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Phenotyping and marker based identification of resistant lines in wheat (*Triticum aestivum*) against spot blotch pathogen (*Cochliobolus sativus*)

DEVENDRA K CHOUDHARY[1], RASHMI AGGARWAL[2], BISHNU MAYA BASHYAL[3], V SHANMUGAM[4] and JASDEEP C PADARIA[5]

ICAR-Indian Agricultural Research Institute, New Delhi 110 012, India

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ABSTRACT

Spot blotch of wheat caused by *Cochliobolus sativus* is an emerging disease in hot and humid climate. Breeding for resistance is most suitable approach for management of this disease. A collection of 55 wheat (*Triticum aestivum* L.) genotypes were phenotyped for spot blotch resistance at seedling and adult plant stages. Based on phenotyping of 10 days old seedling Chirya 7, Chirya 3, Ning 8139, Suzhou, Milan-3, HD 2888, HD 2967, WR 95 were identified as resistant, while genotypes, viz. A-9-30-1, Agra local, C-306, K-68, Sonalika were highly susceptible. Phenotyping of adult plants stage revealed Chirya 7, Chirya 3, Ning 8139, Suzhou, Milan-3, HD 2888, HD 2967, WR 95, HW 3081 as resistant, whereas genotypes A-9-30-1, Agra local, C-306, Sonalika were highly susceptible. Phenotyping in adult and seedling plants showed a strong correlation of r = 0.91. DNA was isolated from all genotypes and was subjected to polymerase chain reaction (PCR) using simple sequence repeat (SSR) primers, viz. Xgwm148, Xgwm374 and Xgwm067, Xgwm371 for QTL Qsb.bhu-2B and Qsb.bhu-5B respectively. Presence and absence of different markers was confirmed by a unique amplification by PCR and visualized in Agarose gel electrophoresis. Two markers Xgwm371 and Xgwm374 showed significant association with resistance to *B. sorokiniana* and established to be linked with resistance since these were absent in highly susceptible genotypes. Hence, these markers could be useful in increasing the efficiency of selection for resistance to *B. sorokiniana* in wheat breeding and can be used in marker assisted selection.

Key words: PCR, Phenotyping, QTL, Spot blotch, SSR

Spot blotch of wheat caused by *Cochliobolus sativus* (anamorph *Bipolaris sorokiniana*) is an emerging disease of wheat (*Triticum aestivum* L.). Pathogen can infect the leaves, coleoptiles, grain and leaf sheath of wheat (Prasanna *et al.* 2013). Globally, an estimated 25 million hectares of wheat cultivated land is affected by spot blotch disease. The Indian subcontinent has 10 million ha of affected lands, out of which India alone has 9 million ha (Nagarajan and Kumar 1998).

At present, many different spot blotch resistance genes/QTL have been reported from wheat and related species by many workers like QSb.bhu-2A, QSb.bhu-2B, QSb.bhu-5B, QSb.bhu-6D and QSb.bhu-7D (Kumar *et al.* 2009, Kumar *et al.* 2010); QSb.cim-1B, QSb.cim-3B and QSb.cim-5A (Zhu *et al.* 2013) further 5 SNP in chromosome 1B, 5A, 5B, 6B, 7B were reported by Gurung *et al.* (2014). SSR markers, viz. Xbarc353, Xgwm445, Xgwm148, Xgwm374, Xgwm067, Xgwm371, Xbarc175, Xgwm732 in a cross between Yangmai 6 × Sonalika; Xgwm148, Xgwm129, Xgwm455, Xgwm815, Xgwm533, Xgwm1037, Xgwm263, Xgwm255, Xgwm111, Xswm008 in cross between resistant parent Ning 8201 and Chirya 3 and susceptible parent Sonalika (Kumar *et al.* 2009, Kumar *et al.* 2010). These SSR markers span a region of approximately 3-37 cM on chromosome 2AL, 2AS, 2BS, 5BL, 6DL, 7DS. They are possibly the candidate markers for MAS of the major spot blotch resistance QTLs in breeding populations. Recently Tembo *et al.* (2017) validated three simple sequence repeat (SSR) molecular markers, viz. Xwgm570, Xgwm544, and Xgwm437 linked with resistance to *B. sorokiniana*, using 66 wheat genotypes comprising 11 parental genotypes and 55 F_2 progenies.

The present study was undertaken to analyze SSR markers associated with spot blotch resistance QTLs, viz. Qsb.bhu-2B and Qsb.bhu-5B in different genotypes of wheat, which further can lead to identification of a resistance donor or resistant parent for commercial release of the variety.

Present address: [1]Ph D Scholar (dvraj400@gmail.com), [2,4]Principal Scientist (rashmi.aggarwal2@gmail.com, shanpatho@yahoo.com), [3]Senior Scientist (bishnumayabashyal@gmail.com), Division of Plant Pathology, ICAR-IARI; [5]Principal Scientist (jasdeep_kaur64@yahoo.com), National Institute for Plant Biotechnology, Pusa, New Delhi.

MATERIALS AND METHODS

Plant material and pathogen: Fifty-five popular and commercially grown cultivars/genotypes of present and recent past were included in the study to identify resistance sources through phenotyping and further confirmation through SSR markers (Prasanna *et al.* 2013). Highly virulent isolates of *B. sorokiniana* (BS-112) was selected for the study (Unpublished data). Spores concentration was maintained 10^4 conidia /ml by using hemocytometer and was inoculated on leaves of wheat cultivars by sprayer (Prasanna *et al.* 2013).

Disease severity assessment: Disease severity in seedling stage was measured based on 0–5 scale given by Adlakha *et al.* (1984) in terms of lesion number per leaf and in adult plant stage disease severity was done under field condition at 77 DAS using Zadoks Scale (Zadoks *et al.* 1974) by using a 0–9 scale as reported by Singh and Kumar (2005).

Infection index was calculated by using the formula:

$$\text{Infection index} = \frac{\text{Class rating} \times \text{Class frequency}}{\text{Total no. of leaves} \times \text{Maximum ratings}} \times 100$$

DNA extraction and polymerase chain reaction: DNA isolation was done by taking wheat leaves (5g) of each genotype of 10 days old seedlings. DNA was extracted by the CTAB method as described by Murray and Thompson (1980). The concentration of DNA was checked for each wheat variety separately by a Nanodrop spectrophotometer (Thermo Fisher Scientific).

Each polymerase chain reaction (PCR) was prepared in a volume of 10 µl consisting of 50 ng of template DNA; 1 µl of each primer; 0.2 mM each of dATP, dCTP, dGTP and dTTP; 0.04 unit of Taq DNA polymerase; and 1 µl of 10X Taq polymerase buffer. The PCR amplification was accomplished in a thermal cycler (Bio-Rad Laboratories, USA). The PCR profile included initial denaturing for 5 min at 95°C, 35 cycles consisting of 30s at 94°C, 30s at 60°C and 1 min at 72°C and a final of 5 min extension at 72°C. The PCR products were electrophorized on 4% of agarose gel containing 8 µl ethidium bromide, at 90 V for 60 min then observed under a UV transilluminator and documented. Bands were counted and the absence and presence of bands were scored as 0 and 1.

Polymorphism of SSR markers linked to the spot blotch resistance QTL: Xgwm67-Xgwm371 and Xgwm148-Xgwm374 SSR markers for QTL Qsb.bhu-5B and Qsb.bhu-2B were selected for screening of 55 wheat genotypes. Primer sequences (Table 1) were obtained from the wheat database GrainGenes, available at http://wheat.pw.usda.gov.

Statistical analysis: All the experiments were performed in randomized block design with three replications per genotypes and five plants per genotype were considered as one replication. Correlation as determined by applying Pearson's method, Pearson correlation coefficient was measured between the severity index in seedling and adult stage. The statistical analysis was performed using OPSTAT software (http://14.139.232.166/opstat/default.asp). Phenotypic variance (R^2) was calculated using QTL/s cartographer software version 2.5.

Table 1 SSR Primer sequences for DNA markers linked to spot blotch resistance genes in wheat

Gene marker	Chro-mosome location	Sequence (5' -3')
Xgwm148	2B	5' GTGAGGCAGCAAGAGAGAAA 3' 5' CAAAGCTTGACTCAGACCAAA 3'
Xgwm374	2B	5' ATAGTGTGTTGCATGCTGTGTG 3' 5' TCTAATTAGCGTTGGCTGCC 3'
Xgwm067	5B	5' ACCACACAAACAAGGTAAGCG 3' 5' CAACCCTCTTAATTTTGTTGGG 3'
Xgwm371	5B	5' GACCAAGATATTCAAACTGGCC 3' 5' AGCTCAGCTTGCTTGGTACC 3'

RESULTS AND DISCUSSION

Phenotyping for spot blotch resistance: Based on phenotyping of ten days old seedling one (Chirya 7), seven (Chirya 3, HD 2888, HD 2967, Milan-3, Ning 8139, Suzhou, and WR 95), twenty, twenty-one and six (A-9-30-1, Agra local, C-306, K-68, Sonalika, and Severe Local) genotypes were classified under five categories, viz. resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible respectively and no plants were immune. Phenotyping in adult stage revealed that four (Chirya 3, Chirya 7, Ning 8139, and Suzhou), five (HD 2888, HD 2967, HW 3081, Milan-3, and WR 95), twenty five, seventeen and four (A-9-30-1, Agra local, C-306, and Sonalika) genotypes were classified under the same five categories (Fig 1, Table 2). The disease severity in seedling plants was ranged from of 8.6 % in Chirya 7 to 91.4% in Agra Local with a mean severity of 34.7%, and in adult plants from 5.6 % in Chirya 7 to 88.9% in Agra Local with mean severity 31.2% (Table 2). The correlation coefficients (r) between the disease severity of seedling and adult plants

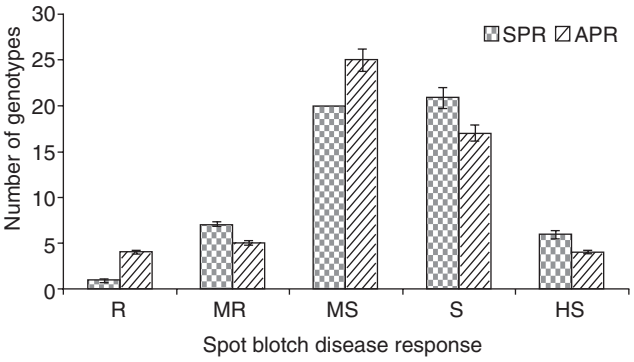

Fig 1 Frequency distribution of various genotypes/cultivars for spot blotch disease severity under the seedling plant and adult plant inoculation with *Bipolaris sorokiniana* strain BS112.

Table 2 Phenotypic and genotypic evaluation of genotypes/cultivars using artificial inoculation of *Bipolaris sorokiniana* strain BS112 and PCR-based Xgwn371 and Xgwn374 SSR markers

Genotype	Infection Index (AP)	Disease response (AP)	Infection Index (SP)	Disease response (SP)	*Xgwn374	*Xgwn371	*Xgwn067	*Xgwn148
A-9-30-1	66.7	HS	71.4	HS	-	-	-	+
Agra local	88.9	HS	91.4	HS	-	-	+	+
C-306	72.2	HS	74.3	HS	-	-	-	+
Chirya 3	5.6	R	11.4	MR	+	-	+	+
Chirya 7	5.6	R	8.6	R	+	+	+	+
DL784-3	44.4	S	48.6	S	-	-	-	+
DL-788-2	27.8	MS	28.6	MS	+	-	+	+
DW1408	27.8	MS	28.6	MS	+	-	-	+
Eagle	22.2	MS	25.7	MS	+	-	-	+
HD 1713	27.8	MS	28.6	MS	+	-	+	+
HD 2329	22.2	MS	28.6	MS	+	-	-	+
HD 2733	22.2	MS	25.7	MS	+	+	+	+
HD 2781	22.2	MS	25.7	MS	+	-	-	+
HD 2833	22.2	MS	22.9	MS	+	-	+	+
HD 2851	27.8	MS	28.6	MS	-	-	+	+
HD 2888	11.1	MR	17.1	MR	+	+	+	+
HD 2894	22.2	MS	25.7	MS	-	-	-	+
HD 2932	22.2	MS	28.6	MS	-	+	+	+
HD 2967	11.1	MR	17.1	MR	+	+	+	+
HD 3059	38.9	S	45.7	S	+	-	+	+
HD 3139	38.9	S	42.9	S	+	+	+	-
HP 1633	38.9	S	40.0	S	-	-	+	+
HW 1085	22.2	MS	25.7	MS	-	-	-	+
HW 2004	33.3	S	34.3	S	+	-	+	+
HW 2045	27.8	MS	28.6	MS	+	+	+	+
HW 3081	16.7	MR	22.9	MS	+	-	-	+
K-68	50.0	S	54.3	HS	-	-	+	+
Karnataka Local	38.9	S	42.9	S	+	-	-	+
Kharchia Local	44.4	S	45.7	S	-	-	-	+
Lal Bahadur	27.8	MS	28.6	MS	+	-	-	+
Lok1	27.8	MS	31.4	S	+	-	-	+
MACS 4145	27.8	MS	31.4	S	+	+	-	+
Milan-3	11.1	MR	14.3	MR	+	+	+	+
Morrocco	44.4	S	48.6	S	+	-	+	+
Ning 8139	5.6	R	11.4	MR	+	+	+	+
PBW 175	38.9	S	42.9	S	+	-	-	+
Severe Local	50.0	S	48.6	S	-	-	+	+
Sonalika	83.3	HS	88.6	HS	-	-	-	+
Sr17	27.8	MS	31.4	S	-	- +	+	+
Sr21	22.2	MS	28.6	MS	+	-	+	+
Sr23	27.8	MS	28.6	MS	-	-	-	+
Suzhou	5.6	R	11.4	MR	+	-	+	+
Trinkaya	22.2	MS	25.7	MS	+	-	+	+

Contd.

Table 2. (*Concluded*)

Genotype	Infection Index (AP)	Disease response (AP)	Infection Index (SP)	Disease response (SP)	*Xgwn374	*Xgwn371	*Xgwn067	*Xgwn148
UP 115	44.4	S	48.6	S	+	-	-	-
UP 2338	27.8	MS	31.4	S	+	-	+	+
Vidish	44.4	S	45.7	S	-	-	-	+
VL-404	22.2	MS	28.6	MS	-	-	-	+
WH 533	22.2	MS	28.6	MS	+	-	-	-
WL 711	44.4	S	45.7	S	+	-	+	+
WR 1753	44.4	S	45.7	S	+	-	+	-
WR 1934	22.2	MS	25.7	MS	+	-	+	+
WR 544	27.8	MS	31.4	S	+	-	-	+
WR 95	16.7	MR	20.0	MR	+	-	+	+
Yr11	22.2	MS	25.7	MS	-	-	-	+
Yr9	33.3	S	37.1	S	-	-	-	+

*Xgwn374, + shows presence of 200bp band size; *Xgwn371, + shows presence of 180bp band size; *Xgwn067, + shows presence of 105bp band size; *Xgwn148, + shows presence of 170bp band size

were calculated and data revealed r-value of 0.91 which showed strong positive correlations.

Molecular marker analysis: Genotypes which showed resistant and susceptible reaction in seedling and adult plant stage were further selected for molecular marker analysis. Marker Xgwm371 yielded three amplification fragments of 130, 180 and 190 base pairs (bp) in length. Marker Xgwm371 amplified PCR product of 180 bp in the two resistant genotypes Chirya 7 and Ning 8139; three moderately resistant genotypes HD 2888, HD 2967 and Milan-3, four moderately susceptible genotypes HD 2733, HD 2932, HW 2045 and MACS 4145; and 1 susceptible genotype HD 3139. Fifteen genotypes showed a 190 bp fragment, Twenty-nine genotypes showed presence of 130 and 190 bp of amplicon and one genotype Sr17 showed 130 and 180 bp fragments upon amplification with marker Xgwm371 (Table 2, Supplementary Fig 1-4).

Marker Xgwm067 amplified PCR product of 95 and 105 bp in length. Marker Xgwm067 amplified PCR product of 105 bp in fifty-one genotypes, and 95 bp amplicon in four genotypes, viz. WH 533, UP 115, HD 3139 and WR 1753 (Table 2, Supplementary Fig 5-8).

Marker Xgwm374 yielded three amplificon of 180, 200 and 220 bp in length. Marker Xgwm374 amplified PCR product of 200 bp in the thirty-six genotypes, out of this one, seven, fifteen and thirteen showed phenotypic responses of resistant, moderately resistant, moderately susceptible and susceptible respectively. Marker Xgwm374 amplified PCR product of 180 and 220 bp in nineteen genotypes (Table 2, Supplementary Fig 9-12).

Marker Xgwm148 amplified PCR products of 150 and 170 bp. Marker Xgwm148 amplified 150 bp amplicon in 25 genotypes, 170 bp amplicon in 28 genotypes, and 150 and 170 bp in two genotypes, Chirya 3 and Morrocco (Table 2, Supplementary Fig 13-16).

Single marker regression analysis showed a significant association ($P < 0{\cdot}05$) between phenotype trait and associated genotyped results for marker Xgwm371 and Xgwm374. Marker Xgwm371, Xgwm374, Xgwm148 and Xgwm067 accounted for 11.8, 22.81, 3.7 and 1% of phenotypic variation (R^2) simultaneously. The percentage of phenotypic variation (R^2) explained by all four markers was 39.31%. Marker Xgwm374 contributed more significantly ($P < 0{\cdot}005$) than molecular markers Xgwm148, Xgwm067 and Xgwn371.

In this study, Chirya 3, Chirya 7, Ning 8139 and Suzhou showed resistant response against *B. sorokiniana* in adult stage, while Chirya 7 showed resistant response in seedling stage. QTL Qsb.bhu-2B and Qsb.bhu-5B mapped by Kumar *et al.* (2009) were used for identification of resistance source in selected wheat genotypes. The resistant and moderately resistant genotypes showed the presence of either one or both SSR markers. Marker Xgwm371 differentiated between resistant and susceptible genotypes by the amplicon size of 190 bp, which amplified in most of the resistant and moderately resistant parental genotypes but not in the susceptible genotypes. However, all the susceptible genotypes showed a 130 bp amplification product, confirming the absence of the gene for *B. sorokiniana* resistance. Marker Xgwm374 differentiated between resistant and susceptible genotypes by the amplicon size of 200 bp, which amplified in most of the resistant, moderately resistant and moderately susceptible genotypes but not in the susceptible and highly susceptible genotypes. However, all the susceptible parental genotypes showed a 180 or 220 bp or both amplification products, confirming the absence of the gene for *B. sorokiniana* resistance. Markers Xgwm067 and Xgwm148 didn't follow any specific pattern regarding phenotyping of different genotypes, but interestingly marker Xgwm148 amplified 170 bp amplicon in all resistant and all moderately resistant genotypes except HW 3081.

The significance of the regression coefficient observed from the regression analysis ($P < 0{\cdot}05$) showed that marker Xgwm371 and Xgwm374 had a relationship with resistance to *B. sorokiniana*. Xgwm371 and Xgwm374 explained 11.8 and 22.8% of the observed total phenotypic variation for resistance simultaneously. This finding confirmed the earlier study of Kumar *et al.* (2009) that Xgwm371 and Xgwm374 were linked to *B. sorokiniana* resistance genes, indicating its usefulness as a tool for identifying resistant genotypes in early breeding generations.

In some of the resistant genotypes only one of the flanking markers was present. This could be due to crossing over between the marker and allele for resistance in these parental genotypes. Additionally, it could also be due to the marker not being tightly linked to the resistant allele in the corresponding genotypes (Gajjar *et al.* 2014) and/or other genetic factors conditioning resistance (Young and Kelly 1997).

Marker Xgwm371 and Xgwm374 may be used in marker-assisted selection; which is potentially useful in breeding for spot blotch resistance and opportunity to select desirable resistant lines on the basis of genotypes rather than phenotype, for combining different resistance genes which would enhance the resistance of the genotypes/ cultivars and provide durable resistance and the developed genotypes would be resistant to spot blotch as well as diseases like leaf, stem and yellow rust of wheat. It can be said that popular rust resistant cultivar will be incorporated with spot blotch resistant QTL in order to combat under climate change situation.

The results suggest that the markers Xgwm371 and Xgwm374 could be useful in MAS in increasing the efficiency for identification of resistant genotypes in the seedling stage even in the absence of the disease epiphytotic conditions.

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REFERENCES

Adlakha K L, Wilcoxson R D and Raychaudhuri S P. 1984. Resistance of wheat to leaf spot caused by *Bipolaris sorokiniana. Plant Disease* 68: 320–1.

Gajjar K, Mishra G, Radhakrishnan T, Dodia S, Rathnakumar A, Kumar N and Kumar A. 2014. Validation of SSR markers linked to the rust and late leaf spot diseases resistance in diverse peanut genotypes. *Australian Journal of Crop Science* 8: 927–36.

Gurung S, Mamidi S, Bonman J M, Xiong M, Brown-Guedira G and Adhikari T B. 2014. Genome-wide association study reveals novel quantitative trait loci associated with resistance to multiple leaf spot diseases of spring wheat. *PLoS One* 9 (9): e108179.

Kumar J, Schafer P, Huckelhoven R, Langen G, Baltruschat H, Stein E, Nagarajan S and Kogel K H. 2002. *Bipolaris sorokiniana*, a cereal pathogen of global concern: cytological and molecular approaches towards better control. *Molecular Plant Pathology* 3:185–95.

Kumar U, Joshi A K, Kumar S, Chand R and Röder M S. 2009 Mapping of resistance to spot blotch disease caused by *Bipolaris sorokiniana* in spring wheat. *Theoretical and Applied Genetics* **118**(4): 783–92.

Kumar U, Joshi A K, Kumar S, Chand R and Röder M S. 2010. Quantitative trait loci for resistance to spot blotch caused by *Bipolaris sorokiniana* in wheat (*T. aestivum* L) lines 'Ning 8201'and 'Chirya 3'. *Molecular Breeding* **26**(3): 477–91.

Murray M G and Thompson W F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research* **8**(19): 4321–6.

Nagarajan S and Kumar J. 1998. Foliar blights of wheat in India: germplasm improvement and future challenges for sustainable, high yielding wheat production, pp 52–8. *Helminthosporium blights of Wheat: Spot Blotch and Tan spot.* Duveiller E, Dubin H J, Reeves J and McNab A (Eds). Mexico, DF, mexico: CIMMYT.

Prasanna K, Aggarwal R, Banerjee R, Gupta S, Sharma S, Bashyal B M, Kumar J and Jindal M. 2013. Basis of resistance in wheat genotypes to *Bipolaris sorokiniana* causing spot blotch disease. *Indian Phytopathology* **66**: 67–75.

Singh D P and Pankaj K. 2005. Method of scoring of leaf blight of wheat caused by *Bipolaris sorokiniana* (Sacc) Shoem on top two leaves at adult plant stage pp. 289–294. *Integrated Plant Disease Management*, pp 289-94. Sharma R C and Sharma J N) (Eds). Scientific Publishers, Jodhpur, India.

Tembo B, Sibiya J, Tongoona P and Tembo L. 2017. Validation of microsatellite molecular markers linked with resistance to *Bipolaris sorokiniana* in wheat (*Triticum aestivum* L). *Journal of Agricultural Science* 155: 1061–8.

Young R A and Kelly J D. 1997. RAPD markers linked to three major anthracnose resistance genes in common bean. *Crop Science* **37**(3): 940–6.

Zadoks J C, Chang T T and Konjak C F. 1974. A decimal code for the growth stages of cereals. *Weed Research* **14**: 415–21.