Identification and Mapping of RAPD Markers Linked to Rice Stripe Virus Resistance Gene, *Stvb-i* , in Rice(O. sativa L.)

Jun Hyeon Cho, D.Y. Kwak, K.H. Yi, Y.J. Weon, J.M. Ko, H.M. Park, S.J. Yang and **Min-Hee Nam**

Yeongnam Agricultural Research Institute, NICS, RDA, Milyang, 627-130, Korea , www.nyaes.go.kr Email nammhee@rda.go.kr

Abstract

Bulked segregation analysis(BSA) was used to identify random amplified polymorphic DNA(RAPD) markers linked to the rice stripe virus resistance gene, *Stvb-i*, in rice. Using two pools of phenotyped lines from a 169 DH population, we identified four RAPD markers positively linked to *Stvb-i* gene. The RAPD markers were mapped to chromosome 11 on the SSLPs map and one RAPD marker, OPO11, showed complete linkage to *Stvb-i*. When the selected specific marker was used on all of *japonica*, indica and tongil type varieties developed in Korea, the positive amplication of about 2500bp fragment generated from OPO11 was observed in only *japonica* varieties in which *Stvb-i* gene was used as a source of RSV resistance gene while the other susceptible *japonica* and all indica as well as tongil varieties showed negative reaction. Therefore, this can be used in the marker-assisted selection of stripe resistant individuals carrying the *Stvb-i* gene.

Media summary

A RAPD marker showing complete linkage to *Stvb-i* gene is being used in MAS breeding and has been converted into SCAR marker.

Key Words

Rice, RAPD marker, Stripe resistance gene, Bulked segregation analysis

Introduction

Rice stripe virus(RSV) transmitted by the small brown planthopper(Laodelphax striatellus) is causal agent of stripe disease. In Korea, the *Stvb-i* gene confers resistance to rice stripe disease was introduced by 'Mineyutake'(japanese *japonica* cultivar) originated from donor parent of indica cultivar 'Modan' to *japonica* variety 'Nakdongbyeo'(Milyang 15) in 1975. And subsequently many *japonica* varieties resistance to RSV was developed using 'Nakdongbyeo' as a donor of *Stvb-i* gene(Washio et al. 1968, Chung et al. 1975). By means of linkage analysis with molecular markers, the *Stvb-i* gene was eventually located on rice chromosome 11(Hayano-Saito et al. 1998). Recently, a SCAR marker 'ST10' derived from a RFLP marker has been developed in Japan(Hayano-Saito et al. 2000) and fine physical mapping of the *Stvb-i* gene was reported(Hayano-Saito et al. 2000). However, there is still no evidence of gene confer the rice stripe disease. To develope a RSV resistant variety, conventional screening methods has been carried out in the field as well as by bioassay with vector insects, but the results involved many technical problems such as controlling of viruliferous vector insects and sometimes remained uncertain reactions. To solve this problem, incorporation of molecular breeding to the conventional breeding has been developed. And MAS(marker-assisted selection) was employed in various fields(Chague et al. 1996).

In the study reported here, we identified a RAPD marker tightly linked to *Stvb-i* gene. And the specific marker identified by BSA analysis was confirmed in *japonica* varieties developed in Korea.

Methods

RAPD analysis was performed on a DH population obtained from a cross between 'HR10624-AC5' and 'Milyang123' carries *Stvb-i* gene. This DH population consisting of 169 lines was derived from anther culture technique and subjected to bioassay for linkage study. At the stage of 2-3 leaves, young seedlings

of each lines were inoculated with viruliferous vector of small brown planthopper for 3 days and score was recorded after 30 days of inoculation.

For BSA analysis, two bulks were prepared : a resistance and a susceptible bulks of DNA consisting each 10 lines were subjected to PCR amplication. A total of 260 single 10-mer oligonucleotide primer(Operon technologies) sets were employed for PCR together with 14 SSR markers to anchor their chromosomal locations. Marker order and map distances were estimated using MAPMAKER version 2.0.

Results

In the bioassay study, the 169 DH population showed distorted segregation of *Stvb-i* gene. The 118 lines showed to carry the *Stvb-i* gene while only 51 lines were susceptible to stripe disease.

In the RAPD study, only four RAPD markers were selected from BSA analysis and those selected markers were subjected to 169 individual lines to confirm the linkage to *Stvb-i* gene. The four markers were mapped onto the same linkage group of chromosome 11 and one RAPD marker OPO11(5'-GACAGGAGGT-3') generating about 2500bp fragment was located on the same locus with the *Stvb-i* gene. To confirm the utility of MAS, various varieties were subjected to the specific marker of OPO11. All the *japonica* varieties sharing the *Stvb-i* gene originated from 'Nakdongbyeo' showed positive reaction while the other susceptible *japonica* varieties and all indica varieties as well as tongil type varieties were negative to the OPO11 marker.

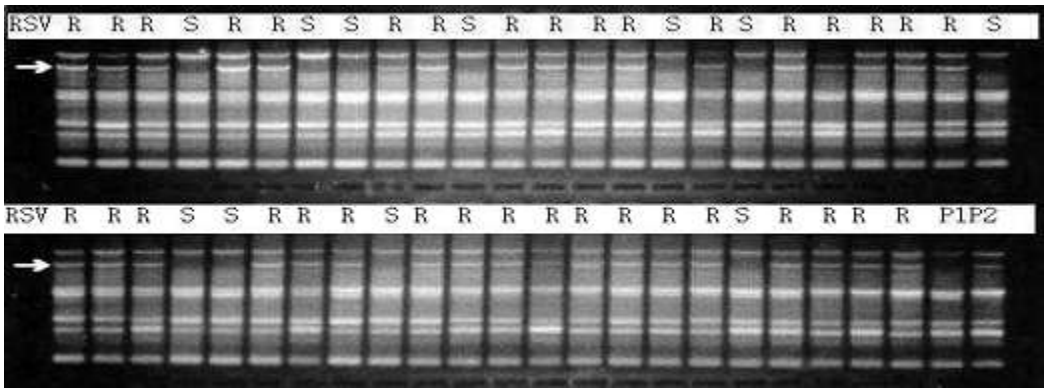

Figure 1. RAPD pattern of OPO11 in the 169 DH population. R, S and P1, P2 represents resistant and susceptible to stripe disease and parents of HR10624-AC5 and Milyang 123, respectively.The arrow indicates the specific DNA fragment of OPO11(about 2500bp) linked to *Stvb-i*

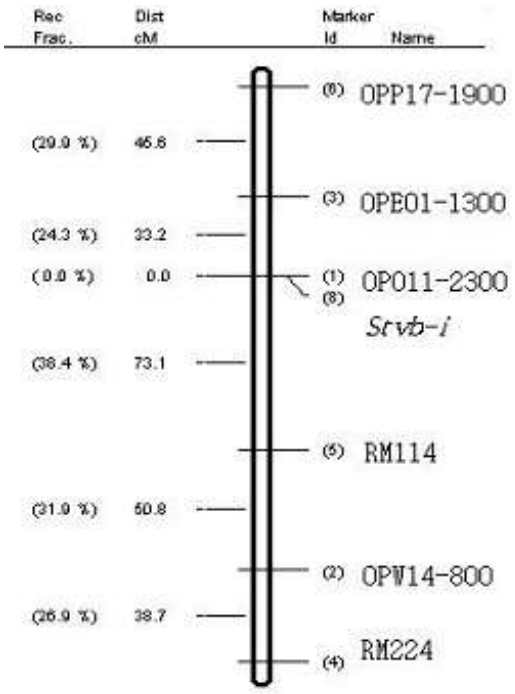

Figure 2. The linkage map of *Stvb-i* gene and RAPD markers on chromosome 11 derived from the cross of HR10624-AC5 and Milyang 123.

Conclusion

The RAPD marker identified in this study can be used in the marker-assisted selection of stripe resistant individuals carrying the *Stvb-i* gene.

References

V. Chague, J. C. Mercier, M. Guenard, A. de Courcel and F. Vedel(1996) Identification and mapping on chromosome 9 of RAPD markers linked to Sw-5 in tomato by bulked segregant analysis. Theor Appl Genet 92:1045-1051.

K.Y. Chung, R. K. Park, K. S. Chung, S. K. Lee, B. T. Jun and Y. D. Jin(1975) A Newly Developed High Yielding Rice Variety 'Milyang 15'. The Research Reprts of the Office of Rural Development Vol. 17(C):17-24.

Hayano-Saito Y, Tsuji T, Fujii K, Saito K, Iwasaki M, Saito A(1998) Localization of the rice stripe disease resistance gene, *Stvb-i* gene, by graphical genotyping and linkage analysis with molecular markers. Theor Appl Genet 96:1044-1049.

Y. Hayano-Saito, K. Saito, K. Fujii, T. Touyama, T. Tsuji, N. Sugiura, T. Izawa and M. Iwasaki(2000) SCAR Marker for Selection of the Rice Stripe Resistance Gene *Stvb-i*, Japan. J. Breed. Res. 2:67-72

Y. Hayano-Saito, K. Saito, S. Nakamura, S. Kawasaki and M. Iwasaki(2000) Fine physical mapping of the rice stripe resistance gene locus, *Stvb-i*. Theor Appl Genet 101:59-63.

Washio, O., K. Toriyama, A. Ezuka and Y. Sakurai(1968) Studies on the breeding of rice varieties resistant to stripe disease . II. Genetic study on resistance to stripe disease in Japanese upland rice. Japan. J. Breed.18:96-101