GENETIC MARKER MEDIATED TRANSFER OF AN ALIEN GENE, *PM21*, INTO WHEAT CONFERRING RESISTANCE TO POWDERY MILDEW

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ABSTRACT

Powdery mildew is one of the most important fungal diseases of wheat in many regions of the world including Turkey. An effective powdery mildew resistance gene, ***Pm21****, originating from* ***Dasypyrum villosum*** *L. Candargy was transferred into the two widely grown common wheat cultivars 'Bezostaja-1' and 'Gerek-79' of Turkey through backcrossing coupled with genetic markers assisted selection. A 6VS/6AL translocation line (92R149) was used as the source of* ***Pm21****. Resistant backcross lines were selected by using C-banding and specific SCAR markers ($SCAR_{1265}$ and $SCAR_{1400}$). Highly resistant BC_4F_2 plants of 'Bezostaja-1' and 'Gerek-79' were produced and the existence of* ***Pm21*** *was confirmed with the specific $SCAR_{1265}$ and $SCAR_{1400}$ markers.*

Powdery mildew caused by *Erysiphe graminis* f. sp. *tritici* is one of the most harmful diseases of wheat in many wheat growing regions of the world. Powdery mildew is also an important disease of wheat in Turkey, causing up to 30 % yield loses under favourable environmental conditions (1, 2).

Disease resistance has been proven to be a very effective and environmentally friendly method for control of wheat diseases including powdery mildew, though, most of resistance provided by major genes were overcome by pathogens in relatively short times, causing difficulties for wheat breeders (2). Twenty-nine resistance genes for powdery mildew (*Pm1* through *Pm29*) have been identified and assigned to specific chromosomal locations (3). Some of these genes like *Pm8, Pm12, Pm13* and *Pm21* were transferred from wheat relatives (4-7). For example, the *Pm8* resistance gene was derived from 1RS of rye and incorporated into a number of wheat cultivars including Bezostaja-1. However, it has been overcome in most wheat growing regions (4-6). The resistance gene *Pm21*, on the other hand, is an effective new gene transferred from *Dasypyrum villosum* as a 6VS/6AL translocation line (5). *Pm21* was found effective in most of the world including Europe and no virulence has been detected so far (8-10).

Backcross breeding method accompanied with genetic marker assisted selection is one of the fastest and most effective breeding methods for single gene trans-

fers and for gene pyramiding in wheat (11, 12). Cytological markers such as C-banding has been used for identification of alien chromosome segments incorporated into wheat, as being in the case of 6VS/6AL translocation lines (5, 13). Various molecular markers have also been used for similar purposes (6, 14-18). SCAR markers are PCR based molecular markers and are very reliable in identifying alien gene transfers. 6VS specific SCAR markers ($SCAR_{1265}$ and $SCAR_{1400}$) were also developed for the *Pm21* gene to enable effective and accurate transfers into wheat (19).

The aim of this study was the transfer of the *Pm21* gene into the most widely produced wheat cultivars of Turkey via the help of genetic markers.

Materials and Methods

***Plant materials*:** The susceptible wheat parents 'Bezostaja-1' and 'Gerek-79' were obtained from the Anatolian Agricultural Research Center in Eskisehir, Turkey. The source of the *Pm21* gene, the 6VS/6AL translocation line (92R-149), was kindly provided by P.D. Chen, Cytogenetics Institute, Nanjing Agricultural University, Nanjing, China. The susceptible wheat parents were crossed with the 6VS/6AL translocation line to produce F_1 plants and were backcrossed to their respective wheat parents four times to produce BC_4F_1 plants. Resistant BC_4F_1 lines were selfed to produce BC_4F_2 lines which are homozygote for the *Pm21* gene.

Disease screening: Plants were inoculated with a population of powdery mildew spores collected from different parts of Turkey at each crossing generation to determine the resistant *Pm21* positive individuals at seedling stage in a greenhouse. Resistant BC lines were used in the subsequent backcrossing cycle. Finally, BC_4F_2 plants from each parental lines were tested in the field for resistance. Totally 500 BC_4F_2 seeds were planted together with their respective parents as 50 seeds/row in Samsun where the disease occurs naturally every year. Plants were inoculated with airborn spores of powdery mildew naturally and also artifically twice in March and in the end of April in 2002.

C-Banding: Randomly selected 70-75 seeds from each backcross generation were germinated on petri dishes and C-banding was performed on root tips according to Cai. et. al (20) to select 6VS chromosome arm carrying plants. In addition, C-banding was used to trace 1BL/1RS translocation chromosome carrying the *Pm8* gene in Bezostaja-1.

DNA isolations: DNA samples were isolated from the fresh leaves of seedlings grown for 10-15 days according to Incirli and Akkaya (21).

SCAR Primers: The sequences of the primers used were the same as reported (19), which were designed as markers linked to the Pm21 gene. For $SCAR_{1265}$ and $SCAR_{1400}$ markers, Pm21 D + Pm21 E and Pm21 C + Pm21 D primer combinations, respectively, were used.

PCR Conditions: PCR reactions were performed in 25 mL reaction volume in a MJ-Reseach PTC-100 thermocycler (Watertown, MA, USA). SCAR amplicons were separated on 1% agarose gels. The amplification reactions contained 100 ng of plant DNA, 1X Qiagen PCR Buffer (1.5 mM Mg^{2+}, 0.2 mM of each dNTP, 5 pmol of each primer, and 1 unit of Taq DNA polymerase). The PCR reactions were incubated at 94 ^{0}C for 3 min until the cycling starts, and followed by 40 cycles of 94 ^{0}C, 55 ^{0}C, 72 ^{0}C denaturation,

annealing and extention steps each for 1 min with a final extention period of 5 min at 72 ^{0}C.

Results and Discussion

Based on the C-banding and disease screening results, 6VS positive (or *Pm21* positive) BC plants were determined and about 10-15 backcross F_1 plants in each BC generation included in the next crossing. In addition, the two specific SCAR markers ($SCAR_{1265}$ and $SCAR_{1400}$) were employed at the BC_4F_1 generation in the experiment. All 6VS positive plants of BC_4F_1 generation determined by C-banding and seedling screening were also checked with SCAR anlyses. Then, *Pm21* carrying plants were selfed to produce BC_4F_2 plants for field tests. Parental cultivars, Bezostaja-1 and Gerek-79, were highly susceptible (80-100S) whereas BC_4F_2 plants contained both highly resistant (0S) and highly susceptible (80-100S) individuals. The translocation line (92R149) was highly resistant (0S). SCAR screenings were performed on the resistant and susceptible BC_4F_2 plants after the field testing (**Fig. 1** and **Fig. 2**). It clearly revealed the presence of *Pm21* in the resistant lines and no recombination was occured between the SCAR markers and *Pm21*. C-banding was applied to BC_4F_3 seeds to confirm the homozygosity for the 6VS/6AL chromosome in the progeny. Moreover, presence of 1BL/1RS translocation chromosome carrying the *Pm8* gene was revealed by C-banding in the resistant BC_4F_3 lines of Bezostaja because there was only one pair of satellited chromosome (6B) instead of two pairs (1B and

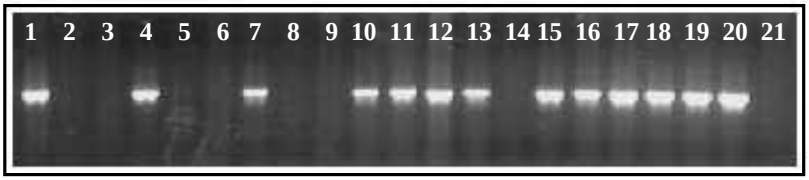

Fig. 1. $SCAR_{1265}$ screening of randomly selected BC_4F_2 plants of Bezostaja-1 and Gerek-79. PCR products from the resistant parent 6VS/6AL translocation line (lane 1), the susceptible parents Bezostaja-1 and Gerek-79 (lanes 2 and 3), resistant BC_4F_2 plants (lanes 4, 7, 10-13 and 15-20) and susceptible BC_4F_2 plants (lanes 5, 6, 8, 9, 14 and 21) amplified by primers *Pm21D* and *Pm21E*.

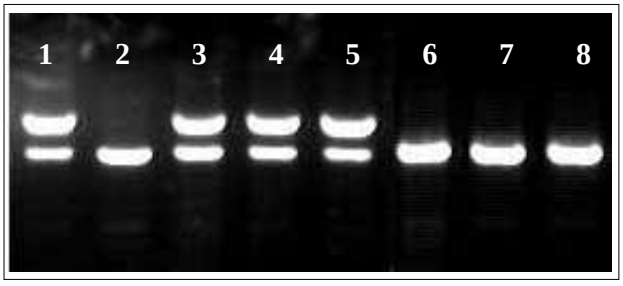

Fig. 2. $SCAR_{1400}$ screening of selected resistant and susceptible BC_4F_2 plants of Gerek-79. PCR products from the resistant parent 6VS/6AL translocation line (lane 1), the susceptible parent Gerek-79 (lane 2), resistant BC_4F_2 plants (lanes 3-5) and susceptible BC_4F_2 plants (lanes 6-8) amplified by primers *Pm21C* and *Pm21D*.

6B) due to the loss of satellited short arm of chromosome 1B in the 1BL/1RS translocation line. This result indicated the pyramiding of *Pm8* + *Pm21* resistance gene complex in the Bezostaja background.

Powdery mildew is one of the most important diseases of wheat in the world. An international survey of facultative and winter wheat breeders about the breeding priorities revealed that powdery mildew was the third most important disease after leaf rust and *Septoria* spp. This survey included the breeders who works on about 90% of the global facultative and winter wheat acreage (22). Similarly, it is also an important disease of Turkey and causes 5-30% yield losses in the Central region and transitional zones of Turkey, the largest wheat production area (1). Unfortunately, breeding against powdery mildew has not got enough attention in this region due to higher degree of damages of other important diseases such as stripe rust and *Septoria* spp. Bezostaja-1 and Gerek-79 are the most widely produced wheat in this region with 3 million ha, which is about 30% of the total wheat production of Turkey. Even the minimum yield loss of 5% due to powdery mildew corresponds to 350,000 ton yield loss per year in expense of farmers directly.

The *Pm21* gene provides very strong resistance and no virulence for *Pm21* has not been found in the world (10) including Turkey and Europe (9). Although transfer of *Pm21* into wheat was achieved as a whole 6VS chromosome arm, due to the lack of recombination between wheat chromosomes and *D. villosum* chromosomes, any unfavourable effects on agronomic traits of the recipients were not observed (5). Therefore, resistant plants produced by this study will provide noticable yield increases in Turkey. In addition, with the assistance of different genetic markers, a gene pyramiding for powdery mildew was established in Bezostaja-1 with the *Pm8* and *Pm21* resistance genes. As a result, more durable and broad spectrum powdery mildew resistance will be possible. Adding new powdery mildew resistance genes into these and some other wheat cultivars is an ongoing research in our breeding program.

Genetic markers are very useful in plant breeding because the presence of a gene can be detected very precisely without waiting for phenotypic expression. We have utilized two different genetic markers to follow up the *Pm21* gene during crossings, along with disease screenings. Based on the results of some previous studies, *Pm21* might always transmit on the translocation chromosome 6VS/6AL in wheat background (23) and behave as a single dominant gene (19). Therefore, C-banding and seedling disease screening were used together in order to detect *Pm21* in the first three backcrossing generations. C-banding seems to be enough to follow up the translocation line because of its very distinct banding pattern (5). However, it is sometimes possible to misidentify or loose a specific chromosome arm (6VS in this case) with C-banding because only three root tips from each plant can be screened under the microscope. If the chromosomes do not spread well on a slide or if staining is not good enough to differentiate chromosomes, misidentification or loosing a specific chromosome is always possible. Therefore, results of seedling disease screening allowed us to identify correct 6VS positive plants. SCAR markers ($SCAR_{1265}$ and $SCAR_{1400}$) linked to *Pm21* were included in our study after BC_3 generation. We have screened BC_4F_1 and BC_4F_2 plants with SCAR markers. Due to no recombination between $SCAR_{1265}$ and $SCAR_{1400}$ and Pm21 (19),

Pm21 positive plants were identified without any doubt and reconfirmed with field disease screening.

We have used backcross breeding method in this study because newly produced lines were required to carry their parental agronomical properties without any major changes. This was also important because the Turkish farmers are very conservatives in terms of changing cultivars. In this way, we managed to produce new powdery mildew resistant cultivar candidates representing Bezostaja-1 and Gerek-79 cultivars, which could be accepted easily by farmers. These new lines could also be used in Balkans and Europe either directly in production or as germplasm resources in where powdery mildew is prevalent.

Acknowledgements

The authors are grateful to Dr. Nevzat Aydin (The Blacksea Agricultural Research Institute) for the field evaluation of powdery mildew infection in Samsun. This project was partly supported by the Scientific and Technical Research Council of Turkey (Project TARP-1804).

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