Genome Wide Association Mapping of Seedling and Adult Plant Resistance to Barley Stripe Rust in India

A. Visioni [1]*, S. Gyawali [1,2], R. Selvakumar [3], O.P. Gangwar [3], P.S. Shekhawat [4], S.C. Bhardwaj [3], A.M. Al-Abdallat [1,5], Z. Kehel [1] and R.P. S. Verma[1]

[1] Biodiversity and Crop Improvement Program, International Center for Agricultural Research in the Dry Areas, Rabat, Morocco,
[2] Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada,
[3] Indian Institute of Wheat and Barley Research, Indian Council of Agricultural Research, Karnal, India,
[4] Rajasthan Agricultural Research Institute, Durgapura, Jaipur, India,
[5] Department of Horticulture and Crop Science, Faculty of Agriculture, The University of Jordan, Amman, Jordan

Abstract

Performing genome-wide association mapping (GWAM), we identified and estimated effects of loci underlying quantitative resistance to barley stripe rust, caused by *Puccinia striiformis f.sp. hordei* (Psh). Barley stripe rust occur worldwide and is a major disease in South Asia. The 261 barley genotypes of the Hi-AM panel consisting of germplasm from North and South America, Europe, Australia and from ICARDA barley breeding program were screened for Psh resistance at seedling and adult plant stages. Seedling resistance was evaluated with the five prevalent Psh races in India. Screening for the adult plant stage resistance was also performed in two different locations by inoculating with a mixture of the five races used for seedling screening. Results of GWAM showed a total of 45 QTL located across the seven barley chromosomes for seedling resistance to the five races and 18 QTL for adult plant stage resistance. Common QTL for different races at seedling stage were found on all chromosomes except on chromosome 1H. Four common QTL associated with seedling and adult plant stage resistance were found on chromosomes 2H, 5H, and 6H. Moreover, one of the QTL located on the long arm of chromosome 5H showed stable effects across environments for adult plant stage resistance. Several QTL identified in this study were also reported before in bi-parental and association mapping populations studies validating current GWAM. However 15 new QTL were found at adult plant stage on all chromosomes except the 4H, explaining up to 36.79% of the variance. The promising QTL detected at both stages, once validated, can be used for MAS in Psh resistance breeding program globally.

Materials and Methods

The 261 spring barley genotypes of the Hi-AM panel (172 two-row and 89 six-row types), that consist of released cultivars, advanced breeding lines, and landraces were used in this study. SRT against five prevalent Psh races in India, namely, Q (5S0), 24 (0S0-1), 57 (0S0), M (1S0), and G (4S0) were performed by the Indian Institute of Wheat and Barley Research (IIWBR), RS Shimla, India. The adult plant stage (APS) screening for Hi-AM were done at IIWBR, Karnal and RARI Jaipur, in India. The panel was genotyped DaRT-Seq platform (Diversity Array Technology Pty Ltd, DaRT P/L). Markers quality control of the initial dataset was conducted by removing heterozygous and monomorphic markers and markers with minor allele frequencies (MAF) < 5% and markers with missing data > 10%. Population structure was firstly determined using STRUCTURE version 2.3.4 (Pritchard et al., 2000). Additionally, the adegenet package for R statistical software (The R Development core team) was used to confirm the number of sub-populations by the Bayesian Information Criterion (BIC). Finally, on the base of PCA results, genotypes were assigned to subgroups or considered admixed on the base of 80% membership criterion. Linkage disequilibrium (LD) was estimated with Tassel software V 5.2.32 (Bradbury et al., 2007) using a subset of 1,577 polymorphic markers with known position selected from the original SNP marker-set. Disease severity scores at seedling and adult plant stages and the genotypic data were used to perform GWAM using Tassel V. 5.2.32. GWAM was performed using both General Linear Model(GLM) and Mixed Linear Model (MLM) methods.

Results

The reaction type of barley genotypes at SRT and APS are summarized in Table 1. The population structure is shown in Figures 1A,B.The first subpopulation (Q1) is mainly composed by ICARDA germplasm (70%). The second group (Q2), located alone in the left side of the PCA chart (Figure 1A) shows the higher degree of diversity and the highest number of entries from South America (37%), followed by ICARDA germplasm (22%), North America (21%), Europe (13%), and Australia (3%). Q3 was again mainly composed by ICARDA germplasm (70%).

TABLE 1 | Summary of Psh reaction types at seedling stage and adult plant stage.

(A) Infection type*	Number of genotypes				
	Q (5S0)**	24 (0S0-1)**	**57 (0S0)**	M (1S0)**	G (4S0)**
'0' ';-' ';'	39 (14.9)	141 (54.0)	73 (27.9)	162 (62.0)	121 (46.4)
'1'	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
'2' '2-' '2+'	2 (0.6)	2 (0.8)	9 (3.4)	0 (0)	3 (1.1)
Resistant	**43 (16.5%)**	**143 (54.8%)**	**82 (31.4%)**	**162 (62.0%)**	**124 (47.5%)**
'3' '3-'	58 (22.2)	22 (8.4)	40 (16.8)	2 (0.8)	25 (9.5)
'33+' '3+'	162 (62.1)	93 (35.6)	135 (51.7)	97(37.1)	111 (42.5)
Susceptible	**220 (84.3%)**	**115 (44.0%)**	**175 (67.0%)**	**99 (37.9%)**	**136 (52.1%)**

(B) Rust severity***	Number of genotypes		
	Durganpura 13	Durganpura 14	Karnal 14
'0, TR, TMR, TMS, TS'	48 (16.9)	34 (12.7)	193 (74.0)
'≤20R, ≤10MR'	43 (16.4)	19 (7.2)	7 (2.7)
'>10MR-40MR, 5MS'	31 (11.9)	20 (7.6)	1 (0.3)
Resistant	**122 (46.7%)**	**73 (27.9%)**	**201 (77.0%)**
'5S-20S, >5MS'	96 (36.8)	130 (49.8)	52 (19.9)
'>20S-60S'	18 (6.9)	27 (10.3)	4 (1.5)
'>60S-100S'	25 (9.5)	30 (11.5)	1 (0.4)
Susceptible	**139 (53.2%)**	**187 (71.6%)**	**57 (21.8%)**

*0; (naught fleck) no visible infection.;- (Fleck minus) slightly necrosis /micro-flecking visible.; (Fleck) no uredia but small hypersensitive flecks present. 1uredia minute, surrounded by distinct necrotic areas. 2 small to medium uredia surrounded by chlorotic or necrotic border. 3 uredia small to medium in size and chlorotic areas may be present. 3+ uredia large with or without chlorosis, sporulating profusely and forming rings. 33+ both 3 and 3+ pustules occur together.
**Race type of *Puccinia striiformis* Westend. f. sp. *hordei* Erikss.
***R no uredia present. TR trace or minute uredia on leaves without sporulation. TMR trace or minute uredia on leaves with some sporulation. MR small uredia with slight sporulation. MR-MS small-to-medium-sized uredia with moderate sporulation. MS-S medium-sized uredia with moderate to heavy sporulation. S large uredia with abundant sporulation.

The 6-row genotypes are spread across the three subgroups representing the 53% (Q1), 26% (Q2), and 45% (Q3) of the total number of genotypes for each subpopulation, respectively. The scatter plots of LD (R2) as a function of the inter-marker distance (cM) within the same chromosome for all genotypes indicated a clear LD decay at 4 cM (R2 = 0.18) with genetic distance.

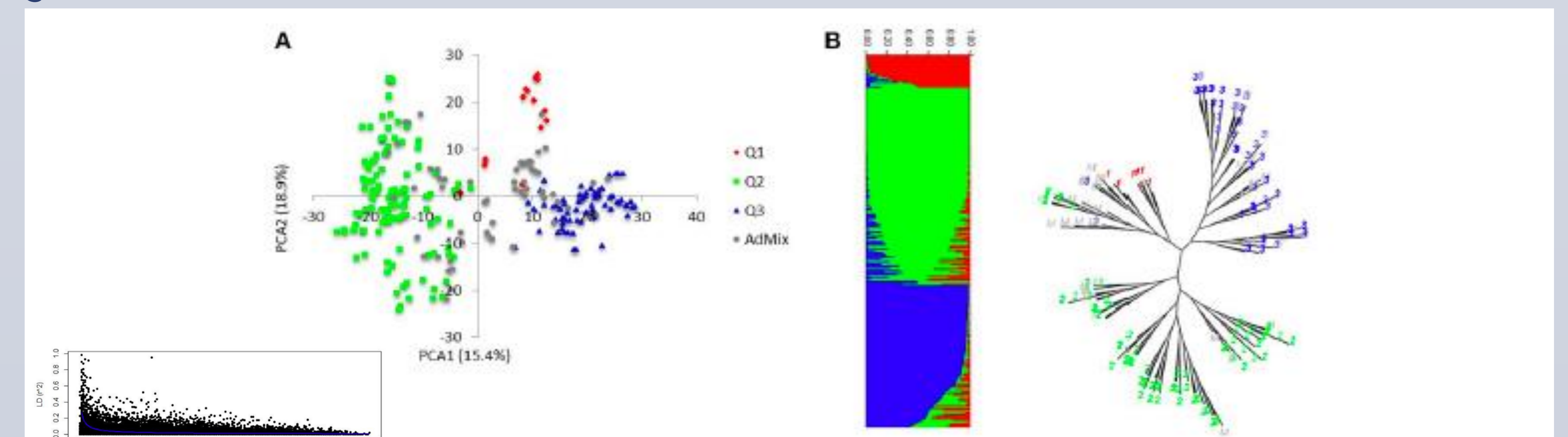

FIGURE 1 | Population structure and linkage disequilibrium. **(A)** Principal component analysis of the HI-AM panel. **(B)** The proportion of the genome of each individual originating from each inferred sub-population, a total of 3 and, each color represent a single sub-population.

The GWAM analyses at SRT identified 45 QTL located across the seven barley chromosomes (Table 2). The marker R^2 ranged from4.25%to 6.56% (Table 2). The race specific QTL detected for SRT explained together the 41.77 % (Race Q),50.1% (Race 24), 36.42% (Race 57), 53.0% (Race G), and 49.84% (Race M) of phenotypic variance, respectively. GWAM for APS showed 18 QTL using phenotypic data from two locations and during two seasons (Table 3). The markers R^2 ranged from 4.54 to 8.11% for APS (Table 3). A QTL located on chromosome 5H was found consistantly stable across seasons and environments. Phenotypic variance explained by QTL detected in case of APS was 15.35% for Dg13, 36.79% for Dg14 and 45.82% for Kr14.

TABLE 2 | GWAM results for seedling resistance test to individual races.

QTL	Marker	Chr.	Pos (cM)	$-\log_{10}(p)$	Marker R^2 (%)	Effect	MAF (%)
RACE Q							
SRT_R0_1	DaRT2415	2H	52.90	3.0248	4.43	1.75	19.92
SRT_R0_2	SNP1425	2H	140.72	3.2218	4.91	−0.50	10.92
SRT_R0_3	SNP1720	3H	51.63	3.1522	4.43	−1.77	31.13
SRT_R0_4	SNP2569	4H	61.12	4.0193	6.05	−0.21	27.53
SRT_R0_5	DaRT8922	5H	151.98	3.1837	4.93	−1.44	35.59
SRT_R0_6	SNP3974	6H	88.51	4.3196	6.41	1.73	26.88
SRT_R0_7	SNP4058	6H	113.24	3.6517	5.47	−2.71	7.72
SRT_R0_8	SNP4090	6H	118.77	3.3578	5.15	1.48	29.54
RACE 24							
SRT_R24_1	SNP50	1H	28.88	3.1535	4.25	−1.94	40.16
SRT_R24_2	DaRT4323	3H	7.01	3.2681	5.03	2.11	23.81
SRT_R24_3	DaRT4492	3H	14.94	3.1554	4.85	2.05	25.70
SRT_R24_4	SNP1579	3H	36.98	3.9825	5.51	3.25	16.60
SRT_R24_5	DaRT4767	3H	42.46	3.7706	6.07	2.56	25.41
SRT_R24_6	DaRT6458	4H	50.99	3.0864	4.73	−2.38	22.40
SRT_R24_7	DaRT7144	4H	111.33	3.1133	5.01	2.51	21.76
SRT_R24_8	DaRT7232	5H	3.02	3.1558	4.74	2.42	14.12
SRT_R24_9	DaRT8907	5H	151.88	3.0918	4.79	2.01	26.03
SRT_R24_10	DaRT11535	7H	24.06	3.3674	5.12	2.26	22.53
RACE 57							
SRT_R57_1	DaRT1073	1H	93.06	3.7433	6.09	2.26	18.30
SRT_R57_2	DaRT3036	2H	94.55	3.3503	5.05	1.98	21.18
SRT_R57_3	SNP1637	3H	45.22	3.1389	4.52	2.30	21.83
SRT_R57_4	DaRT5092	3H	59.63	3.1588	4.64	1.87	25.39
SRT_R57_5	SNP3013	5H	55.62	3.0613	4.28	2.70	11.20
SRT_R57_6	DaRT11344	7H	15.37	3.2730	5.29	1.99	29.66
SRT_R57_7	DaRT11479	7H	23.02	3.9349	6.56	2.04	42.37
RACE G							
SRT_RG_1	DaRT1886	2H	8.85	3.3314	5.18	−1.84	47.56
SRT_RG_2	SNP1434	2H	140.79	3.0564	4.33	2.19	36.36
SRT_RG_3	DaRT3982	2H	146.72	3.3517	5.04	2.33	19.29
SRT_RG_4	DaRT6779	4H	68.98	3.4257	5.57	2.19	22.46
SRT_RG_5	DaRT7132	4H	110.20	3.0167	4.59	2.10	20.33
SRT_RG_6	SNP2949	5H	32.88	3.0373	4.56	−2.65	13.39
SRT_RG_7	DaRT8539	5H	129.65	3.8616	5.88	2.03	30.35
SRT_RG_8	DaRT10087	6H	53.75	3.4969	5.27	3.53	6.64
SRT_RG_9	DaRT11815	7H	46.39	3.8987	6.28	2.04	46.72
SRT_RG_10	DaRT12391	7H	97.10	4.0060	6.29	2.84	13.15
RACE M							
SRT_RM_1	DaRT872	1H	64.02	3.0686	4.55	−3.54	6.61
SRT_RM_2	SNP1972	3H	98.23	3.0077	4.25	2.08	20.24
SRT_RM_3	DaRT6490	4H	51.42	4.0400	6.76	2.42	46.25
SRT_RM_4	DaRT6935	4H	91.50	3.2716	5.00	2.29	21.43
SRT_RM_5	DaRT8426	5H	120.35	3.0441	4.63	1.77	41.06
SRT_RM_6	DaRT8567	5H	133.69	3.0979	4.79	1.82	43.50

TABLE 3 | GWAM results for Psh resistance at adult plant stage.

QTL	Marker	Chr.	Pos (cM)	$-\log_{10}(p)$	Marker R2 (%)	Effect	MAF (%)
DURGAPURA 2013							
*APS_Dg13_1**	DaRT493	1H	40.5	3.0075	4.69	18.70	35.74
*APS_Dg13_2**	DaRT3562	2H	129.09	3.0026	4.92	23.11	8.33
APS_Dg13_3	DaRT8667	5H	137.08	3.7524	5.74	−25.80	9.77
DURGAPURA 2014							
*APS_Dg14_1**	DaRT568	1H	47.52	4.0543	6.73	21.47	24.17
APS_Dg14_2	SNP632	2H	8.85	3.1469	4.79	−16.27	26.56
*APS_Dg14_3**	SNP733	2H	40.08	3.5651	5.34	−15.79	33.06
*APS_Dg14_4**	SNP1886	3H	78.21	3.0723	4.54	−13.43	35.77
APS_Dg14_5	DaRT8661	5H	137.08	3.3738	5.07	−19.21	19.84
APS_Dg14_6	DaRT10854	6H	119.12	3.1978	4.94	13.18	15.98
*APS_Dg14_7**	SNP4572	7H	89.63	3.7015	5.38	−22.30	12.06
KARNAL 2014							
*APS_Kr14_1**	SNP447	1H	103.82	3.5960	5.36	−9.32	16.19
*APS_Kr14_2**	SNP476	1H	109.37	3.3382	4.84	−8.98	16.21
*APS_Kr14_3**	DaRT2151	2H	26.20	3.0050	5.36	8.23	42.13
*APS_Kr14_4**	DaRT2743	2H	64.83	3.2879	5.94	−7.89	22.00
*APS_Kr14_5**	DaRT2798	2H	70.53	4.3200	8.11	−13.42	8.96
APS_Kr14_6	DaRT4067	2H	149.26	3.2942	5.73	−13.77	5.77
APS_Kr14_7	DaRT8668	5H	137.08	3.0786	5.30	−11.44	8.98
*APS_Kr14_8**	SNP3509	5H	159.51	3.4386	5.18	−9.31	17.96

*Putative Adult Plant Resistance (ADP) QTL.

Amongst the QTL detected, 8 QTL were significant for two races. The QTL on chromosome 5H (cM 137.08), overlaps with a QTL for resistance at SRT for the race M. Furthermore, other 2 QTL detected for APS are located on chromosome 2H at the same position of QTL detected for resistance at SRT for race G. Similarly another QTL for APS located on chromosome 6H overlaps with OTL for SRT for race Q (Table 4). Out of the 45 QTL identified for resistance at SRT, 15 were coincident with prior reports Those QTL were reported from different barley germplasm and different Psh races. For Psh resistance at the APS stage using different germplasm and races, only 3 out of the 18 QTL detected, are coincident with previous reports (data not shown).

TABLE 4 | Summary of co-located QTL at seedling adult plant stages.

QTL	Race	Marker	Chr.	Pos (cM)	$-\log_{10}(p)$	Marker R2 (%)	Effect	MAF (%)
(A) SEEDLING STAGE								
Sdl_R0_2	RaceQ	SNP1425	2H	140.72	3.2218	4.91	−0.50	10.92
Sdl_RG_2	RaceG	SNP1434	2H	140.79	3.0564	4.33	2.19	36.36
Sdl_R24_5	Race24	DaRT4767	3H	42.46	3.7706	6.07	2.56	25.41
Sdl_R57_3	Race57	SNP1637	3H	45.22	3.1389	4.52	2.30	21.83
Sdl_R24_6	Race24	DaRT6458	4H	50.99	3.0864	4.73	−2.38	22.40
Sdl_RM_3	RaceM	DaRT6490	4H	51.42	4.0400	6.76	2.42	46.25
Sdl_RG_5	RaceG	DaRT7132	4H	110.20	3.0167	4.59	2.10	20.33
Sdl_R24_7	Race24	DaRT7144	4H	111.33	3.1133	5.01	2.51	21.76
Sdl_RG_7	RaceG	DaRT8539	5H	129.65	3.8616	5.88	2.03	30.35
Sdl_RM_6	RaceM	DaRT8567	5H	133.69	3.0979	4.79	1.82	43.50
Sdl_R24_9	Race24	DaRT8907	5H	151.88	3.0918	4.79	2.01	26.03
Sdl_R0_5	RaceQ	DaRT8922	5H	151.98	3.1837	4.93	−1.44	35.59
Sdl_RM_7	RaceM	SNP3724	6H	51.77	3.1876	4.41	2.72	13.46
Sdl_RG_8	RaceG	DaRT10087	6H	53.75	3.4969	5.27	3.53	6.64
Sdl_R24_10	Race57	DaRT11479	7H	23.02	3.9349	6.56	2.04	42.37
Sdl_R57_7	Race24	DaRT11535	7H	24.06	3.3674	5.12	2.26	22.53
(B) SEEDLING AND ADULT PLANT								
QTL	**Trial/Race**	**Marker**	**Chr**	**Pos (cM)**	**$-\log_{10}(p)$**	**Marker R2 (%)**	**Effect**	**MAF (%)**
Sdl_RM_6	RaceM	DaRT8567	5H	133.69	3.0979	4.79	1.82	43.50
Ap_Dg13_3	DG13	DaRT8667	5H	137.08	3.7524	5.74	−25.80	9.77
Ap_Dg14_5	DG14	DaRT8661	5H	137.08	3.3738	5.07	−19.21	19.84
Ap_Kr13_7	KR13	DaRT8668	5H	137.08	3.0786	5.30	−11.44	8.98
Sdl_RG_1	RaceG	DaRT1886	2H	8.85	3.3314	5.18	−1.84	47.56
Ap_Dg14_2	DG14	SNP632	2H	8.85	3.1469	4.79	−16.27	26.56
Sdl_R0_7	RaceQ	SNP4090	6H	118.77	3.3578	5.15	1.48	29.54
Ap_Dg14_6	DG14	DaRT10854	6H	119.12	3.1978	4.94	13.18	15.98
Sdl_RG_3	RaceG	DaRT3982	2H	146.72	3.3517	5.04	2.33	19.29
Ap_Kr13_6	KR13	DaRT4067	2H	149.26	3.2942	5.73	−13.77	5.77

Conclusions

Barley stripe rust specialization and race structure are poorly defined as compared with wheat rusts and only few studies on the genetic control of the resistance are available in barley. In order to identify genomic regions controlling resistance to five prevalent races of Psh in India at both SRT and APS stages, the study was taken up on barley genotypes from much diverse sources. 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 stripe rust 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 Psh 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 races of Psh existing in other regions of the world to ensure their use for introgression in barley germplasm or for MAS globally.

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