Predicting Success of *Indica/Japonica* Crosses in Rice, Based on a PCR Marker for the *S-5^n* Allele at a Hybrid-Sterility Locus

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ABSTRACT

Exploitation of crosses between the *japonica* and *indica* subspecies of rice (*Oryza sativa* L.) is hindered by hybrid sterility. However, germplasm containing the *S-5^n* wide compatibility allele, derived from tropical *japonica* (*javanica*), can be used as an intermediate in the transfer of traits. A PCR-based DNA marker, STS213, was used to identify the fraction of an F_3 population, segregating for *S-5^n* and the *japonica* allele *S-5^j*, that was most likely to yield fertile progeny from crosses with *indica* rice. Plants carrying the STS213 allele associated with wide compatibility, had significantly higher fertility than plants containing the *japonica* allele. The ability to detect seedlings bearing *S-5^n*, the wide-compatibility allele, will facilitate the introgression of this allele into temperate *japonica* cultivars while eliminating the need to test cross, self and score for fertility a majority of the individuals during introgression.

RICE CULTIVARS are classified into two major subspecies, *indica* and *japonica*. The *japonica* subspecies is composed of two groups, the temperate *japonicas* and the tropical *japonicas* (sometimes referred to as *javanicas*). For clarity, the temperate and tropical types are here referred to as *japonica* and *javanica*, respectively. Because of genes that cause semi-sterility of F_1 hybrids, exchange of desirable traits is limited in wide crosses involving the *indica* and *japonica* subspecies (Kato, 1930; Yanagihara et al., 1992). The hybrid-sterility locus *S-5* on chromosome 6 (Ikehashi and Araki, 1986), is associated with antagonism between the heterozygous *indica/japonica* maternal tissue (*S-5^i/S-5^j*) and female gametes carrying the *S-5^j* allele. As a result, *S-5^j*-bearing spikelets tend to be sterile. However, germplasm carrying a third allele of Indonesian origin, *S-5^n* or the *javanica* wide-compatibility allele, can cross readily with both *S-5^i* and *S-5^j* homozygotes without significant reduction in fertility of the F_1 hybrid (Terao and Mizushima, 1939; Ikehashi and Araki, 1986). Wide-compatibility varieties have been used successfully in rice breeding to produce fertile hybrid progeny (Ikehashi, 1991; Yuan 1994).

At present, the transfer of the *S-5^n* allele into improved *indica* or temperate *japonica* breeding lines is laborious. The presence of this allele can be detected only by first performing testcrosses (for example to an *indica* line if the *S-5^n* allele is being transferred into a *japonica* cultivar) followed by selfing the TC_1 progeny and measuring spikelet fertility or by Southern analysis with a linked RFLP marker, RG213 (Yanagihara et al., 1995). This report describes a PCR-based marker derived from RG213 and its efficient application in marker-assisted selection. This marker for *S-5^n* is applicable to assisting in the transfer of genes from *indica* lines into *japonica* lines, but not the reverse.

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MATERIALS AND METHODS

Plant Material

Four rice cultivars were used in the crossing work. The U.S. southern long-grain cultivar Lemont (PI 475833, Bollich et al., 1985) was previously classified as a tropical *japonica* (*javanica*) cultivar, based on RAPD markers (Mackill, 1995). Lemont was reported to possess the wide-compatibility trait (Zheng et al., 1994). In order to transfer the wide-compatibility allele *S-5^n*, Lemont was crossed in 1992 as a female to two related medium-grain temperate *japonica* cultivars: M-202 (population designated 'DX44') and M-204 (population designated 'DX45'). F_1 plants were grown in the greenhouse during the winter of 1993. The F_2 populations from single F_1 plants were grown in the field during the summer of 1993 at Davis, CA. Seeds were collected and F_3 progeny rows were again grown in the field in 1994.

Fourty-four F_3 plants from the DX44 and DX45 populations were transplanted to the greenhouse. These plants were assayed for the PCR marker STS213 (see molecular manipulations). About 37.5% of these plants were expected to be homozygous for the wide-compatibility allele *S-5^n*, and would be the most useful group for wide crosses. Fourteen selected plants of various genotypes (Table 1) were crossed with an *indica* tester, IR50R (received from Dr. S.S. Virmani of the International Rice Research Institute, Manila, Philippines). A single TC_1 plant from each F_3 parent was grown in the greenhouse and allowed to self pollinate. The percentage of filled grains was measured on 4 to 13 panicles per plant (average 7.5). The average number of spikelets per panicle was 158 [all spikelets were scored per panicle, unlike Yanagihara et al. (1992) who scored the top halves of each panicle]. Progeny of each selfed TC_1 plant were pooled and assayed for the PCR marker STS213 to determine whether the STS allele correlated with the fertility of the TC_1 parent.

Molecular Manipulations

DNA was isolated for use in PCR according to the method of Williams and Ronald (1994). This method requires only 1/3 cm^2 of leaf tissue, which can be harvested as soon as the first leaf is visible on seedlings. The PCR primers were designed from end sequences of the 1.2 kb RG213 Pst1 clone which previously was mapped within 5 cM of the *S-5* locus (Yanagihara et al., 1995). Primer sequences are: RG213f (5′ GAT ACC AGT GGT TAG CAC CAA ATG 3′) and RG213r (5′ AGG AGC GAA CTA GTA AGT TCG ACA 3′). When

Abbreviations: PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; S-5, hybrid sterility locus; *S-5^i*, *indica*-derived allele at the hybrid sterility locus; *S-5^j*, *japonica*-derived allele at the hybrid sterility locus; *S-5^n*; *javanica*-derived wide compatibility allele at the hybrid sterility locus; STS, sequence-tagged site.

Table 1. STS213 genotypes and spikelet fertility data for lines derived from a cross between a source of the wide compatibility allele, *S-5*^{n} and temperate *japonica* cultivars containing *S-5*^{j}.

F_3 Plant		Data from TC_1 (F_3/*indica*)			
Designation	ST213 Genotype†	No. of panicles	Mean no. spikelets per panicle	Filled grains (%)	TC_1 ST213 Genotype
DX45-2-1	j/n	12	155	5.5	j/i
DX45-19-3	j/n	13	209	6.1	j/i
DX44-7-2	j/j	7	179	40.0	j/i
DX44-11-1	j/j	11	135	19.9	j/i
DX45-21-1	j/j	7	196	39.9	j/i
DX45-22-3	j/j	6	179	56.4	j/i
DX45-5-1	j/n	6	143	71.9	n/i
DX45-5-2	j/n	6	136	56.7	n/i
DX44-12-3	n/n	6	164	42.7	n/i
DX45-15-1	n/n	5	104	80.0	n/i
DX45-26-1	n/n	7	104	52.5	n/i
DX45-33-2	n/n	7	182	61.5	n/i
DX45-33-3	n/n	7	160	27.3	n/i
DX45-8-2	n/n	4	171	61.8	n/i

† j = *japonica* allele STS213^j, n = wide-compatibility *javanica* allele STS213^n, i = *indica* allele STS213. The average % filled grains for *japonica* types (28.0%) and the wide compatibility *javanica* types (56.9%) were significantly different by *t*-test (P = 0.013).

the RG213 clone was used as a hybridization probe on Southern blots containing either genomic DNA or STS213 PCR products, only a single band (1.1 or 1.2 kb depending on the allele) was evident in each lane. Each amplification with STS213 primers consisted of 1 to 5 ng rice DNA in a mixture containing 10 m*M* Tris-HCl, pH 8.2; 50 m*M* KCl; 100 μ*M* each of dATP, TTP, dCTP, dGTP; 2 m*M* $MgCl_2$; 400 n*M* of each primer, STS213f and r; and 1 U Taq DNA polymerase per 25-μL reaction volume. PCR began with a denaturation step of 94°C for 1 min followed by 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min. Amplification products were separated on gels composed of 1 × TBE, 1% (w/v) Synergel (Diversified Biotech, Boston), and 0.6% (w/v) Ultra-Pure agarose (Gibco BRL, Gaithersburg, MD). In order to separate alleles of similar size, electrophoresis proceeded for 20 h at 90 V in a 4°C cold room. The amplification products were visualized by staining the gel with ethidium bromide before photographing over UV light.

RESULTS AND DISCUSSION

The use of the PCR-based marker STS213 allowed selection for *S-5*^{n}-containing wide-compatibility plants within a few days of germination (once the first leaf was visible). Amplification of target DNA at the STS213 marker locus resulted in a 1.2-kb product from plants containing the *S-5*^{j} allele, and a 1.1-kb product from plants containing either *S-5*^{i} or *S-5*^{n}. The marker, which is within 5 cM of the *S-5* hybrid sterility locus (Yanagihara et al., 1995), correctly identified *S-5* alleles from cultivars and breeding lines (STS213^j and *S-5*^{j}: Akihikari, S201, M103, M201, M202, M204, M401; STS213^n and *S-5*^{n}: Ketan Nangka, Moroberekan, Lemont, A301, L202, L203; STS213^i and *S-5*^{i}: IR36, IR50, data not shown) and was used to predict the genotype of F_3 individuals at the *S-5* locus (Fig. 1).

F_3 plants were crossed to the *S-5*^{i}-containing *indica* tester and the resulting TC_1 progeny were allowed to self-pollinate. We expected the highest degree of fertility from TC_1 plants that carried the STS213^n allele (genotype: STS213^n, *S-5*^{n}/STS213^i, *S-5*^{i}; Table 1, TC_1 STS213 Genotype n/i), whereas TC_1 plants that carried the STS213^j allele (genotype: STS213^j, *S-5*^{j}/STS213^i, *S-5*^{i}) were expected to have the lowest fertility because of antagonism between the heterozygous maternal tissue and the female gametes carrying the *S-5*^{j} allele. Table 1 summarizes the STS genotypes for the F_3 and TC_1 plants and percent fertility for the TC_1 upon self crossing. PCR results from pooled progeny of the TC_1 plants confirmed the lineage of these plants with respect to the F_3 progenitor genotype. More importantly, the STS genotype of TC_1 families indicated which *S-5* allele was passed from the four heterozygous *S-5*^{j}/*S-5*^{n} F_3 parents to the TC_1 individuals that were scored for fertility. It is clear that the two TC_1 individuals that inherited the *S-5*^{n} allele from their heterozygous F_3 progenitor were more fertile (71.9 and 56.7% filled grains) than the two that inherited the *S-5*^{j} allele (5.5 and 6.1% filled grains). Because the STS213 marker locus resides a few centimorgans from the *S-5* hybrid sterility locus, an occasional lack of correspondence between the marker and its predicted *S-5* allele (such as in the TC_1 plants descended from DX45-22-3 and DX45-33-3, Table 1) may be due to recombination. A second marker on the other side of the *S-5* locus would greatly improve the resolution of this marker-based assay, because a double recombination event would be required to separate both markers from the target allele. Additional PCR-based markers in the region of *S-5* are being developed which provide enhanced resolution when used in combination with STS213 (S. McCouch, 1997, personal communication).

Although the genotype of the F_3 plants at the STS213 locus is not a perfect predictor of fertility in TC_1 plants, it can be used to identify the F_3 plants (37.5% of the F_3 population: genotype *S-5*^{n}/*S-5*^{n}) that are homozygous for the wide-compatibility marker and are most likely to be fertile in crosses with *indica* lines. This eliminates the need to make test crosses followed by selfs and fertility assessments from the remaining 62.5% of the F_3 individuals in order to transfer *S-5*^{n} from *indica* to *japonica* lines. In our tests, four of six TC_1 plants that came from STS213^n/STS213^n F_3 progenitors (Table 1, n/n) had at least 50% filled grains (considered fertile

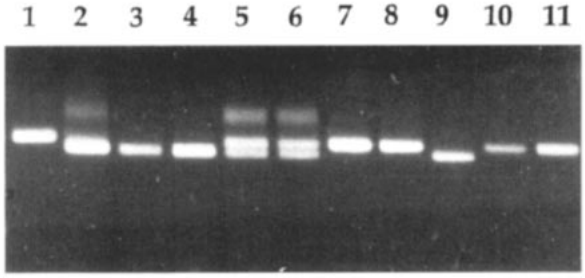

Fig. 1. Gel showing alleles at the STS213 locus. Lane 1, Akihikari, a *japonica* containing *S-5*^{j} and STS213^j; Lane 2, Ketan Nangka, a wide-compatibility *javanica* line containing *S-5*^{n} and STS213^n; Lanes 3 and 4, F_3 plants containing the STS213^n allele; Lanes 5 and 6, F_3 plants heterozygous for the STS213^j and STS213^n alleles; Lanes 7 and 8, F_3 plants containing the STS213^j allele; Lane 9, IR50, the *indica* tester containing *S-5*^{i} and the STS213^i allele; Lanes 10 and 11, M202 and M204, STS213^j-containing *japonica* parents of the F_3 lines.

for this study) after self crossing. Continued selection for the STS213^n allele during line advancement should result in *japonica* lines that contain *S-5^n* and will be able to produce fertile hybrids with *indicas*.

The primers for STS213 generated a band from both *indica* and *javanica* that was indistinguishable in size (Fig. 1). Thus, this marker is appropriate when transferring *S-5^n* into temperate *japonica* cultivars, in which case the test cross would be *japonica/javanica//indica*. When crosses are done in the order *indica/javanica//japonica*, the STS213^i/STS213^j, STS213^n/STS213^j, and STS213^n/STS213^n segregants are indistinguishable because of all three generating a 1.1-kb band. Thus, when transferring *S-5^n* into *indica* lines, the RFLP clone RG213 (from which our primers were derived) or the forthcoming linked STS markers (S. McCouch, 1997, personal communication) should be used as a probe on Southern blots, due to their ability to distinguish all 3 alleles in the *S-5* region (Yanagihara et al., 1995).

Markers for the hybrid sterility locus, *S-5*, are valuable because the ability to correctly evaluate a plant's fertility phenotype is affected by environmental factors as well as genetic background, making *S-5* difficult to use in breeding. These same factors confounded the certainty with which we were able to predict fertility of a TC_1 plant based upon the allele at STS213. This same problem was reported by Yanagihara et al. (1995) who find a continuous distribution of fertility, rather than a fertile and a sterile class of plants, in the population that was used to map RG213 with respect to *S-5*. They suggest that additional hybrid sterility loci, which have been identified in some genetic backgrounds (Yanagihara et al., 1992; Wan et al., 1993), may be present in their lines. In addition, when Yanagihara et al. (1995) applied QTL analysis to the study of hybrid sterility, *S-5* accounted for only 45% of phenotypic variability. However, during our transfer of genes from *indica* into *japonica*, the STS213 marker was predictive of the wide-compatibility genotype and was useful in identifying plants that would most likely lead to sterile progeny in wide crosses. Since STS213 is a PCR-based marker requiring minute quantities of DNA, seedling genotype can be determined within a day or two of germination. Thus, STS213 is useful for early selection of plants for use in wide crosses, eliminating the need to test cross, self and score for fertility, a majority of the plants in a population.

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REFERENCES

Bollich, C.N., B.D. Webb, M.A. Marchetti, and J.E. Scott. 1985. Registration of 'Lemont' rice. Crop Sci. 25:883–885.

Ikehashi, H. 1991. Genetics of hybrid sterility in wide hybridization in rice (*O. sativa* L.). p. 113–127. *In* Y.P.S. Bajaja (ed) Rice. Biotechnology in Agriculture and Forestry, vol 14. Springer, New York.

Ikehashi, H., and H. Araki. 1986. Genetics of F_1 sterility in remote crosses of rice. p. 119–130. *In* Rice genetics. IRRI, Manilla, Philippines.

Kato, S. 1930. On the affinity of cultivated varieties of rice plants, *Oryza sativa* L. J. Dep. Agric. Kyushu Imp. Univ. 2:241–276.

Mackill, D.J. 1995. Classifying *japonica* rice cultivars with RAPD markers. Crop Sci. 35:889–894.

Terao, H., and U. Mizushima. 1939. Some considerations on the classification of *Oryza sativa* L. into subspecies, so-called *japonica* and *indica*. Jpn. J. Bot. 10:213–258.

Wan, J., S. Yanagihara, H. Kato, and H. Ikehashi. 1993. Multiple alleles at a new locus causing hybrid sterility between a Korean *indica* variety and a *javanica* variety in rice (*Oryza sativa* L.). Jpn. J. Breed. 43:507–516.

Williams, C.E., and P.C. Ronald. 1994. PCR template-DNA isolated quickly from monocot and dicot leaves without tissue homogenization. Nuc. Acids Res. 22:1917–1918.

Yanagihara, S., H. Kato, and H. Ikehashi. 1992. A new locus for multiple alleles causing hybrid sterility between an Aus variety and *javanica* varieties in rice (*Oryza sativa* L.). Jpn. J. Breed. 42:793–810.

Yanagihara, S., S. McCouch, K. Ishikawa, Y. Ogi, K. Maruyama, and H. Ikehashi. 1995. Molecular analysis of the inheritance of the *S-5* locus, conferring wide-compatibility in *indica/japonica* hybrids of rice (*Oryza sativa* L.). Theor. Appl. Genet. 90:182–188.

Yuan, L.P. 1994. Increasing yield potential in rice by exploitation of heterosis. p. 1–6. *In* S.S. Virmani (ed.) Hybrid rice technology: New developments and future prospects. International Rice Research Institute, Los Baños, Philippines.

Zheng, K., H. Qian, B. Shen, J. Zhuang, H. Lin, and J. Lu. 1994. RFLP based phylogenetic analysis of wide-compatibility varieties in *Oryza sativa* L. Theor. Appl. Genet. 88:65–69.