Genome Wide Association Study of Spot Blotch at Seedling and Adult Plant stages in Morocco and India

Visioni. A.[1†], Rehman S.[1†], Vaish S.S.[2], Singh S.P.[3], Gyawali S.[1,4], Al-Abdallat A.M.[1,5], and Verma R.P.S[1].

[1] Biodiversity and Crop Improvement Program, International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco
[2] Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India.
[3] Department of Plant Pathology, Narendra Dev University of Agriculture and Technology, Kumarganj, Faizabad, Uttar Pradesh India.
[4] Vegetable Seed Pathology Department, Washington State University, Mount Vernon, USA.
[5] Department of Horticulture and Crop Science, Faculty of Agriculture, The University of Jordan, Amman, Jordan

Abstract

Barley spot blotch caused by *Cochliobolus sativus (Bipolaris sorokiniana)* is one of the major constrains to barley production worldwide in warmer regions. This study was undertaken to identify and estimate effects of loci underlying quantitative resistance to spot blotch at seedling and adult plant stages. A panel of 261 barley genotypes (HI-AM) consisting of released cultivars from North and South America, Europe, Australia, advanced breeding lines, and landraces from ICARDA, was screened for resistance to spot blotch. Seedling resistance screening was conducted using two most virulent isolates from Morocco (ICSB and SB54), while adult plant resistance was assessed at two hot spot locations (Faizabad and Varanasi) in India under artificial inoculation using a mixture of prevalent isolates. Both GLM and MLM model were employed in Tassel using principal component analysis and Kinship Matrix as covariates. Genome wide association mapping indicated a total of 23 QTL at seedling stage (14 for isolate ICSB and 9 for isolate SB54), while 15 QTL were detected for adult plant resistance (6 at Faizabad and 9 at Varanasi). Common QTL at seedling and adult plant stages were found across all barley chromosomes. QTL detected explained together the 73.24% of the variance for seedling resistance to isolate ICSB and 49.26% for isolate SB54. QTL for adult plant resistance explained together 38.32% and 44.09% at Faizabad and Varanasi, respectively. Several QTL identified in this study were also reported before in bi-parental and association mapping populations studies supporting our results. The promising QTL detected at both stages, once validated, can be used for MAS in spot blotch resistance breeding program globally.

Objectives

Spot blotch is major concern for barley in South Asia including China, Nepal, Pakistan, and the humid north eastern regions of India (Kumar et al. 2007; Chand et al. 2008; Singh et al. 2009; Vaish et al. 2011; Prasad et al. 2013). Spot blotch is also considered a serious threat to barley in the upper Midwest of the USA and prairie provinces of Canada (Clark 1979; Ghazvini and Tekauz 2007) and recently in warmer regions of north Africa, especially in Morocco. The main aim of this study was to identify and estimate effects of loci underlying quantitative resistance to spot blotch at seedling and adult plant stages. We used an association mapping panel (Hi-AM) of 261 (172 two-row and 89 six-row types) barley genotypes consisting of germplasm from North and South America, Europe, Australia and from ICARDA barley breeding program. Adult-plant stage (APS) screening was done at two locations (Faizabad and Varanasi) in India using mixture of the prevalent isolates in the region while seedling resistance test done at ICARDA (Rabat, Morocco) using isolates ICSB and SB54).The panel was genotyped with DaRTSeq platform (Diversity Array Technology Pty Ltd, DaRT P/L) and population structure and LD were assessed as already reported by Visioni et al. (2018; see poster PE1056). GWAM was performed using both General Linear Model(GLM) and Mixed Linear Model (MLM) methods.

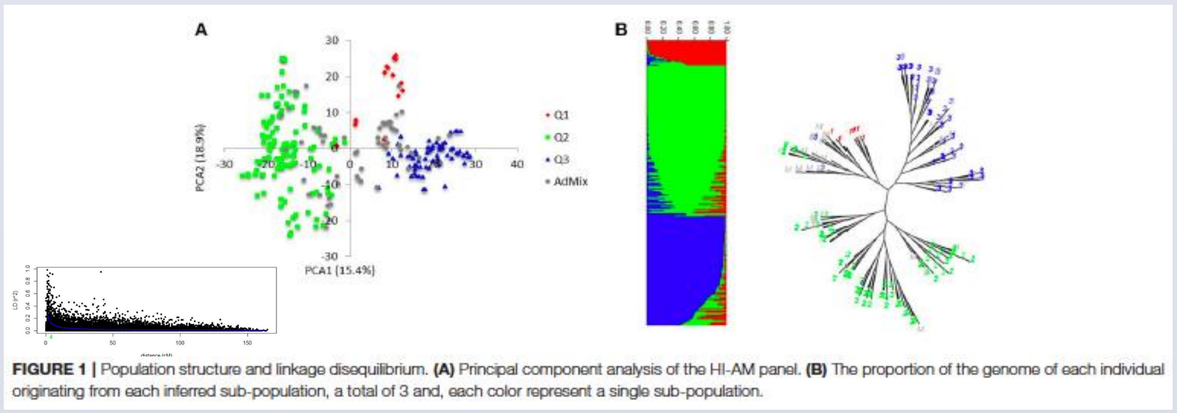

FIGURE 1 | Population structure and linkage disequilibrium. **(A)** Principal component analysis of the HI-AM panel. **(B)** The proportion of the genome of each individual originating from each inferred sub-population, a total of 3 and, each color represent a single sub-population.

Figure 1. Population structure and Linkage disequilibrium decay

Performing GWAS for spot blotch at SRT, the best model fitting was GLM procedure using PCA for accounting population structure and relatedness, when analyzing data for isolate SB54 using both PAVs and SNPs markers sets. On the other hand analyzing data for isolate ICSB GLM + PCA was again the best model fitting using the SNPs marker set, while the MLM procedure using Q+PCA model was the best one fitting using the PAVs marker set.

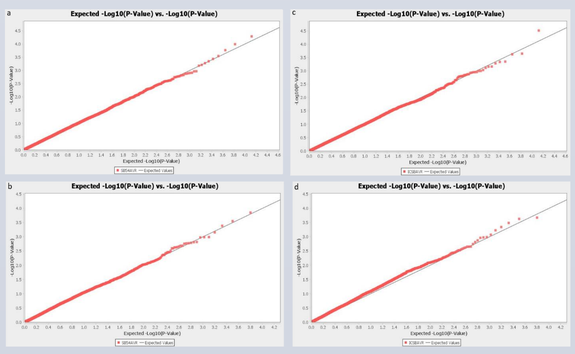

Figure 2. QQ plots of marker-trait association at SRT stage for: (a) Isolate SB54 using PAVs markers set (GLM+PCA); (b) isolate SB54 using SNPs markers set (GLM+PCA); (c) isolate ICSB using PAVs markers set (MLM PCA+K); (d) isolate ICSB using SNPs markers set (GLM+PCA). The black line is the expected line under the null distribution.

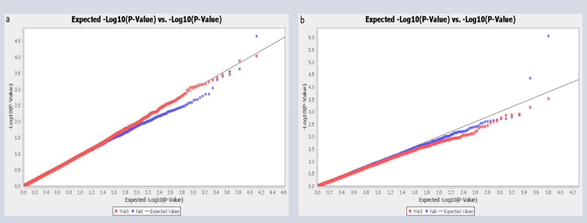

Figure 3. QQ plots of marker-trait association at APS using (a) PAVs markers set and the GLM+PCA procedure (b) SNPs markers set and GLM+ PCA procedure. The black line is the expected line under the null distribution.

Results

Results of genome scans for isolate SB54 showed 9 QTL again located on chromosomes 1H, 3H, 4H, 6H and 7H (Table 1). Markers R^2 for isolate SB54 ranged from 4.53% to 6.82 % and the total phenotypic variance explained by QTL was 49.26%.The GWAM analyses at SRT for ICSB identified 14 QTL located on chromosomes 1H, 3H, 4H, 6H and 7H (Table 1) with markers R^2 ranging from 4.32% to 7.79 % (Table 1). The QTL detected for isolate ICSB at SRT explained together the 72% of phenotypic variance. Performing GWAS for APS resistance to spot blotch using both PAVs and SNPs markers sets the best model fitting was GLM procedure using PCA for accounting population structure and relatedness.

QTL id	Trait	Marker	Chr	cM	-Log10(p)	Marker R²	Mark. Eff.	MAF	Gene Identifier	Description
SRT_ICSB_1	ICSB	DaRT529	1	45	3.16	4.72%	0.73	21.09%	MLOC_14910.1	-
SRT_ICSB_2	ICSB	SNP520	1	120	3.23	4.59%	-1.01	9.84%		-
SRT_ICSB_3	ICSB	DaRT4210	3	2	3.65	5.74%	-0.77	35.10%	AK365963	NBS-LRR disease resistance protein homologue
SRT_ICSB_4	ICSB	SNP1838	3	67	3.08	4.46%	-0.66	35.89%		-
SRT_ICSB_5	ICSB	DaRT5749	3	133	4.51	7.79%	-0.89	23.11%	MLOC_64418.1	NBS-LRR disease resistance protein family-3
SRT_ICSB_6	ICSB	DaRT6694	4	60	3.00	4.32%	1.15	5.81%	AK356118	Glucan endo-1,3-beta-glucosidase 4
SRT_ICSB_7	ICSB	DaRT9634	6	17	3.62	5.86%	0.80	21.28%	MLOC_76542.1	NB-ARC domain-containing disease resistance protein
SRT_ICSB_8	ICSB	DaRT9658	6	25	3.35	5.04%	-0.65	28.69%	AK371644	Glucan endo-1,3-beta-glucosidase 4
SRT_ICSB_9	ICSB	SNP3989	6	93	3.34	4.86%	-1.15	8.00%	MLOC_58499.1	MYB transcription factor
SRT_ICSB_10	ICSB	DaRT11126	7	3	3.28	4.94%	-0.62	34.84%	MLOC_38445.1	NBS-LRR disease resistance protein homologue
SRT_ICSB_11	ICSB	DaRT11173	7	10	3.11	4.78%	-0.64	32.51%	MLOC_22072.1	NBS-LRR disease resistance protein-like protein
SRT_ICSB_12	ICSB	SNP4686	7	116	3.63	5.25%	-0.90	18.50%	MLOC_3420.1	MYB transcription factor
SRT_ICSB_13	ICSB	SNP4986	unk	unk	3.68	5.74%	-1.25	6.78%		-
SRT_ICSB_14	ICSB	SNP5983	unk	unk	3.49	5.14%	-0.70	33.73%		-
SRT_SB54_1	SB54	DaRT266	1	18	3.35	5.09%	0.68	38.59%	MLOC_70910.1	NB-ARC domain-containing disease resistance protein
SRT_SB54_2	SB54	DaRT475	1	38	3.99	5.85%	-0.92	15.75%	MLOC_11791.2	Disease resistance protein
SRT_SB54_3	SB54	DaRT4187	3	2	4.28	6.82%	-1.08	15.25%	AK365963	NBS-LRR disease resistance protein homologue
SRT_SB54_4	SB54	DaRT5749	3	133	3.76	5.89%	-0.86	23.11%	MLOC_64418.1	NBS-LRR disease resistance protein family-3
SRT_SB54_5	SB54	SNP2594	4	68	3.85	5.68%	0.97	13.44%		-
SRT_SB54_6	SB54	SNP2750	4	104	3.39	5.19%	0.89	27.08%		-
SRT_SB54_7	SB54	DaRT9634	6	17	3.27	5.06%	0.81	21.28%	MLOC_76542.1	NB-ARC domain-containing disease resistance protein
SRT_SB54_8	SB54	SNP4686	7	116	3.55	5.16%	-0.99	18.50%	MLOC_3420.1	MYB transcription factor
SRT_SB54_9	SB54	SNP6285	unk	unk	3.16	4.53%	-0.72	48.22%		-

Table 1. Results of GWAM for spot blotch resistance at SRT

GWAM for APS showed a total of 15 QTL using phenotypic data from two locations (Faizabad and Varanasi). Six QTL located on chromosomes 1H, 2H, 4H and 6H were detected analyzing data collected in Faizabad. Markers R^2 ranged from 4.64% and 9.85% explaining 38.32% of the total phenotypic variance. Results from Varanasi show 9 QTLs located on chromosomes 2H, 3H, 4H, 5H and 7H with R^2 between 4.44% and 5.84% and explaining 44.09% of the total phenotypic variance. Overlapping QTL at SRT were found between isolates ICSB and SB54, the QTL were located on chromosomes 3H (2 cM and 133 cM, respectively), 6H (17 cM) and 7H (116 cM). Furthermore we also found the QTL *SRT_ICSB_11* for SRT located on chromosome 7H (10cM;) overlaps with a QTL for APS located on the same chromosome at 12.75 cM (*APS_War_9*).

QTL id	Trait	Marker	Chr	Pos. (cM)	-log(10)p	Marker_R²	Effect	MAF	Gene Identifier	Description
APS_Fai_1	FaiS	SNP340	1	87	6.06	9.85%	0.14	13.62%	MLOC_70659.2	MYB TF
APS_Fai_2	FaiS	DaRT3556	2	127.2	3.04	4.62%	-0.01	28.81%	MLOC_75110.2	-
APS_Fai_3	FaiS	DaRT3981	2	146.72	4.65	6.95%	-0.06	15.42%	MLOC_6943.1	-
APS_Fai_4	FaiS	DaRT6240	4	1.27	3.63	5.37%	-0.07	35.20%	MLOC_10090.4	NBS-LRR disease resistance protein
APS_Fai_5	FaiS	SNP3639	6	30	4.36	6.90%	0.06	6.33%	AK371644	Glucan endo-1,3-beta-glucosidase 4
APS_Fai_6	FaiS	DaRT10608	6	100.42	3.30	4.64%	-0.07	5.79%	MLOC_18670.1	CAP protein
APS_War_1	WarS	DaRT2274	2	40.08	3.29	4.60%	0.07	38.65%	MLOC_73232.1	CsAtPR5 Pathogeneis Response
APS_War_2	WarS	DaRT3041	2	94.72	3.23	4.44%	0.28	16.93%	MLOC_65678.5	-
APS_War_3	WarS	DaRT5301	3	83.07	3.88	5.68%	0.14	42.62%	MLOC_34610.2	-
APS_War_4	WarS	SNP2134	3	128	3.53	4.96%	0.12	26.98%	AK369539/MLOC_64418.1	NBS-LRR disease resistance protein
APS_War_5	WarS	DaRT6861	4	79.76	3.15	4.42%	0.09	20.00%	MLOC_72875.1	-
APS_War_6	WarS	DaRT7465	5	35.1	3.14	4.52%	-0.09	36.78%	MLOC_57279.1	-
APS_War_7	WarS	DaRT7503	5	41.56	3.46	4.87%	-0.03	34.13%	MLOC_59947.3	-
APS_War_8	WarS	DaRT8678	5	137.22	3.45	4.76%	-0.14	44.92%	MLOC_63574.2	Glucan endo-1,3-beta-glucosidase 5
APS_War_9	WarS	DaRT11239	7	12.75	4.03	5.84%	0.11	38.06%	MLOC_22072.1	NBS-LRR disease resistance like protein

Table 2. Results of GWAM for spot blotch resistance at APR

Conclusions

QTL identified in this study may represent an interesting source of quantitative resistance and, if validated they can be introgressed in breeding materials, through MAS, to combine both qualitative and quantitative resistance. Qualitative resistance mechanisms have been extensively studied in terms of genomic location and specificity while mechanisms underlying quantitative resistance still to be clarified. Expanding the catalog of mapped QTL for spot blotch resistance and its validation represent an important step toward the application of MAS for the introgression and pyramiding of resistance genes in new barley cultivars. In this work, novel QTL for spot blotch resistance at SRT and adult plant stages were identified which could be helpful in dissection the resistance mechanism to this pathogen. New QTL need to be validated for their diversity, effectiveness in different genetic background and with more spot blotch isolates existing in other regions of the world to ensure their use for introgression in barley germplasm or for MAS globally.

References

1. Visioni, A. Gyawali S., Selvakumar R., Gangwar O.P., Shekhawat P.S., Bhardwaj S.C., A. Al-Abdallat, Sharma-Poudyal, D., Kehel Z. and Verma R.P.S. (2018). Genome wide association analysis for stripe rust resistance in barley seedlings and adult plant stage in India. Front. Plant Sci. doi: 10.3389/fpls.2018.00520.
2. Kumar D., Chand R., Prasad L.C., Joshi and A.K. 2007. A new technique for monoconidial culture of the most aggressive isolate in a given population of *Bipolaris sorokiniana*, cause of foliar spot blotch in wheat and barley. World J Microbiol Biotechnol, 23(11)**:**1647-51
3. Chand R., Sen D., Prasad K.D., Singh A.K., Bashyal B.M., Prasad L.D. and Joshi A.K. 2008. Screening for disease resistance in barley cultivars against *Bipolaris sorokiniana* using callus culture method. Indian J Exp Biol., **36:**249-253.
4. Prasad R., Prasad L.C., Chand R. and Joshi A.K. 2013. Assessment of diversity for resistance to spot blotch diseases and its association with certain phenotypic traits in barley. Field Crop Res., **154:**195-200.
5. Singh RN, Singh AK, Singh SP (2009) Prevalence and management of spot blotch (*Cochliobolus sativus*) of barley (*Hordeum vulgare* L.) in eastern India. Proceedings of the National Academy of Sciences India. Section B, Biological Sci 79(1):65 –69.
6. Vaish SS, Ahmed SB, Prakash K (2011) First documentation on status of barley diseases from the high altitude cold arid trans Himalayan Ladakh region of India. Crop Protect 30(9):1129–1137.
7. Clark RV (1979) Yield losses in barley cultivars caused by spot blotch. Can J Plant Pathol 1:113 –117.
8. Ghazvini H and Tekauz A (2007) Virulence diversity in the population of *Bipolaris sorokiniana*. Plant Dis 91:814–821

This research was funded by CGIAR research program (CRP) on Dryland Cereals and by CROPTRUST in the frame of DIIVA Project