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Research Article

Inheritance and identification of molecular markers associated with spot blotch (*Cochliobolus sativus* L.) resistance through microsatellites analysis in barley

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Abstract

Spot blotch resistant (IBON 18) and susceptible (RD 2508) lines were crossed to investigate inheritance of resistance and to identify simple sequence repeats (SSRs) associated with resistance. F_1 resistance was intermediate and suggested additive nature of inheritance. Three additive genes was noted in the distribution of F_3, F_4 and F_5 generations. In F_6 and F_{6-7}, the quantitative and qualitative approaches also suggested the control of three resistance genes. The parents and the RILs (F_6/F_{6-7}) were grown in four environments and spot blotch severity recorded. Forty five SSR primers, specific for chromosomes 1 (7H) and 5 (1H), were applied. Of these, 12 were polymorphic between the parents, and between the resistant and susceptible bulks. Three markers BMS 32, BMS 90 and HVCMA showed association with resistance, which was further confirmed through selective genotyping. The co-segregation data on the molecular markers (BMS 32, BMS 90 and HVCMA) and spot blotch severity on 173 RILs was analyzed by single marker linear regression approach. Significant regression suggested linkage among BMS 32, BMS 90 and HVCMA and the three resistant genes (designated as *Rcs-qtl-5H-1*, *Rcs-qtl-5H-2* and *Rcs-qtl-1H-1*.) respectively. These markers explained 28%, 19% and 12% of variation respectively, for spot blotch resistance among the RILs.

Key words: microsatellite markers, molecular marker, spot blotch, *Cochliobolus sativus*, barley.

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Introduction

Spot blotch of barley caused by *Cochliobolus sativus* (Ito and kurib.) Drechsl. ex Dastur *Bipolaris sorokiniana* (Sacc in sorok.) Shoem. *Helminthosporium sativum* Pamm, King and Bakke is responsible for yield and quality reductions in many parts of the world (Piening *et al.*, 1976; Nutter *et al.*, 1985). In susceptible barley cultivars, average yield losses of 16%-33% have been reported (Clark, 1979). In Syria, Van Leur (1991) reported a 40% yield loss in barley due to infection by *C. sativus.* Low resistance to spot blotch in barley cultivars of south Asia causes significant recurring losses to small farmers and recently breeding for spot blotch resistance has assumed significant importance in south Asia.

Several attempts have been made to control spot blotch but no single control measure has been successful. Hence similar to wheat, an integrated approach (Joshi and Chand, 2002) with host resistance as a major component is considered necessary. Inheritance studies on resistance to spot blotch of barley are available but the nature of inheritance still appears to be debatable. Reports indicate presence of monogenic (Arny, 1951; Wilcoxson *et al.*, 1990) and polygenic (Griffee, 1925; Steffenson *et al.*, 1996) types of resistance. Steffenson *et al.* (1996) reported that in barley, different genes control spot blotch resistance at seedling and adult stage. Reports on inheritance of spot blotch resistance using South Asian barley lines is completely lacking. Barley lines with higher levels of resistance are difficult to achieve owing to the influence of environment on disease development (Wilcoxson *et al.*, 1990; Bailey and Wolf, 1994) and the quantitative nature of resistance (Cohen *et al.*, 1969; Kutcher *et al.*, 1994). A clear information regarding inheritance of spot blotch resistance is needed to design suitable strategies to enhance resistance of barley cultivars.

The conventional methods to select resistance genotypes by inoculating plants with spot blotch isolates are

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time consuming, laborious, destructive and are not always reliable because of substantial environmental influence. The number of lines to phenotype can be substantially reduced (and so the cost) by identifying markers that are closely linked to the gene of interest. However, in spot blotch of barley, it would be important to confirm that the progeny of a new cross between the resistant (linked to the marker) and the new susceptible genotype has (in addition to the marker) the resistance phenotype due to possible epistatic or modifying effects (Bilgic *et al.*, 2005). Polymerase chain reaction (PCR) based markers, such as microsatellite (SSRs) (Condit and Hubble, 1991), can reduce the cost of identifying genetic markers and allow large scale genotyping of individuals at any location. The process of identification of molecular markers linked with traits of interest has become more efficient by the use of Bulk segregant analysis (BSA) (Michelmore *et al.*, 1991).

Development of genotype resistant to spot blotch poses serious challenge to the barley breeders. A clear information regarding the inheritance of spot blotch resistance and availability of molecular markers linked to the resistance genes are needed to design suitable strategies to enhance resistance of barley cultivars. Reports on the inheritance and molecular markers of spot blotch resistance in South Asian genotypes is almost non-existent. Therefore, the present study was initiated with two objectives: (i) investigate the inheritance pattern of resistance in the barley accession IBON 18 to spot blotch, and (ii) identify SSR markers associated with spot blotch resistance in the F_6 population of a cross between IBON 18 and RD 2508 to assist barley breeding in South Asia for greater resistance against spot blotch.

Materials and Methods

Plant materials and development of segregating progenies

Single plant selections of resistant (IBON 18) and susceptible (RD 2508) genotypes were multiplied and used in the cross. Resistant parent IBON 18 (a germplasm line introduced form ICARDA/CIMMYT) was crossed with the susceptible RD 2508 (RD 2035/PG 90) which is otherwise higher yielding with good agronomic performance. Parents and F_1 progenies were evaluated for resistance to spot blotch under an induced epiphytotic obtained in the field at Varanasi (North Eastern Plains Zone, India, 25.2° N and 83.0° E) in the year 1999-00 and 2003-04. Plots (*ca.* 60-70 plants) of F_1 generation consisted of two 2-m rows seeded 25 cm apart with 30 cm between plots. The susceptible barley genotype RD 2503 was planted in alleys and borders, two weeks before sowing the experiment to enhance the spread of inoculums.

The progenies of the cross were advanced to the F_3, F_4, F_5 and F_6 generations following the method described by Singh and Rajaram (1991) and Joshi *et al.* (2002, 2004a) where a random plant in each generation from each line was harvested for advancing the generation. F_3 lines were obtained from around 200 randomly chosen space planted F_2 plants grown in the summer crop season (2000) at the off-season nursery, Wellington, Tamil Nadu. Half of the seeds of the F_2 plants were advanced to obtain F_3 generation. The F_3 lines at the off-season nursery were harvested to obtain F_4 families. The F_3 and F_4 lines were evaluated under induced epiphytotic conditions during crop season 2001-02. In both generations, plots of each line consisted of a single 3 m row with 30 cm space between the plots and maintaining around 40-50 plants per row. Sowing was done in the second fortnight of November in order to allow the post anthesis stage to coincide with the relatively warm temperature occurring in March which favors disease development and spread.

In the next crop season (2002-03), 183 progeny lines of F_5 were evaluated under induced epiphytotic conditions at Varanasi. In the F_6 generation, 173 progeny lines were planted in two dates of sowings (third week of November and first week of December) in the year 2003-04. Other details of planting were as described for F_3, F_4 and F_5 generations. However, based on the number of days to maturity of the RILs observed in the F_5 generation, differential sowings were carried out in the F_6 generation to synchronize the growth stages between progeny rows, thereby attempting to nullify the growth stage x disease severity interaction. To further confirm the number of genes controlling resistance, the $F_{6\text{-}7}$ RILs were also evaluated in the year 2004-2005 in two dates of sowings (third week of November and first week of December) following the approach described for previous generation.

Inoculation procedure and disease assessment

Spot blotch disease was induced by inoculating spreader rows and a pure culture of the locally most aggressive isolates of *B. Sorokiniana* (Isolates No. RCBHUBR1857) identified at this center. The isolate was multiplied on barley grains and spores were harvested in water (Misra, 1973). A spore suspension (approximately 10^4 spores/mL) containing the surfactant Tween 20, was uniformly sprayed by using a hand held atomizer at three stages: tillering, flag leaf emergence and anthesis during the evening hours (Joshi *et al.*, 2007b, c).

Disease was measured using spot blotch severity (%) for each genotype such that genotypes that scored less than 30 were considered resistant, between 40 and 50 as moderately resistant; between 60 and 70 as moderately susceptible, and those having higher than 80 as susceptible (Joshi *et al.*, 2007a, b). Spot blotch level was assessed five times; at growth stages 55 (half of inflorescences emerged), 60 (beginning of anthesis), 65 (anthesis half complete), 73 (early milk) and 77 (late milk) (Zadoks *et al.*, 1974). For each line, the disease scores of all the plants, including the most susceptible and most resistant ones, were recorded. For each

scoring date, growth stage was also recorded. Area Under Disease Progress Curve (AUDPC) estimates were based on the plot disease severities at different growth stages (van der Plank, 1963). The lines that showed AUDPC (< 500) were considered resistant and the lines that showed AUDPC (> 2000) were considered susceptible.

Following formula was used for calculating AUDPC.

$$\text{AUDPC} = \sum_{i=1}^{n} \left[\frac{Y_i + Y_{i+1}}{2} \times (t_{i+1} - t_i) \right]$$

where Y_i = disease level at time t_i; $t_{(i+1)} - t_i$ = time (days) between two disease scores; n = number of dates on which spot blotch was recorded.

Estimation of number of genes

To estimate the number of segregating genes in each cross, F_3 lines were grouped in to three classes, (i) homozygous for the resistant parental response, (ii) homozygous for the susceptible parental response, and (iii) segregating or homozygous different than the parents. In the F_4, F_5 and $F_6/F_{6\text{-}7}$ generations, lines were grouped into three classes (Singh and Rajaram, 1991, Joshi *et al.*, 2007a) by merging the last two categories. The observed and expected distributions of F_3, F_4, F_5 and $F_6/F_{6\text{-}7}$ lines in disease severity categories were tested by χ^2 analysis. To confirm the number of genes obtained by χ^2 analysis, a quantitative approach (Wright, 1968) was also followed in the $F_6/F_{6\text{-}7}$ generations. In this method the number of genes controlling spot blotch resistance was verified using the formula (Singh *et al.*, 1995; Joshi *et al.*, 2007a), $n = (GR)^2/R \times \sigma^2 g$, where n = minimum number of genes, GR = genotypic range, $\sigma^2 g$ = genetic variance of the segregating generation, and the factor R = 4.13 in case of F_6 generation. GR was estimated by two different methods (Singh and Rajaram, 1995; Joshi *et al.*, 2007a). In the first method GR was the range of segregating generation line means, while in the second method GR was the range of segregating generation line means multiplied by heritability. Heritability was used in the second method to eliminate the influence of environment on the expression of the disease severity (Mulitze and Baker, 1995).

Analysis of variance was conducted following split plot analysis of the data of four environments of two years (2003-04 and 2004-05) using SAS software (SAS Institute, 1997) to determine the differences for spot blotch severities among the $F_6/F_{6\text{-}7}$ lines. Narrow sense heritability was estimated using the entry mean formula given by Fehr (1987): h^2 = genotypic variance/phenotypic variance; genotypic and phenotypic variances were estimated from the ANOVA table following Comstock and Moll (1963). Although the genetic variance used in the formula to calculate heritability was the total genetic variance of the segregating generation lines, the heritability estimate was considered to be the narrow-sense because dominance variance was negligible and the confounding effect of the additive-by-additive genetic variance could be included in the heritability estimate at the level of inbreeding (Singh and Rajaram, 1995). Phenotypic correlation coefficients of spot blotch severity and AUDPC values among four environments (6 pair-wise combinations) were also calculated using SAS software (SAS, 1997).

DNA isolation and bulked segregant analysis

Leaves were harvested from 15 days old seedlings from the RILs (F_6) in the icebox from the field. Genomic DNA was isolated from the seedling leaves using CTAB method described by Saghai-Maroof *et al.* (1984). DNA concentration was determined through spectrometer and quality of DNA was checked by agarose gel electrophoresis (0.8%). After quantification, the DNA was diluted to a concentration of 30 ng/µL using Tris-EDTA buffer (10 mM Tris, 0.1 mM EDTA, pH 8). DNA from the six to seven most resistant RILs which had disease severity lower than 25% and AUDPC > 500 was pooled at an equal amount to create the resistant DNA bulk. Care was taken to ensure that these RILs possessed similar days to maturity and plant height to avoid the effect of plant growth stages on proper phenotyping of the lines. Similarly, DNA from the six to seven most susceptible RILs, which has disease severity higher than 85% and AUDPC greater than 2000, was pooled to create the susceptible bulk.

SSR markers

Forty five SSRs primers (Table 1), based on the map of Becker and Heun (1995), Liu *et al.* (1996), Ramsay *et al.* (2000) and Li *et al.* (2003), specific for 1H and 5H were used to screen for polymorphism between IBON 18 and RD 2508. Then the polymorphic markers were screened against the two DNA bulks. When the markers were found polymorphic between DNA bulks, they were screened with all 173 lines (F_6 generation) of the `IBON 18 x RD2508'.

PCR reactions were performed as described by Ramsay *et al.* (2000) with minor modifications such that the reaction volume was reduced to 18 µL. DNA amplification was carried out in a 20 well thermocycler (TECHNE, England) each containing 50-100 ng template DNA, 0.2 µM of each primer, 200 µM of each of the dNTPs, 2.5 mM $MgCl_2$, 1X PCR buffer and 1U of *Taq* DNA Polymerase (Bangalore Genei, India; The composition for 10x buffer was = 100 mM Tris-HCl, pH 8.3 at 25 °C; 500 mM KCl; 15 mM $MgCl_2$; 0.5% (vol) Tween 20). The temperature profile for annealing was used according to the information provided for the primers (Becker and Heun, 1995; Liu *et al.*, 1996; Ramsay *et al.*, 2000; Li *et al.*, 2003) (Table 1). The amplification products were separated on 2.5% agarose gels with TBE buffer (100 mM Tris-borate, 2.5 mM EDTA, pH 8). The gels were stained with ethidium bromide, viewed under an ultraviolet transilluminator and then photographed. Single marker QTL analysis using linear re-

gression was performed following the method of Nelson (1997). The marker allele *sr* (spot blotch resistant allele) was coded 1 and the allele *ss* (spot blotch susceptible allele) were coded 0 for conducting regression analysis. Genetic linkage analysis for SSRs was performed using MAPMAKER ver. 3.0 (Lander *et al.*, 1987), with a LOD score of 3.0. Recombination frequencies were transformed to centiMorgans (cM) using the formula of Kosambi (1944).

Table 1 - List of primers for chromosome 1 and 5 of barley used for finding SSR markers for spot blotch resistance.

Chromosome 1	Chromosome 5
HVWAXY	BMS 02
HVCMA	BMS32
HVM 5	BMS 90
HVM 4	HVM 20
HVM 49	HVM 43
HVM 51	HVM 63
Bmac 0063	HVM 64
Bmac 0032	HVM 70
Bmac 0399	GBMS 63
Bmac0213	GBMS 60
EBmac 0783	GBM 68
GBMS 12	GBMS 70
GBMS 37	GBMS 75
GBMS 53	GBMS 106
GBMS 54	GBMS 115
GBMS 62	GBMS 119
GBMS 65	GBMS 156
GBMS 93	GBMS 174
GBMS 143	GBMS 196
GBMS 187	GBMS 219b
GBMS 219a	
Bmag 0382	
Bmag 0154	
Bmag 0579	
Bmag 0345	

Results

Inheritance of spot blotch

Compared with parents, the spot blotch scores of the F_1 (Table 2) appeared to be intermediate, indicating the absence of dominance for the genes governing resistance. This was also suggested by the AUDPC distribution (Figure 1), which has been suggested to be an appropriate parameter to distinguish the resistance of genotypes (van der Plank, 1963). The F_3-line distributions in cross (Figure 1, Table 3) indicated that resistance genes interacted in an additive manner. Very few lines showed a response similar to the resistant or the susceptible parent. In the F_3 progeny rows, the test of goodness of fit suggested segregation at three independent loci (Table 3). As with the F_3 generation, lines having responses similar to the parental types were found to be at a low frequency in the F_4, F_5 as well as the F_6 generations (Table 3). For these generations, these test for goodness of fit also indicated the presence of three genes for resistance (Table 3).

The heritability for the spot blotch severity in the four environments (F_6 date I, F_6 date II, $F_{6\text{-}7}$ date I and $F_{6\text{-}7}$ date II) was moderately high and ranged from 76-83%. The number of resistance genes obtained from the quantitative analysis (Table 4) showed that gene numbers in the resistant genotype were close to three as obtained in the test of goodness of fit. The distribution of F_6 lines (Figure 2) also suggested the role of polygenes in controlling resistance.

ANOVA analysis

The mean disease severity (%) of RILs ranged from 5.0% (Environment I and IV) to 96.8% (Environment I) showing large phenotypic variation in the population. Analysis of variance showed significant variation among the RILs. RIL x environment interaction was also found significant. The six possible combinations of the ranks of RILs in four environments arranged in pairs displayed positive and significant correlations (0.648-0.903) for both dis-

Table 2 - Mean percentage spot blotch scores and AUDPC with standard errors for resistant and susceptible parents, as well as F_1 used in the genetic analysis.

Parents and F_1	Mean disease response to spot blotch			
	% Severity[a]		AUDPC	
	2000-01	2004-05	2000-01	2004-05
IBON18	10.67 ± 3.9	12.92 ± 4.7	2520.51 ± 83.5	2410.3 ± 93.1
RD5208	89.16 ± 4.2	92.58 ± 4.2	463.75 ± 83.7	418.7 ± 88.8
IBON 18 (R) X RD 2508 (S)	45.67 ± 2.9	49.17 ± 3.0	1148.7 ± 55.5	1212.5 ± 63.7

[a]Final disease scoring at late milk stage (Zadoks scale 77).

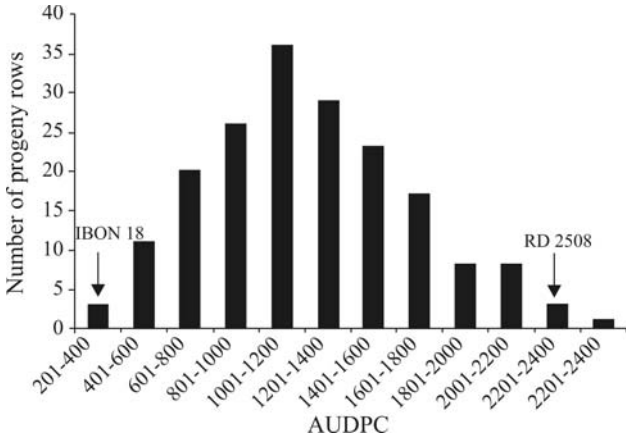

Figure 1 - Distribution of AUDPC of F_3 lines of the cross resistant (IBON 18) x susceptible (RD 2508) for spot blotch severity in barley.

ease severity (%) and AUDPC values. The most resistant and susceptible lines correlated more highly than the intermediate lines. For the two extremes, *i.e.*, the six most resistant and six most susceptible RILs which were used to constitute the bulk, the correlation in the six environments ranged from 0.93 to 0.96.

SSR analysis

Out of a total of 45 SSRs primer pairs used, 12 (26.6%) primer pairs detected reproducible polymorphism between the parental genotypes. Bulked segregant analysis (Michelmore *et al.*, 1991) using these 12 primers showed that three markers, BMS 32, BMS 90 and HVCMA displayed an amplification profile characteristic of resistant and susceptible parent in the corresponding bulks. This suggested an association of these markers with spot blotch resistance. Selective genotyping (Lander and Botstein, 1989) of individual RI lines belonging to the two bulks suggested an association between the BMS 32, BMS 90 and HVCMA markers and spot blotch resistance (Figures 3 and 4). The three SSRs markers had the following primer sequence: BMS 32: forward primer GGATCAAAGTCCG GCTAG, reverse primer TGCGGGCCTCATACTGAC, BMS 90: forward primer ACATCAACCCTCCTGCTC, reverse primer CCGCACATAGTGGTTACATC, and HVCMA: forward primer GCCTCGGTTTGGACATATA AAG, reverse primer GTAAAGCAAATGTTGAGCAA CG (Ramsay *et al.*, 2000 and Li *et al.*, 2003). Subsequently, all (173) F_6 RI lines were genotyped using the three markers and data on segregation of the marker were recorded for conducting QTL analysis.

The regression of spot blotch severity on the BMS 32, BMS 90 and HVCMA markers were highly significant indicating close association between molecular markers and resistance for spot blotch (designated as *Rcs-qtl-5H-1*, *Rcs-qtl-5H-2* and *Rcs-qtl-1H-1*). The two genes (*Rcs-qtl-5H-1*, *Rcs-qtl-5H-2*) were located on chromosome 5H,

Table 3 - Goodness of fit of ratios observed and hypothesized class frequencies for F_3, F_4, F_5, F_6 and F_{6-7} lines from the cross between resistant (IBON 18) and susceptible (RD 2508) parents.

Generation	Resistant[1]	Segregating[2]	Susceptible[3]	Hypothesized ratio	χ^2 value	p-value	Gene number
F_3	3	176	4	1.56:96.87: 1.56	1.54	0.27	3
F_4	9	167	7	5.27:89.45: 5.27	2.29	0.32	3
F_5	15	159	9	8.37:83.25:8.37	1.64	0.44	3
F_6 (Date I)	21	133	19	10.30:79.40:10.30	0.42	0.81	3
F_6 (Date II)	20	131	22	10.30:79.40:10.30	0.14	0.93	3
F_{6-7} (Date I)	22	133	18	10.30:79.40:10.30	0.70	0.71	3
F_{6-7} (Date II)	23	128	22	10.30:79.40:10.30	0.19	0.94	3

[1]Homozygous for resistant parental type (homozygous for all the resistant alleles. [2]Segregating or homozygous for disease levels different from parental level. [3]Homozygous for susceptible parental type (homozygous lacking all the resistant alleles).

Table 4 - Estimate of maximum number of effective genes contributing to spot blotch resistance in crosses between IBON18 x RD 2508 using Wright's (1968) formula modified for F_6 generation (Singh *et al.*, 1995) in four environments.

Generation	Environment	Number of genes			
		Disease severity (%)		AUDPC	
		Method I	Method II	Method I	Method II
F_6 (Date I)	2003-04 (I)	3.98	2.39	4.27	2.67
F_6 (Date II)	2003-04 (II)	3.74	2.27	4.14	2.79
F_{6-7} (Date I)	2004-05 (I)	4.31	2.52	4.61	2.93
F_{6-7} (Date II)	2004-05 (II)	3.34	2.14	3.85	2.65

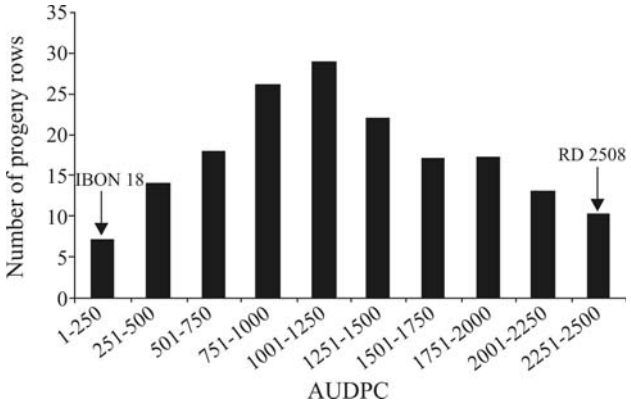

Figure 2 - Distribution of AUDPC of F_6 lines of a cross between resistant (IBON 18) and susceptible (RD 2508) for spot blotch during 2003-2004.

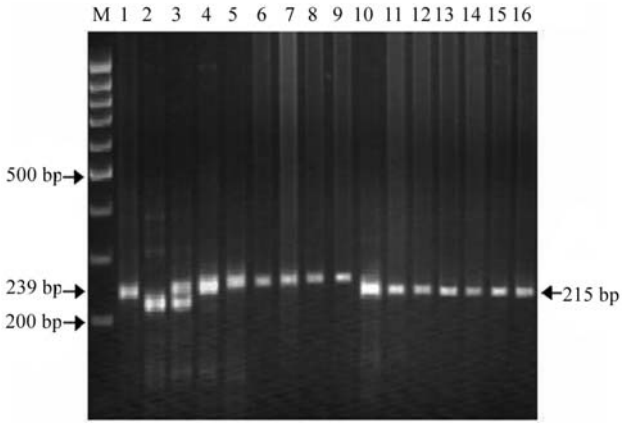

Figure 3 - Selective genotyping of RILs (representing extreme groups) for spot blotch resistance in barley using BMS 32 SSRs primer; Lane M = 100-bp ladder marker; 1 = Resistant parent (IBON18); 2 = Susceptible parent (RD2508); 3-9 = RILs with low disease severity; 10-16 = RILs with high disease severity.

where as the third one (*Rcs-qtl-1H-1)* was on chromosome 1H (Table 5). The R^2-value suggested that the SSRs markers linked with BMS 32, BMS 90 and HVCMA contributed to 28%, 18% and 12% of the total variation present for spot blotch resistance among the RILs.

The SSRs marker BMS32, HVCMA and BMS 90 were found to be linked with a distance of 4.4 cM, 8.4 cM and 12.5 cM, respectively from the resistance locus with a LOD score of 18.49, 12.57 and 8.97 respectively (Table 5), suggesting a good linkage between molecular markers and spot blotch resistance gene.

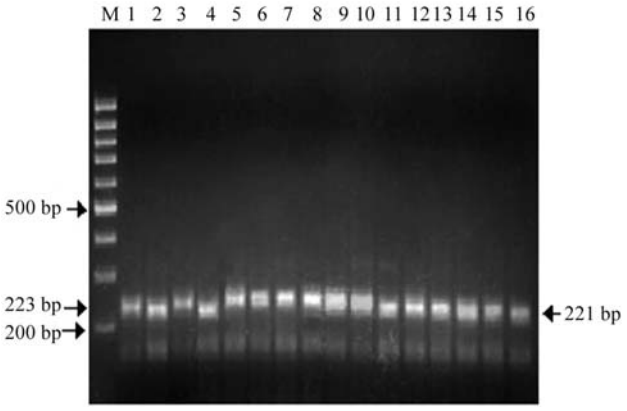

Figure 4 - Selective genotyping of RILs (representing extreme groups) for spot blotch resistance in barley using BMS 90 SSRs primer; Lane M = 100-bp ladder marker; 1 = Resistant parent (IBON18); 2 = Susceptible parent (RD2508); 3 = Resistant bulk; 4 = Susceptible bulk; 5-9 = RILs with low disease severity; 10-16 = RILs with high disease severity.

Discussion

The chi square ratios in the four segregating generations suggested that the spot blotch resistance in the barley line IBON 18 was under the control of probably three genes. In case of single gene control, the F_3 progeny lines are expected to follow a ratio of 1 : 2 : 1. This ratio would be 3 : 2 : 3 and 7 : 2 : 7 in the F_4 and F_5 generations, respectively. As the genes having additive effects will increase, the number of progeny rows similar to parental lines are expected to decrease (Singh and Rajaram, 1995; Joshi *et al.*, 2007a). In all the segregating generations (F_3, F_4, F_5 and F_6) of cross IBON 18 X RD 2508, lines with a disease response equivalent to the parental types were in very low frequencies, and the lines were clearly not distributed in a monogenic (1 : 2 : 1) ratio. The test of goodness of fit suggested segregation at three independent loci.

The inheritance study suggested the role of three genes in controlling resistance to spot blotch in the line IBON 18. The distributions of RILs (F_6 and $F_{6\text{-}7}$) for spot blotch AUDPC also suggested polygenic control. Some of the previous studies concerning inheritance of resistance to spot blotch disease of barley also indicated the control by many genes (Griffee 1925; Cohen *et al.*, 1969; Kutcher *et al.*, 1994). However, the quantitative nature of resistance to spot blotch has not been reported in Indian barley cultivars and lines.

Table 5 - SSRs markers, alleles, chromosome locations and map distance.

Marker	Map distance (cM)	LOD	Chromosome	Annealing temp. (°C)	Reference
BMS 32	4.4	18.89	5 (1H)	60	Stein *et al.*, (2007)
HVCMA	8.4	12.57	1 (7H)	60	Stein *et al.*, (2007)
BMS 90	12.5	8.97	5 (1H)	55	Stein *et al.*, (2007)

The number of effective genes conferring resistance to spot blotch disease in the cross, calculated from the modified formula of Wright (1968), using severity (%) as well as the AUDPC values, showed almost similar gene numbers in the four environments. The gene numbers were influenced by the measure of the genotypic range used in the formula (Singh and Rajaram, 1995). However, the correction of phenotypic range of the F_6 lines by multiplying with heritability (method II) suggested a gene number close to three. In other words, the results were quite similar to that obtained by using χ^2 analyses. Hence, it appeared that probably three additive genes are responsible for spot blotch resistance in the barley IBON 18 investigated in the present study. An earlier report (Hosford *et al.*, 1975) suggested polygenic control for spot blotch resistance in which three and four genes were found to control virulence of *C. sativus* on barley genotypes NDB112 and Larker, respectively. In a study, Griffee (1925) also inferred that three unlinked genes control resistance to spot blotch at the adult plant stage. Bailey *et al.* (1988) reported a polygenic control for resistance to common root rot caused by *Cochliobolus sativus*.

The heritability estimates for the F_6 and $F_{6\text{-}7}$ generations of the cross were moderately high and ranged from 0.77 to 0.83 across four environments. In an earlier study (Kutcher *et al.*, 1994), the estimate of heritability with respect to spot blotch resistance were reported to be moderate. The spot blotch severity increases with plant maturation (Joshi *et al.*, 2002). Therefore, for best evaluation of resistance of a line or plant, comparison must be done when the disease has just reached maximum severity in the susceptible parent (Joshi *et al.*, 2004b). The additive interactions of only a few (three) genes observed in this study suggested that spot blotch resistance in barley can be realized by growing fairly large segregating populations and selecting for low scoring genotypes at appropriate growth stages under high inoculum pressure. Further, since only a few additive genes were able to display substantially high level of resistance and heritability was moderately high, effective selection could be applied in the early segregation generations as well. For further gains, strong selection pressure could be applied in advanced generations when high homozygosity has been achieved, as also suggested in case of wheat by Joshi *et al.*, (2004a) for spot blotch and Singh and Rajaram (1995) for resistance to scab.

SSR analysis

In barley, molecular mapping for different traits has been an area of active research, which led to the identification of many markers for several important traits (Steffenson *et al.*, 1996; Tuberosa and Salvi, 2004; Bilgic *et al.*, 2005; Stein *et al.*, 2007). In this study we found that with a large population and precise phenotypic characterization it is possible to detect molecular markers for a trait such as spot blotch resistance that is under polygenic control, even though some loci display large effects. The SSR markers had significant additive effects on spot blotch resistance and appear to display major effect consistently over four environments. These markers were named as QTLs *Rcs-qtl-5H-1*, *Rcs-qtl-5H- 2* and *Rcs-qtl-7H-1.* The two QTLs, *Rcs-qtl-5H-1*, *Rcs-qtl-5H- 2* contributed to around 28.40% and 18.97% variation respectively and were located on chromosome 5 (1H). The other QTL *Rcs-qtl-7H-1,* contributing 12.52% variation, was found to be located on chromosome 1(7H). Thus, *Rcs-qtl-5H-1* contributing 28.40% variation and which was located on chromosome 5 (1H) appeared to be a major SSR marker. Steffenson *et al.* (1996) also reported the presence of the largest QTLs for spot blotch resistance in chromosome 5 (1H). A second QTL having lesser magnitude was also mapped in the chromosome 1H. Using microsatellite markers, Mesfin *et al.* (2003) identified three QTLs for *Fusarium* head blight on chromosome 2 (2H). We mapped microsatellite markers (BMS32, BMS 90 and HVCMA) linked to the spot blotch resistance genes at map distances of 4.4 cM, 12.5 cM and 8.4 cM, respectively. The two QTLs on chromosome 5 (1H) obtained in the present study contributed a total phenotypic variation of 47.37% (*Rcs-qtl-5H-1* = 28.40% and *Rcs-qtl-5H-2* = 18.97%) for spot blotch resistance. Since the association of HVCMA located on chromosome 1 (7H) (*Rcs-qtl-1H-1*) explained only 12.52% of phenotypic variation, we conclude that the marker HVCMA may either be linked to a QTL with a small effect or is loosely linked to a QTL with a large effect (Melchinger, 1998). With moderately high heritability expressed for spot blotch resistance, the markers detected in this study explained around 59.89% of phenotypic variation.

The identification of three genes that probably control spot blotch resistance and the detection of closely linked markers, should now make marker assisted selection for spot blotch resistance a promising approach in barley. Mapping of additional markers should even result in better genetic resolution and more tightly linked markers for spot blotch resistance genes. Successful MAS and cloning of the major resistance QTLs will crucially depend on the generation of new flanking markers on chromosomes 5H and 1H.

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