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Full Length Article

Development and Verification of Wheat Germplasm Containing *Pm21*

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Abstract

Powdery mildew, a devastating disease that affects wheat, is caused by a *Blumeria graminis* f. sp. *tritici*. Powdery mildew is the obstacle in the cultivation of high yielding cultivars especially for wet climatic regions throughout the world. Developing new cultivar with high disease resistance genes is a commercially feasible and environmentally safe approach to reduce yield loss. In order to develop a marker associated with *Pm21* gene, a pair of primers (STPK–F and STPK–R) was designed according to the sequence of a serine/threonine kinase gene (accession: HQ864471.1) using Primer Premier 5.0 software. To screen and characterize *Pm21* gene, we used 37 recombinant inbred lines (RIL) of 02P67//Aizao–781/Yangmai158 along with five susceptible cultivars. Initially, these RIL were screened at both seedling and adult plant stages with *B. graminis* f. sp. *tritici* for visible scoring of powdery mildew resistant lines. It was observed that only a particular (*Pm21*) gene governs the resistance to disease in all the 43 set of genotypes. © 2018 Friends Science Publishers

Keywords: *B. graminis*; *Pm21*; Powdery mildew; *T. aestivum*

Introduction

Common wheat (*T. aestivum* L.) is one of the major crop in the world, but its production is continuously reduced by powdery mildew disease (Costanzo and Bàrberi, 2014). One of the species involved in the disease is *Blumeria graminis* f. sp. *tritici*, which is the causal agent of powdery mildew that rapidly reduce yield in a short period of time (Hao *et al*., 2015). Petersen *et al*. (2015) and Wang *et al*. (2015) reported that host resistance could be the most reliable method to control a disease, which is also economically and biologically efficient. Several genes were associated to powdery mildew resistance, as a result, increasing grain production in wheat. *Pm21* has been found to be the most effective gene in conferring host resistance to disease (An *et al*., 2013). The accumulation of *Pm21* gene in a susceptible variety is a substantial aspect in both experimental breeding and commercial cultivation. Several studies were reported on the efforts to develop and exploit molecular markers to tag *Pm21* (Qi *et al*., 1996; Liu *et al*., 1999; Cao *et al*., 2006; Song *et al*., 2009; Cao *et al*., 2011; He *et al*., 2013; Bie *et al*., 2015) and *PmV* (Lin *et al*., 2013).

Numerous resistance genes have been found conferring powdery mildew resistance in closely related wild species. These species are considered to be a good source to overcome the obstacles in developing resistant wheat varieties (Cowger *et al*., 2012). For example, *Pm21* gene has successfully transferred from a wild plant (*Haynaldia villosa*) to common wheat (Cao *et al*., 2006). The objectives of this study were to screen RIL in response to *B. graminis* f. sp. *Tritici* at both seedling and adult plant stages and to develop a new molecular marker associated to *Pm21* gene.

Materials and Methods

RIL were developed from the cross of 02P67//Aizao–781/Yangmai158 in the National United Engineering Laboratory for Crop Stress Resistance Breeding, Hefei, China. 02P67 was a susceptible variety, while Yangmai–158 was a resistant variety to powdery mildew. After continuous selfing, thirty–seven RIL were randomly selected along with three parental lines and three other susceptible cultivars Y14, Yang00–126 and Shengxuan3 (used as control, Table 1).

Screening for Powdery Mildew

A set of 43 genotypes comprising both resistant and vulnerable cultivars were inoculated at both seedling and

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adult plant stages for visible scoring. At the initial stage, the plants were inoculated with fresh *B. graminis* f. sp. *tritici* spores using dusting method, when the plants reached to one to two leaf stage, and then transferred to a greenhouse at 18°C/12°C (day/night) with a photoperiod of 10–12 h of light per day. Infection types were scored 12–16 days after the inoculation when visible spots were completely established on the vulnerable controls (Y14, Yang00–126, and Shengxuan3). The infection type of each plant was documented on 0–4 scale, whereas 0 represents no visible symptom; 1 represents small visible symptom with the sporulation diameter of colonies less than 1 mm; 2 represents moderate visible symptom with the sporulation diameter of colonies less or equal to 1 mm; 3 exhibits dense sporulation with the diameter of colonies more than 1 mm; and 4 shows plentiful sporulation with more than 80% of the leaf area covered with aerial hypha. The plants with an infection type score of 0–2 were considered resistant, while those with an infection types score of 3–4 were considered as susceptible (An *et al*., 2013). The field inoculation was considered as adult plant stage (more than six leaf stage), a set of 43 genotypes including RIL and susceptible cultivars were inoculated in field condition using a mixture of *B. graminis* f. sp. *tritici*s train. The evaluation of powdery mildew symptoms with the mixture of the strain was conducted at the National United Engineering Laboratory for Crop Stress Resistance Breeding Hefei, China. The infection types were recorded on 0–9 scale, of which 0–4 was considered as resistant, while 5–9 was considered as vulnerable to disease.

Primer Design and PCR Amplification

Total DNA from each wheat line mentioned in Table 1 was extracted from 40 g of fresh leaf, ground in liquid nitrogen with a mortar and pestle, using modified CTAB method previously described by (Porebski *et al*., 1997). Initially, nine pairs of primers were used for the screening of *Pm21* gene on the set of 43 lines (Table 2). A new pair of PCR primers was designed based on the published sequence of *Pm21* gene accession number JF439306.1 (Cao *et al*., 2011) (http://www.ncbi.nlm.nih.gov/genbank) STPK–F (5′–AGGACAAGTGACACGGAAGT–3′) and STPK–R (5′–TTGCGACAACAATGGAGA–3′) using Primer Premier 5.0 software. The primers were synthesized commercially by Gene based Biotechnology Co. Ltd (Shanghai, China). Polymerase chain reaction was performed in 25–µL volumes containing 50 ng of template DNA, 1 X PCR buffer, 5 pmol of each primer, 200 lM (each) of deoxyribonucleotides and 1 U of Taq DNA polymerase. PCR conditions were performed as follows: 94°C for 4 min, followed by 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 2 min, with a final extension at 72°C for 10 min. The final PCR products were separated on 8% non-denaturing polyacrylamide gel in 1 × TBE buffer.

Extraction of DNA from Agarose Gel

Final PCR products were separated on 2% agarose gel, and observed under UV light. The amplified fragments were purified with Easy Pure Quick Gel Extraction Kit, (Beijing, China), and the extracted DNA was then get sequenced by Sangon Biotechnology Shanghai Co. Ltd, China. For both the forward and reverse primer readings, a full length nucleotide assembly sequence was aligned using the software DNAMAN Version 8 (Lynnon Biosoft, Quebec, Canada). The BLAST algorithm was used for similarity search, and sequence similarity was analyzed through the NCBI website (https://blast.ncbi.nlm.nih.gov) using the nucleotide BLAST program (Zhang and Madden, 1997).

Results

Cloning of *Pm21* Gene

The genomic DNA of the 43 genotypes was amplified for *Pm21* gene using the STPK–A primer. Nucleotide blast sequence alignment exhibited 98% identity with STPK–A (accession: JF439306.1), 96% identity with STPK–D (accession: JF439307.1), and 96% identity with STPK–B gene (accession: JF439308.1).

Molecular Marker STPK–A is Linked with Powdery Mildew Resistance Gene *Pm21*

In order to identify *Pm21* gene, PCR with the primer STPK–A was conducted using the genomic DNA of the 43 genotypes including RIL and susceptible cultivar. A fragment of 933 bp, as decided by the sequencing in this study, was only amplified by resistant lines (Fig. 1). A 900 bp fragment was detected in Annong–1245, Annong–1439, Annong–1431, Annong–1121, Annong–1122, Annong–1123 and 22 genotyping of the Annong–1124 and Yangmai–158 while Y14, Aizao–781, Yang00–126 and Shengxuan–3 did not produce *Pm21* gene fragment (Fig. 1). Similarly, WS–1 marker was found to be associated with *Pm21* gene in the resistant cultivars of the RILs (Fig. 2). Thus, regarding the results for powdery mildew evaluation in the field and in the green house, 37 RILs were symptomless to powdery mildew, while five cultivars (Aizao781, O2P67, Y14, Yang00–126, and Shengxuan–3) were infected by powdery mildew.

Discussion

The most promising strategy for the management of powdery mildew disease is utilizing and developing wheat varieties with diseases tolerance or resistance. *Pm21* resistance gene is important in wheat breeding programs to obtain resistant varieties against powdery mildew. The field performance of 43 genotypes including RIL and susceptible cultivars were inoculated at both seedling and adult plant stages for visible infection of powdery mildew.

Table 1: Pedigree of common wheat genotypes used as parental lines for producing RIL

Lines/Varieties	Pedigree	Lines/Varieties	Pedigree
Annong1121	O2P67//Aizao781/Yangmai158	Annong1124–19	02P67//Aizao781/Yangmai158
Annong1122	O2P67//Aizao781/Yangmai158	Annong1124–20	02P67//Aizao781/Yangmai158
Annong1123	O2P67//Aizao781/Yangmai158	Annong1124–21	02P67//Aizao781/Yangmai158
Annong1124	O2P67//Aizao781/Yangmai158	Annong1124–22	02P67//Aizao781/Yangmai158
Annong1124–1	02P67//Aizao781/Yangmai158	Annong1124–23	02P67//Aizao781/Yangmai158
Annong1124–2	02P67//Aizao781/Yangmai158	Annong1124–24	02P67//Aizao781/Yangmai158
Annong1124–3	02P67//Aizao781/Yangmai158	Annong1124–25	02P67//Aizao781/Yangmai158
Annong1124–4	02P67//Aizao781/Yangmai158	Annong1124–26	02P67//Aizao781/Yangmai158
Annong1124–5	02P67//Aizao781/Yangmai158	Annong1124–27	02P67//Aizao781/Yangmai158
Annong1124–6	02P67//Aizao781/Yangmai158	Annong1124–28	02P67//Aizao781/Yangmai158
Annong1124–7	02P67//Aizao781/Yangmai158	Annong1124–29	02P67//Aizao781/Yangmai158
Annong1124–8	02P67//Aizao781/Yangmai158	Annong1124–30	02P67//Aizao781/Yangmai158
Annong1124–9	02P67//Aizao781/Yangmai158	Annong1245	02P67/Annong0419//Yang00–126
Annong1124–10	02P67//Aizao781/Yangmai158	Annong1439	Y14/Annong1124
Annong1124–11	02P67//Aizao781/Yangmai158	Annong1431	Shengxuan3/Annong1124
Annong1124–12	02P67//Aizao781/Yangmai158	Shengxuan3	Unknown
Annong1124–13	02P67//Aizao781/Yangmai158	Yangmai158	Unknown
Annong1124–14	02P67//Aizao781/Yangmai158	Aizao781	Unknown
Annong1124–15	02P67//Aizao781/Yangmai158	Y14	Unknown
Annong1124–16	02P67//Aizao781/Yangmai158	Yang00–126	Unknown
Annong1124–17	02P67//Aizao781/Yangmai158	Shengxuan3	Unknown
Annong1124–18	02P67//Aizao781/Yangmai158	O2P67	Unknown

Table 2: Specific molecular markers and new developmental marker STPK–A associated with *Pm21* gene in RIL of wheat

Marker	Primer sequence	Annealing temperature	Reference
CINAU92–F	CCAGTCGGAGAGGATCTCAA	55	Chen and Chen (2010)
CINAU92–R	TGGGCCTCTTGATCTTGACT		
CINAU276–F	AATGTGTTCCGTTCCCTGAG	60	
CINAU276–R	GTAAACCGGAACGTCATGCT		
CINAU277–F	CTCTTCCCCTCTCTCGTCCT	60	
CINAU277–R	GCTCCAAATCTTCACCAAGC		
CINAU91–F	TGGCTGATGATTCTGCTTCA	55	Chen and Chen (2010)
CINAU91–R	CCACAAGGTTCAGCCAAGTT		
CINAU90–F	AGGTCCTCCATGACCTCGAC	55	Chen and Chen (2010)
CINAU90–R	GCATCATCTTCCTGGACTGC		
CINAU16–F	CATGGCCCGCACCAAGCAGA	55	Chen *et al.* (2006)
CINAU16–R	TTGGCGTGGATGGCGCAGAG		
CINAU18–F	TAGTTCCCTGACGCTGCTTT	55	Wang *et al.* (2007)
CINAU18–R	TGTTGACCGCTCATACGTTC		
CINAU278–F	CCGTTTCAGGTTGCCGTGTC	55	Chen and Chen (2010)
CINAU278–R	TTGCCATCGCTTTGATTGTT		
WS–1–F	TTGGTGTTTTGCTTCTGGA	55	Zheng et al. (2014)
WS–1–R	CTGATATTGCGGTGAATGTT		
STPK–A–F	AGGACAAGTGACACGGAAGT	56	
STPK–A–R	TTGCGACAACAATGGAGA		

Hence, molecular markers are important tools in recognizing resistance genes against a disease (Xu *et al*., 2008). A PCR–based marker STPK–A reported in the current study was found to be a valuable marker for the identification of *Pm21* gene in wheat breeding programs. This newly designed marker (STPK–A) will play an effective role in the identification and characterization of *Pm21* resistance gene in a wheat population. In previous studies, several PCR–based markers associated with *Pm21* gene were reported (Cao *et al*., 2006; Song *et al*., 2009; Chen *et al*., 2013; He *et al*., 2013; Bie *et al*., 2015) as mentioned in Table 2, but many of them have shortcomings and complications for breeding, such as unpredictability, dominance rather than co-dominance and low visual determination. Among them, WS–1 marker was potentially associated with *Pm21* gene as described by Zheng *et al*., (2014). For the aforementioned reason, we developed a new marker that was potentially associated to *Pm21* gene in a resistant population. For evaluation, RIL sand susceptible cultivars were evaluated by artificial inoculation with *B. graminis* f. sp. *tritici* strain during seedling stage, while RIL plants were amplified by STPK–A, and the testing results and infection types were analyzed to confirm the accuracy of STPK–A. A total of 43 wheat cultivars were detected by STPK–A to analyze the distribution of *Pm21*, and the materials carrying *Pm21* were further tested the resistance to powdery mildew under field conditions. In order to further prove the accuracy of STPK–A, the marker WS–1 developed by Zheng *et al.* (2014) was used to amplify the lines with *Pm21* and without *Pm21*.

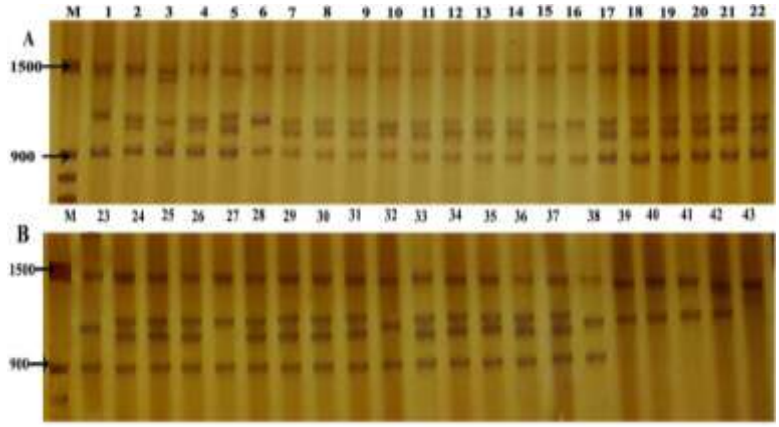

Fig. 1: The genomic DNA of the 43 genotypes including parental lines and susceptible cultivars were analyzed for *Pm21* gene using STPK–A as a primer and an expected fragment of 933 bp in length was generated

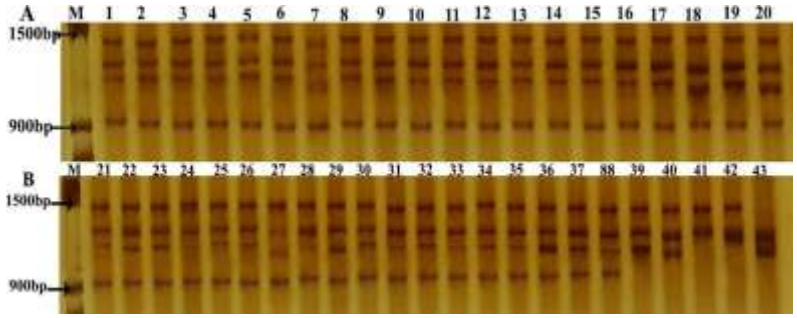

Fig. 2: The genomic DNA of the 43 genotypes including parental lines and susceptible cultivars were analyzed for *Pm21* gene using WS–1 as a primer

Table 3: Pedigree and powdery mildew responses of RIL used in this research

Entry No.	Lines/Varieties	Result	Entry #	Lines/Varieties	Result
1	Annong1121	+	23	Annong1124–19	+
2	Annong1122	+	24	Annong1124–20	+
3	Annong1123	+	25	Annong1124–21	+
4	Annong1124	+	26	Annong1124–22	+
5	Annong1124–1	+	27	Annong1124–23	+
6	Annong1124–2	+	28	Annong1124–24	+
7	Annong1124–3	+	29	Annong1124–25	+
8	Annong1124–4	+	30	Annong1124–26	+
9	Annong1124–5	+	31	Annong1124–27	+
10	Annong1124–6	+	32	Annong1124–28	+
11	Annong1124–7	+	33	Annong1124–29	+
12	Annong1124–8	+	34	Annong1124–30	+
13	Annong1124–9	+	35	Annong1245	+
14	Annong1124–10	+	36	Annong1439	+
15	Annong1124–11	+	37	Annong1431	+
16	Annong1124–12	+	38	O2P67	–
17	Annong1124–13	+	39	Yangmai158	+
18	Annong1124–14	+	40	Aizao–781	–
19	Annong1124–15	+	41	Y14	–
20	Annong1124–16	+	42	Yang00–126	–
21	Annong1124–17	+	43	Shengxuan3	–
22	Annong1124–18	+			

+ = represented as resistance, – = exhibited as susceptible

In conclusion, the newly developed marker could be an efficient molecular tool for the recognition and screening of wheat breeding population carrying *Pm21* gene. In a previous study, Robe *et al*. (1996) reported that the reaction of young vernalized seedlings toward an inoculation of synthetic mildew population closely resembled that of an adult plant, when evaluated under field conditions. In the present study, we defined an alternative molecular method

for the screening of population against powdery mildew, allowing an early recognition and assessment at the seedling and at the five or six leaf stage (considered as adult plant mildew response). The inoculation of the resistant progenies of 02P67//Aizao–781/Yangmai158 and the susceptible cultivars at five or six leaf stage provided the best assumption of the adult plant resistance to powdery mildew infection. Annong–1245, Annong–1439, Annong–1431, Annong–1121, Annong–1122, Annong–1123 and 22 genotyping of the Annong–1124 were highly resistant under natural field epidemic conditions. They were also resistant at the seedling stages under controlled conditions (Table 3). The evaluated data against *B. graminis* f. sp. *tritici* are presented in Table 3, which indicated that the resistance was completely associated to *Pm21*. Similar result was concord well with those obtained by Ma *et al*. (2011). In addition, *Pm21* gene are closely associated to molecular markers STPK–A and it will be useful to combine the diversity of the genetic resistance to powdery mildew in wheat.

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