Transferring, mapping, cloning of powdery mildew resistance gene of *Haynaldia villosa* and its utilization in common wheat

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ABSTRACT

The powdery mildew resistance gene *Pm21* has been transferred from *Haynaldia villosa* into common wheat through development of 6VS/6AL translocation line, and mapped in the region of FL0.00-FL0.58 of the short arm of 6V using the alien deletion addition line. To precisely map *Pm21* gene, γ-Ray irradiation of the mature female gametes of the 6VS/6AL translocation line was employed with higher dosage to induce interstitial translocations with small alien chromosome segment. More than 20 new translocations and deletions involved in different regions of the short arm of 6V have been obtained, and *Pm21* was further mapped in a smaller region by genomic *in situ* hybridization and molecular marker analysis. A microarray analysis using the barley Affymetrix Gene-Chip was conducted to clone candidate genes of *Pm21*, and a full length candidate clone was transformed into the powdery mildew susceptible receptor variety Yangmai 158, whose resistance was then highly improved. TAC-FISH using the TAC clone containing the whole candidate gene as probe was conducted, and the result indicated that this clone was located in the same region of *Pm21*, i.e. FL0.45-0.58 of the 6VS. The 6VS/6AL translocation line has been used as parent in breeding programs and a number of new varieties with high yield and good disease resistance, such as Nannong 9918, Neimai 8~10 and Shimai14, have been developed and released.

INTRODUCTION

Wheat powdery mildew, caused by *Erysiphe graminis* DC.f. sp. *Tritic* Marchl., is one of the most serious wheat diseases in China and worldwide. Introduction and utilization of new resistant genes has proved to be an efficient strategy for controlling the disease. *Haynaldia villosa* Schur. (syn. *Dasypyrum villosum* Candargy, 2n=14, VV), a related species of wheat, has been identified to be resistant to powdery mildew, rusts, take all and eyespot diseases, and tolerant to drought and cold stresses. The powdery mildew resistance gene *Pm21* has been transferred from *H.villosa* into common wheat through development of 6V addition, 6V(6A) substitution and 6VS/6AL translocation lines by Cytogenetics Institute of Nanjing Agricultural University(CINAU), China (Chen et al.,1995), and mapped in the region of FL0.00-0.58 of the short arm of 6V using the alien deletion addition line(Qi et al., 1998). However, the chromosomes of *H. villosa* in the wheat background rarely pair and crossover with wheat chromosomes since they have a wide genetic distance. Therefore, further development of small fragment translocation, especially small interstitial translocation with powdery mildew resistance, and cloning the gene is important for better utilization of *Pm21* in wheat improvement.

MATERIALS AND METHODS

Plant materials *Triticum aestivum-Haynaldia. villosa* 6V addition, 6V(6A) substitution, 6VS deletion addition , 6VS/6AL translocation lines were developed and conserved in CINAU. *Triticum aestivum* c.v. Chinese spring and its nulli-tetrasomic lines, deletion lines were introduced from WGRC, KSU, USA.

Irradiation treatment The mature female gametes of 6VS/6AL translocation line 92R137 were irradiated by $^{60}C_O$ γ- ray using the dosages of 1600, 1920 and 2240 Rad 2-3 days before flowering. The spikes irradiated were emasculated at same day and pollinated with normal fresh matured pollens of common wheat cv. "Chinese Spring" after 2-3 days, and the produced hybrids were named M_1.

Cytogenetic and molecular analysis The structural aberrant chromosomes involved in the short arm of 6V of *H.villosa* were detected by genomic *in situ* hybridization (GISH). GISH and FISH techniques were followed as in Mukai and Gill (1991). The FISH using TAC clone as probe followed Cheng et al. (2001) methodology. The PCR procedure was done according to Cao et al. (2006).

RESULTS AND DISCUSSION

Induction of chromosome translocation and deletion involved in the small segment of 6V short arm by irradiating mature female gametes of translocation line 6VS/6AL The structural aberrant chromosomes involved in the short arm of 6V of *H.villosa* were detected by genomic *in situ* hybridization (GISH). Among the 534 M_1 plants, 97 plants were identified with 192 structural changes on chromosome 6V short arm. Of those changes, 57 were terminal translocations, 80 were interstitial translocations and 55 were deletions (Fig.1). The frequency of plants with small fragment structural changes of 6V was as high as 18.3%. The highest frequency of terminal translocations (14.0%), interstitial translocations (21.0%) and deletions (14.7%) occurred in the treatment of 2240 Rad dosage. The backcross seed-set rate using Chinese spring fresh pollen was 70.2% - 82.5%. Most of the structural changes observed in the M_1 chromosomes were re-discovered in the M_2 generation which should be useful materials for

chromosome based physical mapping. Two heterozygous interstitial translocation lines with a segment of 6VS (FL0.40-FL0.70) and high resistance to powdery mildew were obtained (Fig.2).

Mapping the powdery mildew resistance gene introduced from *H. villosa* Eight interstitial translocation lines with different breakpoint location and segment size of 6VS were used for physical mapping *Pm21*. The results showed that the *Pm21* was located in the region between FL 0.45 and FL0.58 (Fig 3). The markers, Xcinau15-902, X6BS28-386 and X6DS38-730, linked with the *Pm21* were screened.

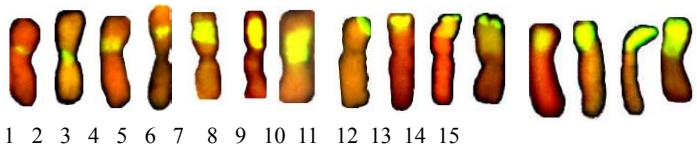

Figure1 Part of structurally changed chromosomes involving the short arm of 6V chromosome of *H. villosa* detected by GISH in M_1 plants. 1-7: interstitial translocation chromosomes with small fragments, 8-11: terminal translocations chromosomes, 12-15: deletion chromosomes of 6VS.

Figure2 NJ2-2 with a small interstitial translocation segment of 6VS showed high powdery mildew resistance.
Note: Arrow shows translocation chromosome segment of 6VS

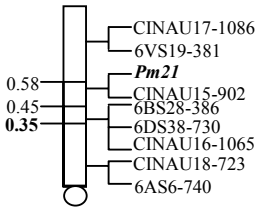

Figure3 Physical location of gene *Pm21* on 6VS and molecule markers linked to *Pm21*

Cloning and transformation of the candidate gene for powdery mildew resistance of *H. villosa*

A microarray analysis using Barley Affymetrix Gene-Chip was conducted to identify differentially expressed genes induced by the infection of *Erysiphe graminis*. A full length gene named *Hv-S/TPK*, which contains a serine/threonine kinase domain, was cloned. Transformation using the constructed recombinant vector pAHC-*Hv-S/TPK* with a *bar* gene as selective marker through gene-gun bombarding was conducted . Ninety seven positive T_0 plants with both the marker and the target genes in a powdery mildew susceptible receptor variety Yangmai 158 were obtained. The positive plants showed high resistance, indicating good compensate function of the candidate gene. Resistant plants were also identified through T_1 and T_2 generations. Four lines introduced *Hv-S/TPK* gene and high resistance to powdery mildew has been obtained.

According to the sequence of *Hv-S/TPK*, a pair of primers, CINAU15, were designed, and a co-dominant marker Xcinau15-902 linked to *Hv-S/TPK* was developed and further mapped in the region of FL0.45~0.58 of the 6VS by using *T.aestivum-H.villosa* addition lines 1V-7V, translocation line 6VS/6AL, deletion lines of 6VS and Chinese spring nulli-tetrasomic lines. Xcinau15-902 was further used to screen a TAC library of translocation 6VS/6AL line 92R137, and a 30kb long positive clone and a 5kb long sub-clone were obtained. Sequencing of the sub-clone indicated that it contains 4 exons and 3 introns. The combined sequence of the exons were completely homologous to the original cDNA sequence of *Hv-S/TPK*. FISH analysis using the positive TAC clone as probe indicated that when using the gDNA of wheat as blocking, the signals were observed through the whole length of 6VS, while when using the gDNA of both *H. villosa* and common wheat as blocking, the dominant signal was only located at the region around FL0.56-0.60 of 6VS (Fig. 4).

From the above results we concluded that the powdery mildew resistance gene of *H. villosa* was located in the region of FL0.45-0.58.

Utilization of the translocation with *Pm21* In order to understand the effects of the 6VS/6AL translocation on agronomic and quality traits in different wheat background, 19 isogenic lines and varieties derived from the translocation lines 6VS/6AL and their parents were evaluated and compared. No obvious disadvantage effects were observed. Up to now, using the translocation lines as parents, new varieties including Nannong 9918, Neimai 8~10, Shimai 14, Shimai 15, Zhongyu 6 and Yuanzhong 175 etc, have been developed and released from different breeding institutes of China. A number of elite lines have been integrated in to the regional yield test. The new developed small fragment translocation lines and the transgenic lines will play important roles as useful genetic resources in wheat improvement.

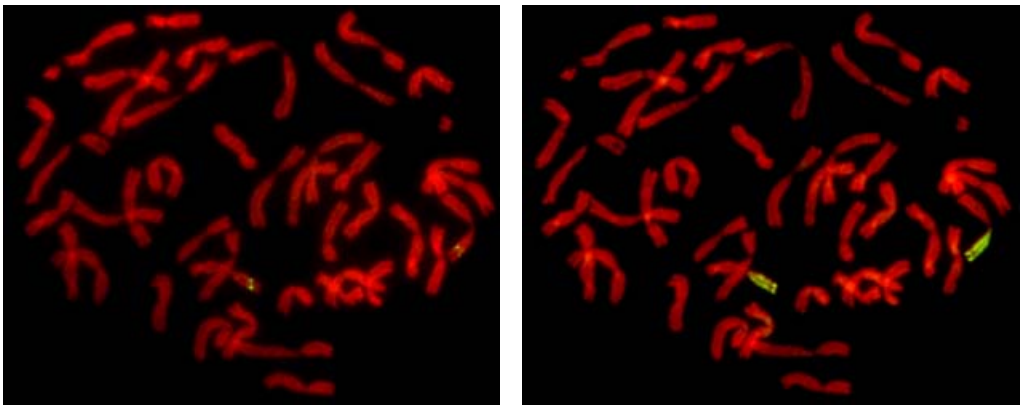

Figure4 Sequencial TAC-FISH (left) and GISH (right) at same mitotic metaphase preparation of the translocation line 6VS/6AL using TAC15 and genomic DNA of *H.villosa* as the probe respectively.

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