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IDENTIFICATION OF GENOMIC REGIONS ASSOCIATED WITH LEAF BLIGHT RESISTANCE IN BARLEY

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ABSTRACT

Leaf blight or Spot blotch, caused by *Bipolaris sorokiniana*, is a serious foliar disease of barley (*Hordeum vulgare* L.) which may cause a significant yield loss of more than 30% at adult plant stage. The Quantitative Trait Loci (QTLs) studies in barley have concluded debatable complex inheritance of leaf blight resistance due to different genetic background of mapping populations with very few reports coming from India. An integrated approach is recommended with host resistance as a major component to identify QTLs and specific markers targeted for genomic regions involved in leaf blight resistance in Indian barley lines. Leaf blight resistant (DWR49) and susceptible (RD 2503) lines were crossed to investigate inheritance of resistance and to identify QTLs associated with resistance. 283 SSR and STS primers specific to all the seven chromosomes were used to screen the parental lines, of which 50 showed polymorphism over resistant and susceptible bulks and used for genotyping of 142 RILs (Recombinant Inbreed Lines) of the cross DWR49 x RD2503. Eleven molecular markers (Bmac 213, ABG 058, Bmag 125, Hv5s, Gbm1208, GBM1444, GBM1506, GMS1, GBM5012, Ebmac827, and Bmag369) specific to chromosome 1H, 2H, 3H, 5H, 6H and 7H were found closely linked during chi- square analysis. The findings may be useful in developing a marker-assisted selection strategy for leaf blight resistance in barley.

Key words: Barley, *Bipolaris sorokiniana*, Molecular markers, Chi-square analysis

INTRODUCTION

Barley is an important cereal grain crop, grown in about 100 countries worldwide. Foliar and seed borne diseases are prevalent in all barley growing areas. One of the most severe foliar diseases of barley in many part of world is leaf blight caused by necrotrophic fungal pathogen, *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*). Leaf blight or spot blotch in barley occurs worldwide in warmer and humid growing conditions. Repeated fungicide application can be used to control leaf blight, but the use of resistant cultivars offers the most economically and environmentally sound means of control.

Leaf blight, has been controlled for over 40 years through the deployment of resistant cultivars. But still many commercial cultivars are vulnerable to leaf blight epidemics. In barley, resistance is often controlled by multiple genes on different chromosomes with additive effects. Marker-assisted selection (MAS), in combination with field selection, can accelerate the identification of progeny with multiple genes for resistance in the breeding process. Using DNA-based markers, a large number of barley genes or QTLs (Quantitative Trait Loci) for important traits have been mapped, including resistance to spot blotch. Study on genetics of adult plant resistance to spot blotch in Steptoe x Morex doubled haploid (DH) population reported two QTLs on chromosome 1H and 7H explaining 70.1% phenotypic variance [14]. In another study for adult plant resistance, a QTL at or near Rcs5 on chromosome 7H explained nearly all the phenotypic variance (75%) for the disease severity in Harrindton x Morex population. In Dicatoo X Morex (D/M) population Three QTLs conferred seedling resistance: one near Rcs5 on chromosome 7H explaining 30%, a second near the centromere of the chromosome 7H explaining 9% and third on short arm of chromosome 3H explaining 19% of phenotypic variation. For adult plant resistance in the D/M population, three QTLs explained most of the variation: one on the short arm of chromosome 3H explaining 36%, a second on the long arm of chromosome 3H explaining 11%, and a third at or near Rcs5 on chromosome 7H explaining 20% of the phenotypic variation [1]. Two most significant SNP markers were reported during the association mapping of 767 lines of Barley at or near *Rcs5*, a major gene on the short arm of chromosome 7H conferring seedling resistance to spot blotch [17].

In India, barley is grown chiefly in the northern plains and hilly areas. Previously, Leaf blight was commonly occurred in north eastern plains zone (NEPZ) of India but now it has become a serious disease of north western plains zone (NWPZ) also. Current Indian barley varieties are largely susceptible to this disease and attempts are being made to introduce sources of resistance in barley lines. Previous study reported scanty resistance in Indian barley for leaf blight under controlled conditions [16]. In another study, Indian barley germplasm lines were assessed for fungal toxin of *B. Sorokiniana* in standing field and *in vitro* conditions and only few genotypes were found resistant to leaf blight disease [4]. Inheritance studies of spot blotch resistance in cross IBON 18/RD 2508 reported three resistance genes *Rcs-qtl-5H1*, *Rcs-qtl-5H-2* and *Rcs-qtl-1H1* closely linked to SSR markers BMS 32, BMS 90 and HVCMA explaining 28%, 19% and 12% of variation, respectively [16].Not much work has been done so far on leaf blight disease of barley at molecular level. Therefore, present study is proposed to identify the QTLs involved in leaf blight resistance in Indian barley.

MATERIAL AND METHODS

Plant material

Leaf blight resistant parent DWR49 was crossed with leaf blight susceptible parent RD2503 to build F1 generation. The progenies of F1 generation were advanced up to F_9 generation to obtain RILs (Recombinant Inbreed Lines). DWR-49 is a 2 rowed variety which shows a high level of resistance to leaf blight, a genetic stock registered with NBPGR, New Delhi. It carries resistance up to 13 scales that is considered as the best observation for leaf blight in NWPZ and

NEPZ regions. RD2503 is a high yielding and best Indian malt variety among 6 rowed barley lines but it is highly susceptible to leaf blight.

Pathogen inoculation and phenotypic disease assessment

RILs of cross DWR49 X RD2503 were screened under epiphytotic conditions during the crop season (2010-13) at Directorate of Wheat Research (DWR), Karnal. Leaf blight disease incidence was induced by inoculating infector rows (RD2503) with a pure culture of isolates of *B. sorokiniana.* The infector was grown at right angle to the direction of test lines. The inoculum (monosporidial culture) was multiplied on autoclaved sorghum seeds in the laboratory. The spores were harvested in water. A spore suspension (approximately 10^4 spore/ml) containing 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 [8,9]. For each crop season, the inoculation of pathogen was started after mid of January and continued till the first week of March. First symptoms for leaf blight appeared in first week of March on the infector plants (RD2503). Disease data were recorded following the double digit system, indicating the % area covered on the flag leaf and on the next below to flag leaf on 1 to 9 scale [10,11]. The third observation was recorded on overall reaction of the plant on 1-9 scale for classification [5] and plants were categorised as resistant (up to 3), moderately resistant (3-5), moderately susceptible (5-7) and susceptible (>7).

Genotyping using molecular markers

Barley lines of F_8 generation (142 RILs) were used for genotyping study. Fresh leaves from 14 days old seedlings were used for DNA extraction using CTAB mini-prep method [13]. The DNA samples were analyzed both qualitatively and quantitatively using 0.8% agarose gel electrophoresis. 283 SSR/STS markers covering all the seven chromosomes of barley were used to screen parental lines. Five most resistant and five most susceptible RILs was pooled at an equal amount to create the resistant and susceptible DNA bulks, respectively. All the markers found polymorphic on the parental lines were further screened with these two bulks. Molecular markers found polymorphic between both the parents and two bulks were used to genotype individual RILs and the frequencies of parental alleles were estimated for each locus.

PCR reactions were conducted in a reaction volume of 20 µl containing 1X PCR buffer, 200 mM dNTPs, 0.25 µM of primer, 2Mm $MgCl_2$, 1u Taq polymerase and 50 ng template DNA. PCR amplification was performed using BIORADS 1000 thermocycler. PCR products were resolved by electrophoresis on 2 % agarose gels (HiMedia) at 4v/cm in 0.5 X TBE buffer. Fragment sizes were approximately calculated by interpolation from the migration distance of marker fragments of 100-bpDNA ladder (Invitrogen, USA) and corroborated with the reported amplified fragment size of respective molecular marker. Gels were stained with ethidium bromide (0.5ug/ml). DNA banding patterns were visualized with UV light and recorded by imagining system (Syngene Synoptics Ltd. USA).

RESULTS AND DISCUSSION

Phenotypic data and Molecular characterization

The artificial inoculation created very high incidence of disease on the test material and indicated good distribution of leaf blight. Leaf blight infection on plants was uniform across all experiments, allowing clear and

unambiguous classification of resistant and susceptible plants (Figure 1).

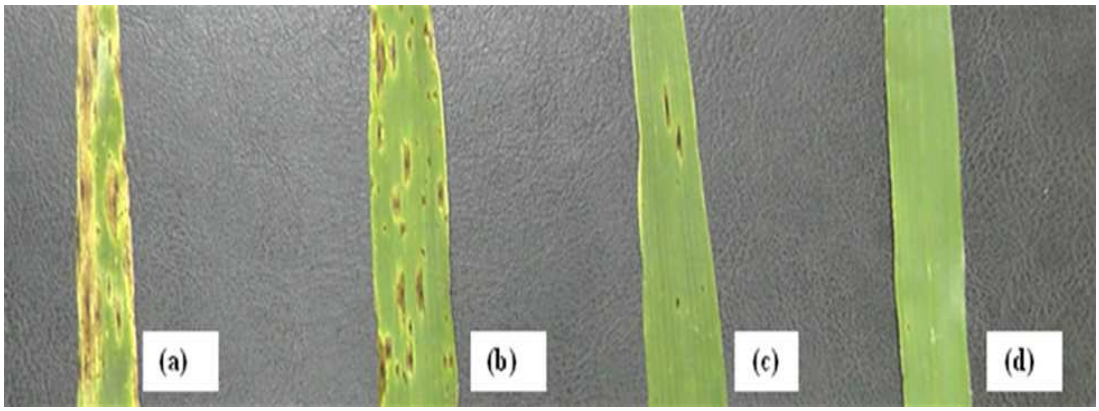

Figure 1: Symptoms of leaf blight disease on susceptible (a), moderately susceptible (b), moderately resistant(c) and resistant (d) RILS of cross DWR49 X RD 2503.

The controls reacted as expected to leaf blight infection. Resistant control, DWR 49 exhibited very low infection response (IR) whereas the susceptible control, RD2503 exhibited a high IR. During phenotypic screening, out of 142 barley lines observed, 59 RILs were found resistant and 83 RILs were found susceptible. The RILs grouped as resistant were able to keep the disease level at very low level. Out of 283 SSR/STS markers used for genotyping, 50 markers gave reproducible polymorphism between the parents and contrasting bulks (Figure 2). These polymorphic markers were used to genotype individual RILs of cross DWR49 x RD2503 to identify genomic regions associated with leaf blight resistance.

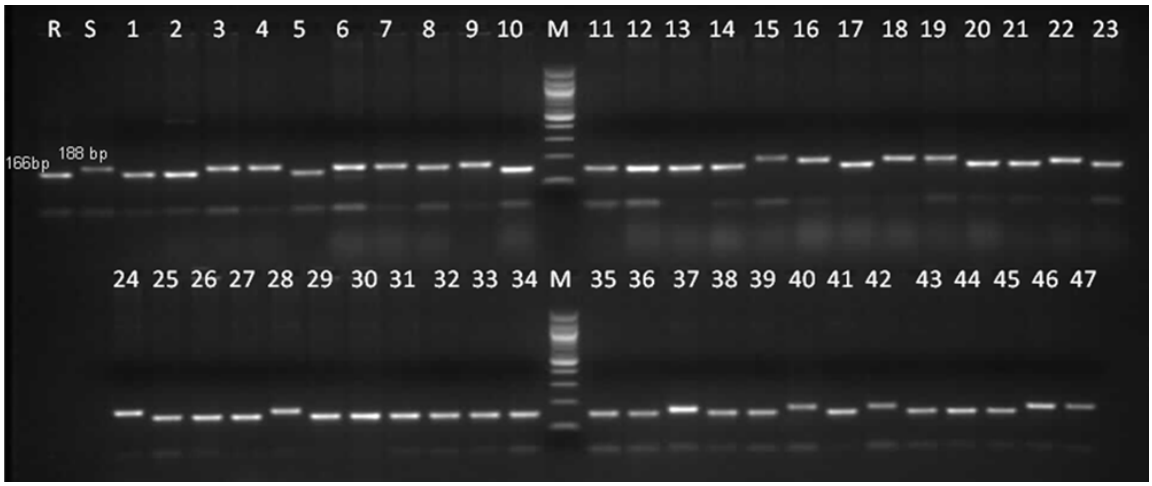

Figure 2: Genotyping of RILs with marker Bmac213 located on 1H chromosome for leaf blight resistance in DWR 49/ RD 2503 barley population of F_8 generation (M- Ladder, R- Resistant parent, S- Susceptible parent, RILs 1-47).

Inheritance of resistance

Out of 50 polymorphic markers, eleven molecular markers (Bmac 213, ABG 058, Bmag 125, Hv5s, Gbm 1208 , GBM1444, GBM1506, GMS1, GBM5012, Ebmac827, Bmag369) present on chromosome 1H, 2H,

3H, 5H and 7H were found to be closely linked during chi square test analysis for leaf blight resistance as p values for these markers were found greater than 0.05 and χ^2 values less than the significant χ^2 value (for P>0.05, significant χ^2 value is 3.84) (Table 1). This suggested that the resistance to leaf blight is controlled by polygenic inheritance which is also supported by previous research studies.

Table1: Segregation pattern of leaf blight resistance in DWR 49 x RD 2503 RIL population (F_8 generation)

	Resistant	**Susceptible**	**χ^2 value**	**P value**
Expected values	71	71		
Observed values				
DWR 49 X RD 2503	59	83	0.0440058	0.8338
Molecular markers with Chromosome location				
Bmac 213 (1H)	69	73	0.7371178	0.3906
ABG 058(2H)	69	73	0.7371178	0.3906
Bmag 125 (2H)	70	72	0.8667121	0.3519
Hv5s (2H)	69	73	0.7371178	0.3906
Gbm 1208 (2H)	73	69	0.7371178	0.3906
GBM1444 (3H)	70	72	0.8667121	0.3519
GBM1506 (5H)	73	69	0.7371178	0.3906
GMS1 (5H)	70	72	0.8667121	0.3519
GBM5012 (6H)	69	73	0.7371178	0.3906
Ebmac827 (7H)	71	71	1	0.317311
Bmag369 (7H)	72	70	0.8667121	0.3519

An earlier report also suggested polygenic control for spot blotch resistance three and four genes were found to control virulence of *C. sativus* on barley genotypes NDB112 and Larker, respectively [7]. Polygenic control for resistance to common root rot caused by *Cochliobolus sativus* was also reported [2]. In another study, three unlinked genes were found to control resistance to spot blotch at the adult plant stage [6]. Previous researches demonstrated that spot blotch at adult plant stage in the field is controlled primarily by a major effect QTL on chromosome 5(1H), along with a minor effect QTL on chromosome 1(7H) [14]. In another study, Thirteen QTLs were identified using DArT and SNP markers on chromosomes 1H, 2H, 3H, 5H, and 7H explaining 2.3 to 3.9% phenotypic variation during association mapping of spot blotch resistance in wild barley [12]. Genetic study of four resistant lines of two rowed barley reported adult plant resistance QTLs on chromosome 3HS and 7HS explaining 8% to 42% phenotypic variance and a single QTL on 7HS chromosome for seedling resistance explaining 62% phenotypic variance [3].

Previous Indian barley study also inferred that three genes control the inheritance of resistance to spot blotch. Two QTLs (*Rcs-qtl-5H-1* = 28.40% and *Rcs-qtl-5H-2* = 18.97%) on 5H chromosome region (BMS 32 and BMS90) was accounted for a total phenotypic variation of 47.37% and one QTL (*Rcs-qtl-1H-1*) on 1H chromosome region (HVCMA) was accounted for 12% phenotypic variation

for spot blotch resistance in RIL population IBON18 X RD2508 [15]. The markers found associated with these loci might prove very useful for selection of spot blotch resistance within Indian and international barley breeding programs.

CONCLUSION

Molecular markers found closely linked with leaf blight resistance in this study would facilitate mapping of QTLs and ensure efficient screening of large populations during marker assisted studies in barley breeding program for leaf blight.

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