	5	6	7	8	N. Selfed plants
BC_6	IR68897 x Usen	20	8	RM311	AB	AB	AA	AA	AB	AB	AA	AB	
				RM1335	AB	AB	AB	AA	AB	AA	AB	AA	
				RM3773	AB	AB	AB	AA	AA	AB	AA	AB	
				RM131	AA	AA	AA	AA	AA	AA	AB	AB	
				RM1003	AB	AB	AB	AB	AA	AA	AA	AB	
				RM151	AB	AB	AB	AB	AB	AA	AB	AA	
				RM302	AB	AB	AB	AB	AA	AA	AA	AB	
				RM1146	AB	AB	AB	AA	AA	AB	AA	AB	
				RM118	AA	AA	AB	AA	AA	AA	AB	AA	
				RM1335	AB	AB	AA	AB	AB	AB	AA	AA	
				Result	NS	NS	NS	NS	NS	NS	N	N	0 (0%)

S: selfed; NS: non-selfed. Allele A came from recurrent parent (P_2) and allele B came from donor parent (P_1).

after selfing, either by pollens from same spikelet due to non-careful emasculation or by pollens from emerged panicles in the same plant. Altogether, results indicated that selfing rate in this cross was up to 46%.

In BC_6 generation for which crossing was dine under high-stringent controlled conditions, eight out of 20 progenies were randomly selected and amplified by 32 polymorphic markers. Here again, one can expect that all BC_6 plants should show AB or AA genotype. At all marker loci none of 8 BC_6 progenies showed BB genotype (ten of which depicted in Table 3). These results obviously indicated that all BC_6 plants received their alleles due to segregation after backcrossing to recurrent parent, because its maternal BC_5 parent (with AB genotype) was backcrossed under high-stringent controlled conditions to recurrent Usen parent (with AA genotype), and as expected AB x AA cross gave AA or AB genotypes (Table 3).

CONCLUSION

Our experience shows that unwanted self-pollination in crossing programs of rice under low-stringent conditions ranges between 17 to 50%, averaging as high as unexpected value of 40% (26 off-type seeds among 65 studied BC seeds) indicating that making crosses under uncontrolled conditions interferes with trait improvement and sometimes makes longer the breeding period. Thus, for overcoming the problem, controlling the crossing conditions guaranties the production of hybrid seeds, as we showed that making crosses under controlled conditions results into production of completely true hybrid seeds.

REFERENCES

[1] A. Ahmadikhah, *Novin Genet.*, **2009a**, 3(4), 65-74 (in Persian).
[2[A. Ahmadikhah, *Afr. J. Biotechnol.*, **2009b**, 8(2), 234-238.
[3] P.S. Devanand, J. Wan, M. Rangaswamy and H. Ikehashi, *Crop Sci.*, **1999**, 39, 831-835.
[4] M. Ichii, D.L. Hong, Y. Ohara, C.M. Zhao and S. Taketa, *Euphytica*, **2003**, 129, 249-252.
[5] C.X. Mao, S.S. Virmani, and I. Kumar, In: S.S. Virmani et al. (Eds.), Advances in hybrid rice technology (Directorate of Rice Research, Hyderabad, India, **1996**) 111–128.
[6] N. Nandakumr, A. Singh, K. Sharma, R.K. Mohapara, T.K.V. Prabhu and F.U. Zaman, *Euphytica*, **2004**, 136, 257-264.
[7] M.A. Saghai-Maroof, K.M. Soliman, R.A. Jorgensen and R.W. Allard, *Proc. Natl. Acad. Sci.*, **1984**, 81, 8014–8018.
[8] M.M. Verma, *Seed Technol. Newsl.*, **1996**, 26, 1–4.
[9] S.S. Virmani, Z.X. Sun, T.M. Mou, A. Jauhar Ali and C.X. Mao; Two-line Hybrid rice breeding manual, International Rice Research Institute, Los Baños (Philippines), **2003**, 324.
[10] S. Wang and Z. Lu, *Plant Breeding*, **2006**, 125, 606-612.
[11] Y. Wang, Y. Xue and J. Li, *Trends Plant Sci.*, **2005**, 10, 610-614.
[12] W. Yan, Crop heterosis and herbicide. *United States Patent*, **2000,** #6,066,779.
[13] J. Yashitola, T. Thirumurgan, R.M. Sundaram, M.K. Naseerullah, M.S. Ramesha, N.P. Sarma and R.V. Sonti, *Crop Sci.*, **2002**, 42: 1369-1373.