ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 10, Issue, 04(D), pp. 31897-31900, April, 2019

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Paper

MOLECULAR MARKERS AND QTLS ASSOCIATED WITH LEAF BLIGHT RESISTANCE IN BARLEY

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DOI: http://dx.doi.org/10.24327/ijrsr.2019.1004.3359

ARTICLE INFO

Article History:
Received 6th January, 2019
Received in revised form 15th February, 2019
Accepted 12th March, 2019
Published online 28th April, 2019

Key Words:

Barley, Bipolaris sorokiniana, Leaf blight, Molecular markers, Quantitative Trait Loci, Spot blotch

ABSTRACT

Spot blotch or leaf spot, most common form of leaf blights of barley, caused by Bipolaris sorokiniana (earlier Helminthosporium sativum), 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 backgrounds of mapping populations with very few reports coming from all over the world. An integrated approach is recommended with host resistance as a major component to identify QTLs and specific markers targeted for genomic regions involved in spot blotch resistance in barley lines. Spot blotch resistant (DWR49) and susceptible (RD2503) lines were crossed 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 (D/R). Two QTLs, Rcs-qtl-1H-1 and Rcs-qtl-1H-2 on chromosome 1H explaining phenotypic variance of 62.2% and 7.4% were found to be associated with spot blotch resistance.

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INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cereal grain crop, grown in about 100 countries worldwide for feed, forage and malt. Spot blotch 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 (Nutter *et al.* 1985). In susceptible barley cultivars, average yield losses up to 40% have been reported (Van Leur 1991). Repeated fungicide application can be used to control spot blotch, but the use of resistant cultivars offers the most economically and environmentally sound means of control. Spot blotch, has been controlled for over 40 years through the deployment of resistant cultivars. But still many commercial cultivars are vulnerable to spot blotch epidemics. 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.

Disease resistance in barley 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. Resistance for spot blotch follows quantitative inheritance and several investigations have been conducted to determine the genetic basis of this durable spot blotch resistance. Initial study for the genetics of this disease resistance postulated one or two genes regulated adult plant resistance in barley (Wilcoxson et al. 1990). Later, Steffenson *et al.* (1996) reported a seedling resistance gene (Rcs5) on the short arm of chromosome 7H, a major effect quantitative trait locus (QTL) conferring adult plant resistance on the long arm of chromosome 1H, and a minor effect QTL conferring adult plant resistance at or near Rcs5 on chromosome 7H in doubled haploid (DH) population of cross Steptoe x Morex. Afterward, Bilgic *et al.* (2005) validated the large effect of Rcs-qtl-1H-6-7 on chromosome 1H as well as the one Rcs-qtl-7H-2-4 on chromosome 7H at the Rcs5 locus, and identified two additional QTLs, one conferring seedling resistance (Rcs-qtl- 3H-11-12) on the long arm of chromosome 3H and the other conferring adult plant resistance on the short arm of chromosome 3H (Rcs-qtl-3H-3-5) in Morex genotype. An another QTL at or near Rcs5 on chromosome 7H explaining 75% phenotypic variance have been categorized in Harrington x Morex population (Bilgic *et al.* 2005). Similarly,

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genetic study of four resistant lines of two rowed barley postulated adult plant resistance QTLs on chromosome 3HS and 7HS and a single QTL on 7HS chromosome for seedling resistance (Bowill *et al.* 2010). Two most significant SNP markers were reported during the association mapping of 767 lines of US barley lines (Zhou *et al.* 2009). In another study, thirteen QTLs were identified using DArT and SNP markers on chromosomes 1H, 2H, 3H, 5H, and 7H during association mapping of spot blotch resistance in wild barley (Roy *et al.* 2010). Recently, Zhou *et al.* (2013) reported three quantitative trait loci (QTL), Rcs-qtl- 1H-11_10764, Rcs-qtl-3H-11_10565 and Rcs-qtl-7H- 11_20162, for both seedling and adult plant resistance for spot blotch during genome wide association mapping of US barley germplasm lines. The genetic study for spot blotch resistance in cross IBON 18/RD 2508 reported three resistance genes *Rcs-qtl-5H1*, *Rcs-qtl-5H-2* and *Rcs-qtl-1H1* for north east sowing region of India (Tyagi *et al*. 2008). Still not much has been categorized for genetics and inheritance of spot blotch resistance. The present study has been conducted with the focus to identify the loci involved in durable spot blotch resistance in barley lines.

MATERIAL AND METHODS

Plant Material

DWR49 is a 2 rowed variety which shows a high level of resistance to spot blotch, a genetic stock registered with NBPGR, New Delhi. It carries resistance up to 13 scales that is considered as the best observation for spot blotch resistance. RD2503 is a 6 rowed high yielding malt variety released under All India Coordinated Wheat and Barley Improvement Program (AICW&BIP) and is highly susceptible to spot blotch disease. These two genotypes were screened as highly resistant (DWR49) and susceptible (RD2503) for spot blotch resistance for three consecutive years (2003-07) at four different hotspot regions for foliar blight in north western and north eastern regions of India. Recombinant Inbreed Lines population was developed from the cross between resistant parent DWR49 and susceptible parent RD2503 and used to map loci conferring resistance to spot blotch. In this study, 142 progeny from the D/R population were phenotyped for their reaction to the spot blotch pathogen.

Pathogen Inoculation and Phenotypic Disease Assessment

RILs of cross DWR49 X RD2503 were screened under controlled conditions during the crop season (2010-13) at Directorate of Wheat Research (DWR), Karnal. Spot blotch disease incidence was induced by inoculating infector rows (RD2503) with a pure culture of isolates of *B. sorokiniana.* The experimental design was a randomized complete block with two replications. The barley entries were planted in paired 1 m rows (15 to 25 seeds per row) spaced 0.3 m apart. The infector was grown at right angle to the direction of test lines. The inoculum (monoconidial culture of BS-13 highly virulent isolate) was multiplied on autoclaved sorghum seed and 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 (Joshi *et al*. 2007b, c). 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 spot blotch appeared in first week of March on the infector plants (RD2503). Disease data were recorded in three observations on overall reaction of the plant following 1-9 scale of classification (Fetch and Steffenson 1999) and plants were categorised as resistant (up to 3), moderately resistant (3-5), moderately susceptible (5-7) and susceptible (>7).

Single Sequence Repeat (SSR) Analysis

Fresh leaves from 14 days old seedlings were used for DNA extraction using CTAB mini-prep method (Saghai-Maroof *et al.* 1984). 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 in this study. Primer sequences for SSR markers were obtained from GrainGenes (http://wheat.pw.usda.gov/GG2/index.shtml) and a standard protocol was used to amplify the markers. PCR reactions were set up 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. The following polymerase chain reaction (PCR) cycle profile was used: 7 min at 95°C, followed by 35 cycles at 94°C for 30 s, 50–60°C (depending on annealing temperature) for 30 s and 72°C for 30 s and one cycle at 72°C for 10 min. 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). DNA samples of five each homozygous resistant and homozygous susceptible RILs of F_6 were pooled in an equal quantity to make the homozygous resistant and susceptible DNA bulks, respectively. The contrasting bulks and parental lines were screened with total 283 SSR/STS markers during BSA to identify polymorphic markers for spot blotch resistance trait. Total 50 markers found polymorphic were used for genotyping F_8 generation of RIL population at individual recombinant line and the frequencies of parental alleles were estimated for each locus.

QTL Analysis

QTL analysis was conducted using a reference map of barley. Composite interval mapping (CIM) was undertaken using Windows QTL Cartographer version 2.5 (Wang *et al*. 2007), employing model 6 with a 10 cm window. Forward–backward regression was done to select cofactors before performing QTL detection by CIM. One thousand (1000) permutation tests at 1.5 cM intervals were executed to determine significance LOD (Logarithm of odds) thresholds for QTL detection. Recombination frequencies were transformed to centiMorgans (cM) using the Kosambi function.

RESULTS

Phenotypic data and Molecular Characterization

The artificial inoculation created very high incidence of disease on the test material and indicated good distribution of spot

blotch. Spot blotch infection on plants was uniform across all experiments, allowing clear and unambiguous classification of resistant and susceptible plants (Figure 1).

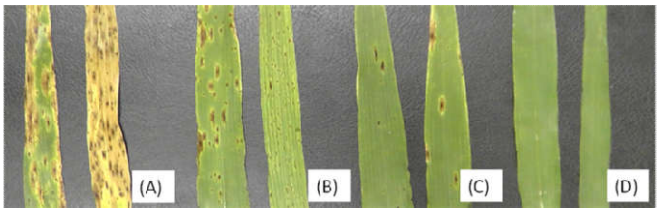

Figure 1 Symptoms of spot blotch on susceptible (a), moderately susceptible (b), moderately resistant(c) and resistant (d) RILS of cross DWR49 X RD 2503.

The controls reacted as expected to spot blotch infection. Resistant control, DWR49 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. These polymorphic markers were used to genotype individual RILs of cross DWR49 x RD2503 to identify genomic regions associated with spot blotch resistance (Figure 2).

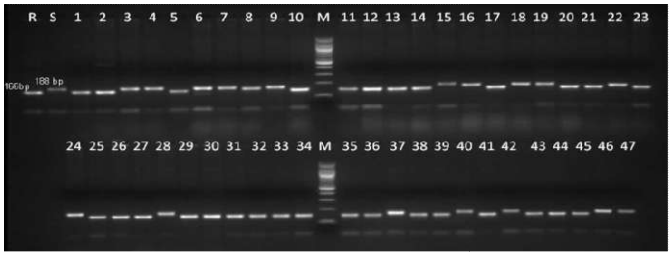

Figure 2 Genotyping of RILs with marker Bmac213 located on 1H chromosome for spot blotch resistance in DWR49 X RD2503 barley population of F_8 generation (M- Ladder, R- Resistant parent, S- Susceptible parent, RILs 1-47).

QTL Identification

Whole genome genetic linkage map for RILs population of DWR49 X RD2503 was available and used in the QTL analysis. Permutation test indicated that 2.5 was the critical logarithm of odds (LOD) score for a QTL to be significant. QTL analysis revealed that resistance to spot blotch is contributed by 2 QTLs on chromosome 1H in RIL populations of cross DWR49 X RD2503 (Table 1).

Table 1 Details of QTLs identified for spot blotch resistance in RIL population of DWR49 X RD2503

Chromosome	Closest marker	LOD	R^2
1H	Bmac 213	22.7	62.2
1H	Abg059-Bmag872	3.6	7.4

One major QTL, Rcs-qtl-1H-1 was found on IH chromosome at 3 cM near Bmac 213 with LOD score 22.7 which explained 62.2 % of phenotypic variation. One minor QTL, Rcs-qtl-1H-1 on chromosome 1H was found at 10.5 cM in Abg 059 and Bmag 872 interval with LOD score 3.6 which explained 7.4% of the phenotypic variation (Figure 3).

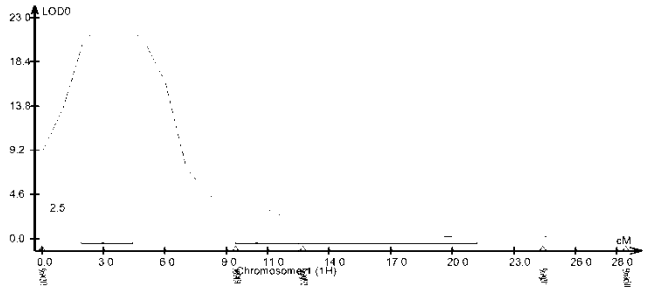

Figure 3 LOD score profiles of QTLs on 1H chromosome for spot blotch resistance in the DWR49 X RD2503 population.

DISCUSSION

During this study, our primary objective was to identify QTLs for spot blotch resistance and to characterize the genetic basis and expression of durable spot blotch resistance in barley lines. This study identified two QTLs on chromosome 1H: Rcs-qtl-1H-1 and Rcs-qtl-1H-12 having LOD score 22.7 and 3.6 respectively. These two QTLs explained 69.6 % of phenotypic variation collectively.

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) (Steffenson *et al.* 1996). In earlier study, Two QTLs (Rcs-qtl-1H-bPb-2813 and Rcs-qtl-1H-bPb- 3089) on 1H chromosome region (bPb-2813 and bPb- 3089, respectively) explained 2.7 and 3.9% of the phenotypic variation respectively (Roy *et al.* 2010). Likewise, one QTL (Rcs-qtl-1H-6-7) with major effect (r2=0.62) mapped in between ABG500A-ABG452 interval on chromosome 1H and another QTL (Rcs-qtl-5H-10-11) was mapped on chromosome 5H with minor effect (r2=0.05) for regulation of spot blotch resistance in barley (Bilgic *et al*. 2005). It was also reported that spot blotch resistance in the cultivar Svanhals was generally correlated with the vrs1 (formerly V/v) locus on chromosome 2(2H), the Blp (formerly B/b) locus on chromosome 5(1H), and the raw1 (formerly R/r) locus on chromosome 7(5H) (Griffee, 1925). Resistance to spot blotch conferred by a single QTL (Rcs6- QTL) mapped in cer-yy/Hor-2 interval on short arm of chromosome 1H. This major effect QTL explained 93% of phenotypic variation (Bilgic *et al.* 2006). Over the past 40 years, breeders have been very successful in retaining the chromosome 5 (1H) and 7 (5H) QTLs for spot blotch resistance in their six-rowed malting germplasm, presumably by fixing the resistance allele in elite parents and practicing occasional phenotypic selection. In earlier study reported, 1H and 5H chromosome regions (BMS 32, BMS90 and HVCMA) was accounted for 12 to 28% phenotypic variation and for leaf blight resistance in RIL population IBON18 X RD 2508 (Tyagi *et al*., 2008). This shows that chromosome 1H is exclusively involve in spot blotch resistance in barley lines.

CONCLUSION

SSR markers prove to be very useful tool for identification of genomic regions associated with spot blotch resistance in barley. 2 QTLS (Rcs-qtl-1H-1 & Rcs-qtl-1H-2) on chromosome 1H explaining 69.6 % of phenotypic variation

collectively were reported. This shows that chromosome 1H is exclusively involve in spot blotch resistance in barley.

Acknowledgement

Authors are thankful for the financial support from DBT to carry this research work under the project "Identification of genomic regions for leaf blight resistance in barley".

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How to cite this article:

Dr Neha Jain *et al*., 2019, Molecular Markers and Qtls Associated with Leaf Blight Resistance in Barley. *Int J Recent Sci Res.* 10(04), pp. 31897-31900. DOI: http://dx.doi.org/10.24327/ijrsr.2019.1004.3359

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