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Identification of a Notched Kernel Gene Associated with Pre-Harvest Sprouting Using *Oryza glumaepatula* Introgression Lines in Rice

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ABSTRACT

Pre-harvest sprouting in rice is related to the lack of a normal dormancy level during seed development and maturation. The prominent effects of pre-harvest sprouting are lower yield due to harvest losses and end-product quality reduction. A single novel gene (nk2) for notched kernel was identified at backcross segregating population (BC4F2) of *Oryza glumaepatula* to *Oryza sativa* cv. Taichung 65 as recurrent parent. The nk2 gene was closely associated to pre-harvest sprouting character, and mapped on the long arm of chromosome 5 with 3.5 cM and 3.6 cM distance to G1103 and R521 RFLP markers, respectively.

Key words : Notched kernel, pre-harvest sprouting, genetic map, rice

INTRODUCTION

Precocious germination of cereal grain is a serious problem in crop production. Pre-harvest sprouting is the condition where germination of grains occurs in the spike before harvest. Prolonged rainfall and high humidity contribute to premature germination of grains before harvest. In south-east Asia, due the long spell of rainy weather in early summer and autumn, pre-harvest sprouting frequently happens and leads to great loss of yield and low quality in rice. Lack of adequate seed dormancy is the major reason for pre-harvest sprouting in the field especially under wet weather conditions (Seshu and Sorrells, 1986; Li, Ni *et al.*, 2004).

In many cereal crops, such as wheat, barley, pre-harvest sprouting is usually controlled by multiple genes. Although this trait is governed by multiple genes it is also highly heritable. The recent development of molecular markers has made it possible to identify individual genetic factors controlling such traits. Several QTLs conferring pre-harvest sprouting have been identified in wheat (Kulwal *et al.*, 2004, 2005; Gross *et al.*, 2002), in barley (Li, Ni *et al.*, 2004).

In rice, however, no information for pre-harvest sprouting has been reported. Recently, several rice molecular linkage maps have been developed by independent research groups (McCouch *et al.*, 1988; 2002; Causse *et al.*, 1994; Harushima *et al.*, 1998). These linkage maps have facilitated analyses of genetic controlling several traits, such as heading date (Yamamoto *et al.*, 2000; Yano *et al.*, 2000), grain quality (Aluko, 2004; Li, Xiao *et al.*, 2004), blast resistance (Zenbayashi *et al.*, 2002; Fjellstrom *et. al.*, 2004). By backcrossing and marker assisted selection methods, a series of *O. glumaepatula* introgression lines with *O. sativa*, cv. Taichung 65 genetic back-ground was developed (Sobrizal *et al.*, 1999). A single novel gene for pre-harvest sprouting in rice found during the development of this introgression lines was reported in this paper.

MATERIALS AND METHODS

Plant materials

A cultivated rice *Oryza sativa* L. cv. Taichung 65 as a female and a wild rice *O. glumaepatula* Steud. (Acc. IRGC 105668) as a male were used as the parents in the original cross. The resultant F_1 plants served as female parents and were continuously backcrossed with Taichung 65 to generate BC_4F_1 populations. One hundred eighty four BC_4F_1 plants were genotyped using 106 RFLP markers distributed evenly on the rice genome in order to select the candidate plants for a series of *O. glumaepatula* introgression lines (Sobrizal *et al.*, 1999). Based on these data, the plant BC_4F_1 274-3 carried heterozygous alleles at *R2289*, *R1553* and *C246* on chromosome 5 (Fig. 1). The self progenies of this plant (BC_4F_2 population) consisted of 59 plats segregated for notched kernels. This BC_4F_2 population was employed for genetic analysis.

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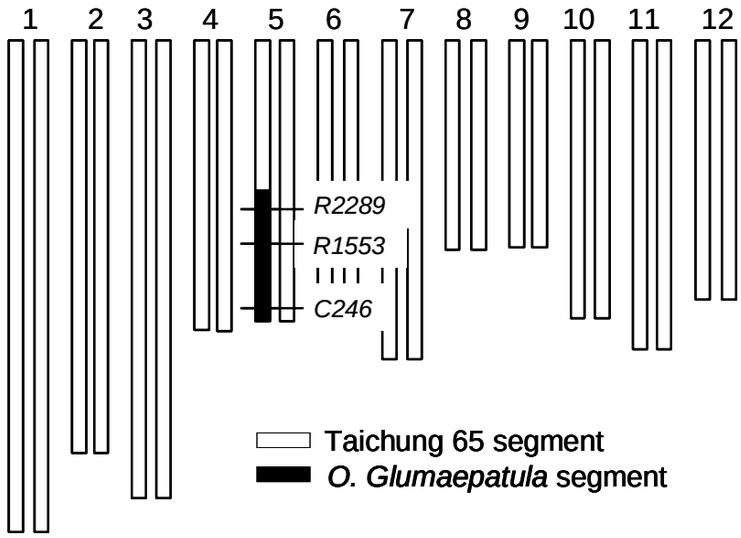

Figure 1. Graphical genotype of plant, BC_4F_1 274-3, carrying heterozygous alleles at *R2289*, *R1553* and *C246* on chromosome 5. The self progenies of this plant (BC_4F_2 population) were used in this study.

DNA extraction and RFLP analysis

DNA was extracted from frozen leaf samples using the CTAB method (Murray and Thompson, 1980). The isolated DNA (2.0 µg) was digested with restriction enzymes (*Apa* I, *Bam*H I, *Bgl* II, *Dra* I, *Eco* RI, *Eco* RV, *Hind* III, *Kpn* I), separated by 0.8% agarose-gel electrophoresis and blotted onto Hybond N^+ membranes (Amersham) by capillary transfer on 0.4 N NaOH solution. The blotted membranes were rinsed in 2 X SSC, dried and backed at 120 ^oC for 20 min. DNA clones, previously mapped by Tsunematsu *et al.* (1996) and Harushima *et al.* (1998), were used as DNA markers. DNA labeling, DNA hybridization and signal detection were conducted using the ECL detection system (Amersham).

Data analysis

Recombination values were estimated with the maximum likelihood equation (Allard, 1956). Obtained values were converted into map distances (cM) using the Kosambi function (Kosambi, 1944).

RESULTS AND DISCUSSION

In the process to develop *O. glumaepatula* introgression lines with *O. sativa* cv. Taichung 65 genetic background, we found plants with notched kernel (Fig. 2) segregated in a BC_4F_2 population. Notched kernels were characterized by wrinkled, brownish and white-cored, notched at the belly. When these notched seeds were incubated at 25 ^oC for 7 days, they germinated starting from 21 days after pollination, and the germination rate reaches 70% at 28 days after pollination (Ishibasi *et al*., 2005), while the normal seeds of Taichung 65 germinated only about 20% when they were treated by the same treatment. So, this notched kernel was higly associated with pre-harvest sprouting character.

Figure 2. Phenotype of notched kernel (left) and normal kernel (right) segregated in BC_4F_2 population.

Based on whole genome survey of candidate *O. glumaepatula* introgression lines, the BC_4F_2 population used in this study has retained *O. glumaepatula* chromosomal segment on chromosome 5. To determine the notched kernel gene location on the rice chromosome, RFLP analysis was conducted using this BC_4F_2 population. Several RFLP markers located on chromosome 5 were used in linkage analysis.

Phenotype of each BC_4F_2 plant was determined by observing BC_4F_3 seeds. Seeds were classified into normal, notched and segregating phenotypes. Linkage analysis between notched kernels and markers on chromosome 5 revealed that notched kernel co-segregated with *R1607* RFLP marker on the long arm of chromosome 5 (Table 1). Six plants with notched kernel phenotype were homozygous for *O. glumaepatula* allele, 24 plants with normal phenotype were homozygous for Taichung 65 allele, and the others 29 plants with segregating phenotype were heterozygous. These results indicate that a single recessive gene controlling notched kernel was closed to *R1607* on the long arm of chromosome 5.

Table 1. Relationship between notched kernel and genotype at *R1607* RFLP marker in BC_4F_2 population.

Phenotype	Genotype *R1607* [1)]		
Normal	24	0	0
Segregation	0	29	0
Notched	0	0	0

1) TT, TG and GG are Taichung 65 homozygous, heterozygous and *O. glumaepatula* homozygous alleles, respectively.

So far, only one dominant gene for notched kernel in rice (*Nk*) has been reported (Misro *et al*., 1966; Pavithran, 1977), however, there is no information whether this gene related to pre-harvest sprouting. *Nk* was linked *gl1* (glabrous hull 1) on chromosome 5 with a recombination value of 23% (Misro *et al*., 1966; Pavithran, 1977), and *gl1* mapped on the short arm of chromosome 5 (Yoshimura *et al*., 2001) was far from the position of the notched kernel gene identified in this study. *Nk* appeared to be different from notched kernel gene identified in this study, since it distances to the others and allelic interactions of these genes were differed. Therefore, the gene identified in this study was designated as *nk2*. The gene *nk2* was mapped between RFLP markers *G1103* and *R521* on the long arm of chromosome 5, with map distance of 3.5 cM and 3.6 cM, respectively (Fig. 3).

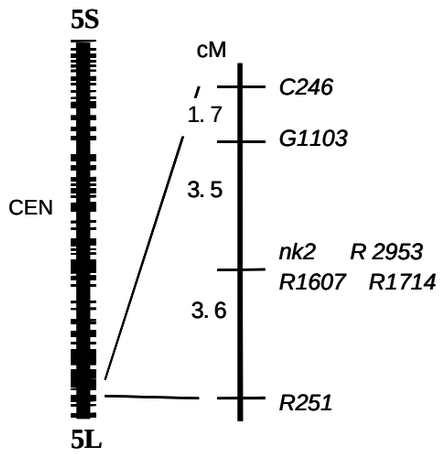

Figure 3. Linkage map of chromosome 5 showing the location of *nk2*. Framework map on the left was quoted from Harushima *et al*. (1998).

Pre-harvest sprouting results in significant economic loss for the grain industry around the world. Lack of adequate seed dormancy is the major reason for pre-harvest sprouting in the field under wet weather conditions (Seshu and Sorrells, 1986). On the other hand, very high level of dormancy proves undesirable in raising two to three crops a year of the same cultivar by using seed from one crop to raise the succeeding crop. It is also a problem for plant breeders to grow breeding materials in quick succession, and for seed technologists to test seed perform after harvesting.

A major QTL controlling both pre-harvest sprouting and seed dormancy has been identified on the long arm of barley chromosome 5H, and explained over 70% of the phenotypic variation (Li, Ni *et al*., 2004). Further more, Li, Ni *et al*. (2004) observed that the region of barley seed dormancy and pre-harvest sprouting QTL showed good synteny with the terminal end of the long arm of rice chromosome 3. Using molecular markers, researchers have identified several putative QTL for rice seed dormancy (Wan *et al*., 1997; Lin *et al*., 1998; Cai and Morishima, 2000). Molecular mechanisms that are involved in dormancy and pre-harvest sprouting along with quantitative and qualitative genetics will provide new strategies to produce crop varieties with desired level of dormancy. In addition, development of plant materials and RFLP map associated with pre-harvest sprouting in this study are the preliminary works to perform the isolation of gene controlling pre-harvest sprouting. Furthermore, this will be important in elucidating the stable biological functions of the pre-harvest sprouting.

CONCLUSIONS

Based on the results of this study, some conclusions were summarized as follow;

1. By using *Oryza glumaepatula* introgression lines, a novel gene for notched kernel was identified.
2. The gene was designated as *nk2* and was mapped between RFLP markers *G1103* and *R521* on long arm of chromosome 5.

REFERENCES

Allard, R.W. 1956. Formulas and tables to facilitate the calculation of recombination values in heredity. Hilgardia. 24:235-278.

Aluko, G., C. Martinez, J. Tohme, C. Castano, C. Bergman, J.H. Oard. 2004. QTL mapping of grain quality traits from the interspecific cross *Oryza sativa X O. glaberima*. Theor. Appl. Genet. 109:630-639.

Cai, W.H., H. Morishima. 2000. Genomic regions affecting seed shattering and seed dormancy in rice. Theor. Appl. Genet. 98:281-284.

Cause, M.A., T.M. Fulton, Y.G. Cho, S.N. Ahn, J. Chunwongse, K.S. Wu, J.H. Xio, Z.H. Yu, P.C. Ronald, S.E. Harrington, G. Second, S.R. McCouch, S.D. Tanksley. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. Genetics. 138:1251-1274.

Fjellstrom, K., C.A. Conaway-Bormans, A.M. McClung, M.A. Marchetti, A.R. Shank, W.D. Park. 2004. Development of DNA markers suitable for marker assisted selection of three Pi genes conferring resistance to multiple *Pyricularia grisea* pathotype. Crop Sci. 44:1790-1798.

Groos, C., G. Gay, M.R. Perretant, L. Gervais, M. Bernard, F. Dedryver, G. Charmet. 2002. Study of the relationship between pre-harvest sprouting and grain color by quantitative trait loci analysis in a white x red grain bread-wheat cross. Theor. Appl. Genet. 104:39-47.

Harushima, Y., M. Yano, A. Shomura, M. Sato, T. Shimano, Y. Kuboki, T. Yamamoto, S.Y. Lin, B.A. Atonio, A. Parco, H. Kajiya, N. Huang, K. Yamamoto, Y. Nagamura, N. Kurata, G.S. Khush, T. Sasaki. 1998. A high-density rice genetic linkage map with 2275 markers using a single F_2 population, Genetics. 148: 479-494.

Ishibashi, Y., T. Morita, R. Sueyoshi, A. Yoshimura, M. Fukuyama, M.I. Inoue. 2004. Early identification of pre-harvest sprouting in rice seeds by using ^{1}H-NMR. http://www.cropscience.org.au/icsc2004/poster/5/1/1/774_ishibashi.htm.

Kosambi, D. 1944. The estimation of map distance from recombination values. Ann. Eugen. 12:172-175.

Kulwal, P.L., R. Singgh, H.S. Balyan, P.K. Gupta. 2004. Genetic basis of pre-harvest sprouting tolerance using single-locus and two-locus QTL analyses in bread wheat. Funct. Integr. Genomics. 4:94-101.

Kulwal, P.L., N. Kumar, A. Gaur, P. Khurana, J.P. Khurana, A.K. Tyagi, H.S Balyan, P.K. Gupta. 2005. Mapping of a major QTL for pre-harvest sprouting tolerance on chromosome 3A in bread wheat. Theor. Appl. Genet. 111:1052-9.

Li, C., P. Ni, M. Francki, A. Hunter, Y. Zhang, D. Schibeci, H. Li, A. Tarr, J. Wang, M. Cakir, J. Yu, M. Bellgard, R. Lance, R. Appels. 2004. Genes controlling seed dormancy and pre-harvest sprouting in a rice-wheat-barley comparison. Funct Integr Genomics. 4: 84-93.

Li, J., J. Xiao, S. Grandillo, L. jiang, Y. Wan, Q. Deng, L.P. Yuan, S.R. McCouch. 2004. QTL detection for rice grain quality tarits using an interspecific backcross population derived from cultivated Asian (*O. sativa* L.) and African (*O. glaberrima* S.) rice. Genome. 47:697-704.

Lin, S.Y., T. Sasaki, M. Yano. 1998. Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. Theor. Appl. Genet. 96:997-1003.

McCouch, S.R., G., Kochert, Z.H. Yu, Z.Y. Wang, G.S. Khush. 1988. Molecular mapping of rice chromosomes. Theor. Appl. Genet. 76:815-829.

McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck. D. Schneider, S. Cartinhour, D. Ware, L. Stein. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res. 9:199-207.

Misro, B., R.H. Richharia, R. Thakur. 1966. Linkage studies in rice (*Oryza sativa* L.) VII. Identification of linkage groups in indica rice. Oryza. 3:96-105.

Murray, M., W.F. Thompson. 1980. Rapid isolation of high-molecular-weight plant DNA. Nucleic Acid Res. 8:4321-4325.

Pavithran, K. 1977. Inheritance and linkage relationship of notched kernel in rice (*Oryza sativa*). Can. J. Genet. Cytol. 19:483-486.

Seshu, D.V., M.E. Sorrels. 1986. Genetic studies on seed dormancy in rice. In: Rice Genetics. IRRI, Philippines, p. 369-382.

Sobrizal, Ikeda, K., P.L. Sanchez, K. Doi, E.R. Angeles, G.S. Khush, A.Yoshimura. 1999. Development of *Oryza glumaepatula* introgression lines in rice, O. sativa L. Rice Genet. Newsl. 16:107-108.

Tsunematsu, H., A. Yoshimura, Y. Harushima, Y. Nagamura, N. Kurata, M. Yano, T. Sasaki, N. Iwara. 1996. RFLP framework map using recombinant inbred lines in rice. Breed. Sci. 46:279-284.

Wan, J., T. Nakazaki, K. Kawaura, H. Ikehashi. 1997. Identification of marker loci for seed dormancy in rice. (*Oryza sativa* L.). Crop Sci. 37:1795-1763.

Yamamoto, T., H. Lin, T. Sasaki, M. Yano. 2000. Identification of heading date quantitative trait locus *Hd6* and characterization of its epistatic interactions with *Hd2* in rice using advanced backcross progeny. Genetics 154:885-891.

Yano, M., Y. Katayose, M. Ashikari, U. Yamanouchi, L. Monna, T. Fuse, T. Baba, K. Yamamoto, Y. Umehara, Y. Nagamura, T. Sasaki. 2000. *Hd1*, a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the arabidopsis flowering time gene *CONSTANS*. The Plant Cell. 12:2473-2483.

Yoshimura, A., N. Takano-Kai, T. Eguchi, M. Ashikari, O. Ideta, K. Doi, H. Yasui. 2001. RFLP mapping of 95 morphological trait loci in rice. Breed. Res. 3 (Suppl. 1): 94.

Zenbayashi, K., T. Ashizawa, T. Tani, S. Koizumi. 2002. Mapping of the QTL (quantitative trait locus) conferring partial resistance to leaf blast in rice cultivar Chubu 32. Theor. Appl. Genet. 104:547-552.