



Identification of a Large Number of Stripe Rust Resistance Loci in U.S. Spring and Winter Wheat Cultivars and Breeding Lines Using Genome-wide Association Mapping and Yr Gene Markers



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Abstract

Stripe rust (or yellow rust), caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most destructive diseases of wheat worldwide, especially in the United States. To identify loci for effective stripe rust resistance in U.S. wheat, a genome-wide association study (GWAS) was conducted using two panels of 616 spring wheat and 857 winter wheat cultivars and breeding lines. The accessions in these panels were phenotyped for stripe rust response at seedling stage in a greenhouse with multiple predominant and highly virulent races of the pathogen and at adult-plant stage in multiple field environments. The accessions were genotyped using a genotyping by multiplexed sequencing (GMS) platform. In the spring panel, a total of 37 loci on 15 chromosomes (1A, 1B, 2A, 2B, 2D, 3B, 3D, 4A, 4B, 5A, 5B, 6B, 6D, 7A, and 7D) for resistance to stripe rust were detected including 10 new loci. In the winter panel, a total of 51 loci on 19 chromosomes (1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 5A, 5B, 5D, 6A, 6B, 7A, 7B, and 7D) were associated to stripe rust resistance, including at least 4 new loci. The frequencies of the resistance genes or QTL in various regional nurseries were determined, indicating different use intensities of these genes or QTL in breeding programs in different regions. The resistance loci and the information on their markers, effectiveness, and distributions should be useful for improving stripe rust resistance in wheat cultivars.

Introduction

Wheat is one of the most import crops worldwide. Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) is one of the most destructive diseases in most wheat production regions in the world, especially in the U.S. (Chen 2005, 2020). Among all management approaches, planting resistance cultivars is the most effective, economical, easy-to-use, and environment-friendly strategy to control the disease. Breeding stripe rust resistant cultivars is a top priority in breeding programs throughout the U.S. However, resistance genes in most cultivars and breeding lines are not clear. The objective of this study was to identify and map stripe rust resistance genes in genetic stocks, cultivars, and breeding lines used and developed in the U.S. The information should be useful for control of stripe rust by select cultivars with effective resistance and further developing new cultivars with durable and adequate resistance.

Materials and methods

Wheat materials. A total of 616 spring wheat and 857 winter wheat entries of various nurseries tested in our program in 2017 were used in the separate spring and winter wheat studies, respectively. The entries were classified into genetic stocks, varieties trials, and uniform nurseries from various regions in the U.S.

Stripe rust phenotyping. The accessions in the spring wheat panel were phenotyped for stripe rust response in the greenhouse at seedling stage with five predominant and highly virulent races of *Pst* and at adult-plant stage in Pullman and Mount Vernon in 2017 and 2018. The accessions in the winter wheat panel were phenotyped for stripe rust response in the greenhouse at seedling stage with six races and at adult-plant stage in Pullman and Mount Vernon in 2018 and 2019.

Genotyping. The spring panel was tested with 23 markers for 16 previously reported stripe rust resistance *Yr* genes/QTL and the winter panel was tested with 31 markers for 18 genes/QTL. The markers were SSR, STS, or KASP types depending upon the *Yr* gene/QTL. The accessions in both spring and winter panels were also genotyped through genotyping by multiplexed sequencing using an Ion Proton system (Life Technologies Inc., Carlsbad, CA, USA) according to the protocol developed by Ruff et al. (2020).

Genetic diversity, population structure, and linkage disequilibrium (LD)

analyses. Genetic diversity was assessed by gene diversity (GD) and polymorphism information content (PIC) using POWERMARKE v3.25 (Liu and Muse 2005). The population structure of the winter wheat panel was analyzed using Bayesian model-based clustering and distance-based hierarchical clustering in software STRUCTURE v2.3.4 (Pritchard et al. 2000). LD, measured as squared allele-frequency correlation (r^2) between marker pairs, was obtained using software Haploview v4.2 (Barrett et al. 2005).

Genome-wide association study (GWAS). GWAS was conducted with BLINK (Huang et al. 2018) and markers were identified as significant threshold of 5% after Bonferroni correction (Bland and Altman 1995), which is equivalent to the threshold p value 2.44E-05 and $-\log_{10}(p)$ 4.61 (0.05/2052 SNPs). Manhattan plots were drawn using the Cmplot package in the R program (<https://github.com/YinLiLin/R-Cmplot>).

Results

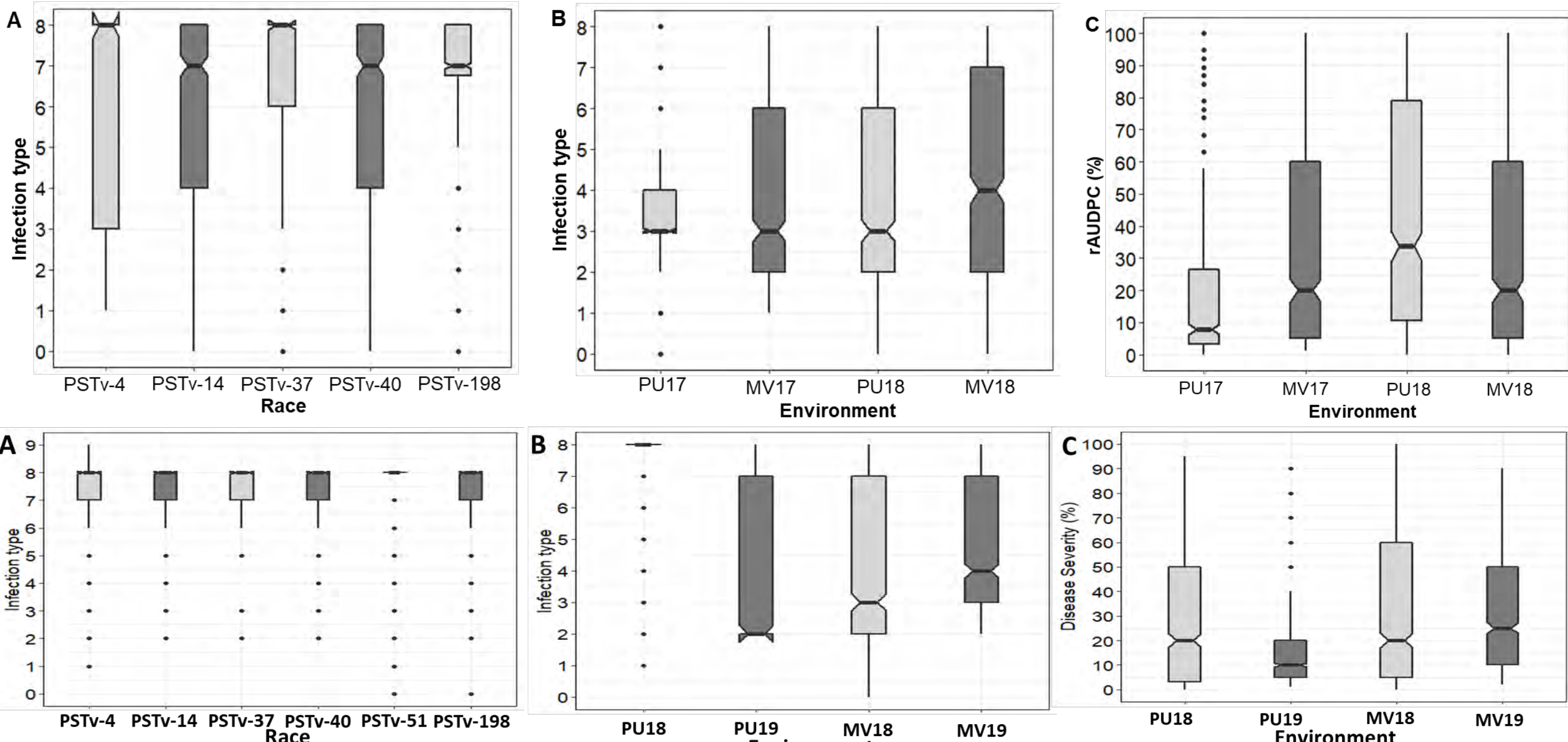


Fig. 1. Stripe rust phenotypic distributions in the spring (top row) and winter (bottom row) wheat panels. PU = Pullman, MV = Mount Vernon, 17 = 2017, 18 = 2018, and 19 = 2019.

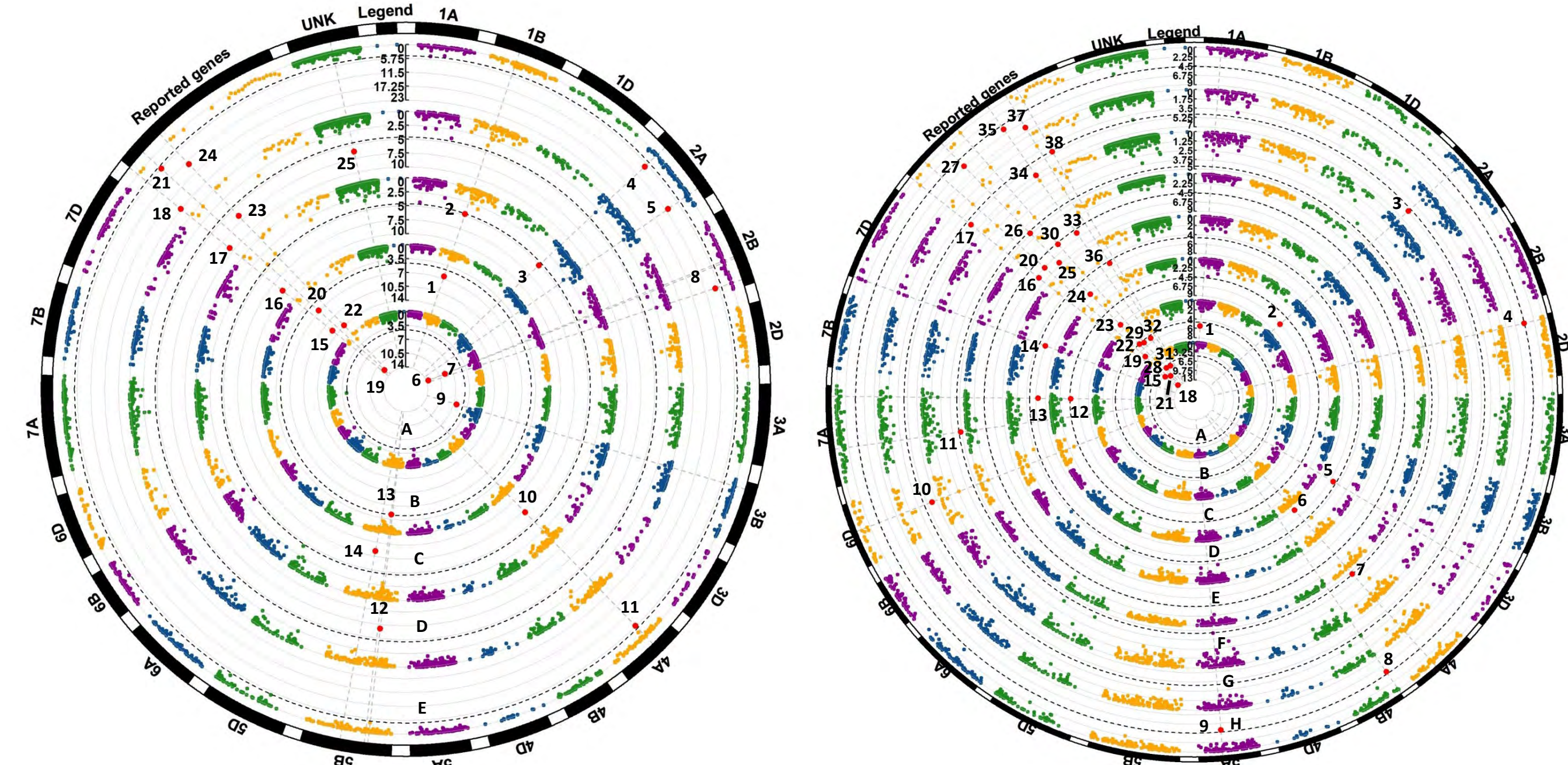


Fig. 2. Manhattan plots of significant p values for markers associated with all-stage resistance (left) detected in the greenhouse seedling tests and resistance detected in the field experiments (right). In the left figure, each circle represent one greenhouse test with race PSTv-4 (A), PSTv-14 (B), PSTv-37 (C), PSTv-40 (D), or PSTv-198 (E). In the right figure, Each circular represents one field test for Pullman 2017 IT data (A), Pullman 2017 rAUDPC data (B), Mount Vernon 2017 IT data (C), Mount Vernon 2017 DS data (D), Pullman 2018 IT data (E), Pullman 2018 rAUDPC data (F), Mount Vernon 2018 IT data (G), and Mount Vernon 2018 DS data (H). Significant markers are marked in red and numbered. “Reported genes” stands for the group of marks used to represent previously reported genes and QTL; “UNK” stands for the group of markers without specified chromosomes and positions. The black dash line shows the threshold $-\log_{10}(p)$ value of 4.61.

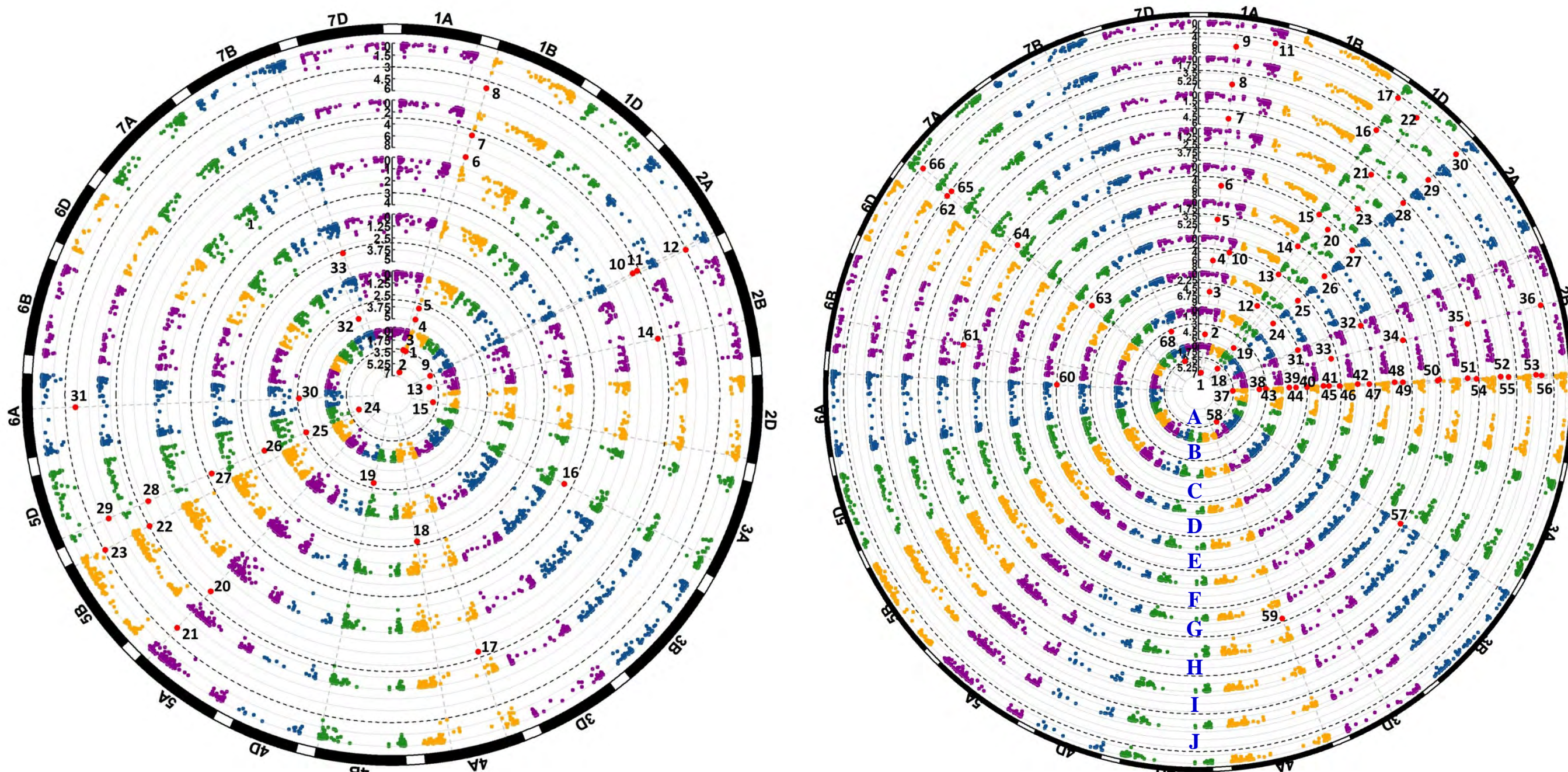


Fig. 3. Manhattan plots of significant p values for markers associated with all-stage resistance to stripe rust detected in the greenhouse seedling tests (left) and at the adult-plant stage in the field experiments (right). In the left figure, each circular represents one seedling test with race PSTv-4 (A), PSTv-14 (B), PSTv-37 (C), PSTv-40 (D), PSTv-51(E), and PSTv-198 (F). In the right figure, each circular represents one field data set of Pullman 2018 IT data (A), Pullman 2018 DS data (B), Mount Vernon 2018 IT data (C), Mount Vernon 2018 DS data (D), Pullman 2019 IT data (E), Pullman 2019 DS data (F), Mount Vernon 2019 IT data (G), Mount Vernon 2019 DS data (H), BLUE-ALL-IT data (I), and BLUE-ALL-DS data (J). The black dash line shows the threshold $-\log_{10}(p)$ value of 3. Significant markers are enlarged in red dots and numbered.

Table 2. Frequencies of resistance genes or QTL in the spring wheat panel, different categories, and nurseries

Yr genes/QTL	Whole panel		Category* (%)				Nursery ^b (%)							
	No.	Freq. (%)	B	C	G	SCDN	SRDN	SGLN	SEDN	SWCMN	SRCN	SMRN		
			(234)	(222)	(160)	(191)	(167)	(77)	(74)	(50)	(38)	(19)		
Yr5	13	2.1	3.4	1.4	1.3	0.0	3.6	2.6	1.4	4.0	5.3	0.0		
Yr9	10	1.6	1.3	1.4	2.5	1.6	0.0	2.6	4.1	4.0	0.0	0.0		
Yr10	11	1.8	2.1	1.4	1.9	0.5	2.4	3.9	1.4	2.0	2.6	0.0		
Yr15	26	4.2	6.8	4.1	0.6	0.0	7.2	1.3	9.5	4.0	10.5	0.0		
Yr16	6	1.0	0.0	0.9	2.5	1.0	0.0	3.9	0.0	2.0	0.0	0.0		
Yr17	26	4.2	5.6	4.5	1.9	0.5	3.6	2.6	14.9	8.0	5.3	0.0		
Yr18	115	18.7	25.6	21.2	5.0	11.0	25.1	6.5	21.6	22.0	28.9	47.4		
Yr27	407	66.1	65.4	66.7	66.3	80.6	62.3	37.7	68.9	60.0	76.3	52.6		
Yr29	107	17.4	18.4	14.4	20.0	23.0	14.4	2.6	21.6	16.0	15.8	36.8		
Yr30	105	17.0	23.1	18.0	6.9	11.5	21.6	9.1	18.9	16.0	36.8	21.1		
Yr36	19	3.1	3.0	2.3	4.4	1.0	3.6	6.5	1.4	8.0	2.6	0.0		
Yr39	37	6.0	5.6	5.9	6.9	8.9	7.8	2.6	4.1	0.0	5.3	0.0		
Yr46	28	4.5	0.0	0.0	17.5	14.7	0.0	0.0	0.0	0.0	0.0	0.0		
YrTr1	5	0.8	0.4	1.4	0.6	1.6	0.6	0.0	1.4	0.0	0.0	0.0		
QYrMa.wgp-6BS	7	1.1	2.1	0.5	0.6	1.0	1.2	0.0	2.7	0.0	2.6	0.0		
QYr.wgp-1B.1	113	18.3	16.2	18.5	21.3	17.3	9.6	22.1	24.3	34.0	15.8	31.6		
QYrSW.wgp-1A	231	37.5	41.9	33.8	36.3	29.3	28.7	27.3	39.2	58.0	78.9	94.7		
QYrSW.wgp-1B	47	7.6	8.5	5.9	8.8	5.2	9.6	10.4	6.8	6.0	13.2	0.0		
QYrSW.wgp-2A.1	20	3.2	0.4	8.6	0.0	1.0	0.6	0.0	1.4	32.0	0.0	0.0		
QYrSW.wgp-2A.2	312	50.6	54.3	55.4	38.8	50.8	59.3	27.3	62.2	62.0	39.5	15.8		
QYrSW.wgp-2A.3	173	28.1	36.3	29.7	13.8	25.1	35.9	6.5	31.1	16.0	55.3	42.1		
QYrSW.wgp-2B.1	137	22.2	30.3	23.9	8.1	15.7	35.9	10.4	20.3	18.0	34.2	10.5		
QYrSW.wgp-2B.2	164	26.6	15.8	19.4	52.5	34.6	14.4	40.3	20.3	40.0	18.4	5.3		
QYrSW.wgp-2D	135	21.9	19.7	15.8	33.8	30.9	16.8	14.3	45.9	2.0	5.3	0.0		
QYrSW.wgp-3B	259	42.0	46.6	33.8	46.9	38.7	46.7	40.3	50.0	26.0	63.2	10.5		
QYrSW.wgp-3D	127	20.6	29.1	21.2	7.5	13.6	32.3	3.9	27.0	20.0	21.1	31.6		
QYrSW.wgp-4A.1	34	5.5	6.4	5.0	5.0	3.1	7.2	6.5	6.8	0.0	15.8	0.0		
QYrSW.wgp-4A.2	335	54.4	66.2	51.4	41.3	51.8	71.3	27.3	78.4	20.0	71.1	5.3		
QYrSW.wgp-4B	369	59.9	52.6	61.3	68.8	68.6	47.9	51.9	62.2	74.0	71.1	42.1		
QYrSW.wgp-5A	171	27.8	20.1	33.3	31.3	32.5	21.6	27.3	23.0	44.0	34.2	0.0		
QYrSW.wgp-5B.1	83	13.5	15.4	8.1	18.1	6.8	18.0	23.4	24.3	0.0	10.5	0.0		
QYrSW.wgp-5B.2	33	5.4	5.6	7.2	2.5	6.3	4.2	1.3	10.8	4.0	5.3	5.3		
QYrSW.wgp-5B.3	40	6.5	10.3	6.3	1.3	3.7	9.0	1.3	16.2	2.0	10.5	0.0		
QYrSW.wgp-6D	167	27.1	27.4	35.6	15.0	20.9	31.7	19.5	31.1	52.0	21.1	10.5		
QYrSW.wgp-7A.1	308	50.0	44.9	45.0	64.4	58.6	36.5	51.9	45.9	62.0	63.2	31.6		
QYrSW.wgp-7A.2	214	34.7	30.3	34.2	41.9	41.4	24.0	22.1	31.1	64.0	36.8	47.4		
QYrSW.wgp-7D	298	48.4	50.0	38.7	59.4	56.0	45.5	36.4	62.2	30.0	60.5	15.8		
QYrSW.wgp-1D	155	25.2	28.6	16.2	32.5	33.0	25.7	10.4	36.5	4.0	26.3	10.5		

^a B: breeding line; C: cultivar; and G: genetic stock.

^b SCDN = Spring Cereal Disease Nursery including genetic stocks for monitoring stripe rust epidemics and virulence changes; SRDN = Spring Regional Disease Nursery including historically and currently grown spring wheat cultivars in the western U.S.; SGLN = Single Gene Line Nursery including spring wheat near-isogenic or single-gene lines for stem rust, leaf rust, and stripe rust resistance genes; SEDN = Spring Extension Disease Nursery including spring wheat advanced breeding lines and major commercially grown cultivars in the western U.S.; SWCMN = Spring Wheat Cultivar Monitoring Nursery including major commercially grown spring wheat cultivars from different states in the U.S.; SRCN = Spring Regional Cooperation Nursery including breeding lines from the western U.S.; and SMRN = Spring Midwest Region Nursery including breeding lines mainly from the Midwest states).

Table 3. Frequencies of resistance genes or QTL in the winter wheat panel and nurseries

Gene/QTL	Frequencies (%) of genes or QTL in nurseries									
	Panel (857)	1711_WCDN (165)	1709_WWCMN (204)	1701_WRDN (185)	1702_WEDN (92)	1726_WURN (16)	1712_WHWN (86)	1715_WEWN (36)	1716_WSWN (30)	1718_WESR (43)
Genes/QTL identified by markers										
Yr5	0.12	0.61	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yr9	8.05	4.85	5.88	10.27	10.87	0.00	15.12	19.44	0.00	0.00
Yr10	22.75	31.52	20.59	31.35	0.00	25.00	18.60	16.67	30.00	18.60
Yr15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yr17	29.05	39.39	23.53	15.14	34.78	12.50	60.47	36.11	0.00	20.93
Yr18	10.62	7.88	8.82	7.03	0.00	81.25	0.00	5.56	46.67	41.86
Yr27	2.22	3.03	0.98	4.32	1.09	0.00	0.00	0.00	6.67	2.33
Yr30	4.90	4.24	15.69	0.54	0.00	0.00	2.33	0.00	0.00	0.00
Yr46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Yr5P	2.92	3.64	1.47	2.70	3.26	0.00	8.14	2.78	0.00	0.00
YrTr1	1.75	1.82	0.00	3.78	1.09	12.50	2.33	0.00	0.00	0.00
Yr76	1.75	1.82	0.98	3.78	3.26	0.00	0.00	0.00	0.00	0.00
Yr78	9.33	4.85	2.94	20.00	29.35	0.00	2.33	0.00	0.00	0.00
QYrMa.wgp-1AS	17.85	12.73	13.73	43.78	1.09	25.00	12.79	5.56	0.00	11.63
QYr.wgp-1B.1	17.85	12.73	14.71	32.97	13.04	31.25	15.12	13.89	6.67	9.30
QYrel.wgp-2BS	38.86	66.06	16.18	51.35	3.26	100.00	48.84	33.33	26.67	34.88
QYrsk.wgp-3BS	34.42	18.18	42.16	53.51	2.17	56.25	43.02	22.22	26.67	37.21
QYrsk.wgp-4BL	7.23	15.15	3.92	3.78	7.61	12.50	10.47	0.00	13.33	0.00
Genes/QTL identified by GMS-GWAS										
QYrww.wgp.1A-1	11.09	6.06	5.88	18.38	0.00	50.00	18.60	0.00	20.00	20.93
QYrww.wgp.1A-2	32.32	11.52	39.71	35.68	39.13	56.25	32.56	30.56	30.00	41.86
QYrww.wgp.1A-3	6.42	4.24	2.45	13.51	0.00	31.25	8.14	8.33	10.00	0.00
QYrww.wgp.1B	15.17	4.24	3.43	53.51	6.52	31.25	3.49	0.00	6.67	2.33
QYrww.wgp.1D-1	9.45	7.88	12.25	14.05	0.00	6.25	4.65	13.89	20.00	2.33
QYrww.wgp.1D-2	55.78	52.73	42.65	41.08	51.09	93.75	100.00	100.00	90.00	39.53
QYrww.wgp.1D-3	6.65	6.06	3.43	3.78	3.26	12.50	15.12	19.44	13.33	9.30
QYrww.wgp.2A-1	23.92	4.85	20.59	20.54	25.00	31.25	56.98	30.56	56.67	27.91
QYrww.wgp.2A-2	6.77	7.88	2.94	10.27	5.43	18.75	6.98	5.56	3.33	6.98
QYrww.wgp.2B-1	53.44	55.15	65.69	26.49	41.30	37.50	66.28	66.67	73.33	86.05
QYrww.wgp.2B-2	12.14	19.39	15.69	9.73	0.00	6.25	12.79	8.33	13.33	6.98
QYrww.wgp.2B-3	14.12	0.00	44.12	14.05	1.09	0.00	2.33	5.56	0.00	0.00
QYrww.wgp.2B-4	6.10	3.40	3.40	6.50	1.20	0.00	1.20	8.30	23.3	4.70
QYrww.wgp.2D-1	26.60	9.09	33.82	26.49	39.13	0.00	50.00	41.67	3.33	0.00
QYrww.wgp.2D-2	27.54	8.48	24.02	24.86	39.13	50.00	50.00	69.44	0.00	34.88
QYrww.wgp.2D-3	10.62	9.70	3.43	6.49	6.52	43.75	11.63	0.00	60.00	34.88
QYrww.wgp.2D-4	4.43	0.00	0.00	2.70	0.00	12.50	8.14	44.44	0.00	18.60
QYrww.wgp.3A	5.37	3.03	0.00	4.32	10.87	0.00	22.09	11.11	0.00	0.00
QYrww.wgp.3B	3.38	0.00	0.00	4.32	0.00	43.75	0.00	8.33	16.67	13.95
QYrww.wgp.3D	26.60	12.12	10.78	81.08	26.09	0.00	5.81	19.44	0.00	0.00
QYrww.wgp.4A-1	7.00	6.06	4.90	3.78	0.00	37.50	11.63	22.22	0.00	20.93
QYrww.wgp.4A-2	13.19	4.85	10.78	27.57	20.65	6.25	6.98	0.00	10.00	6.98
QYrww.wgp.4A-3	21.47	29.70	25.00	25.41	27.17	18.75	10.47	0.00	0.00	0.00
QYrww.wgp.4B	6.77	3.64	5.88	5.41	3.26	25.00	12.79	0.00	16.67	16.28
QYrww.wgp.5A	6.88	5.45	8.33	2.16	9.78	12.50	4.65	8.33	10.00	18.60
QYrww.wgp.5B	9.92	9.09	2.45	5.95	19.57	50.00	11.63	11.11	20.00	18.60
QYrww.wgp.5D	7.58	3.64	4.41	1.62	13.04	43.75	17.44	11.11	10.00	13.95
QYrww.wgp.6A-1	41.07	63.03	42.65	40.54	38.04	25.00	32.56	13.89	23.33	16.28
QYrww.wgp.6A-2	48.07	59.39	49.02	51.35	53.26	50.00	32.56	44.44	30.00	20.93
QYrww.wgp.6B	7.70	7.27	0.49	2.16	14.13	43.75	0.00	0.00	33.33	44.93
QYrww.wgp.7A-1	5.37	3.64	2.45	1.62	9.78	43.75	2.33	22.22	3.33	11.63
QYrww.wgp.7A-2	7.35	14.55	7.35	3.24	4.35	0.00	12.79	0.00	10.00	0.00
QYrww.wgp.7B-1	7.00	0.00	0.00	15.68	3.26	12.50	12.79	19.44	16.67	6.98
QYrww.wgp.7B-2	13.30	13.94	6.86	24.32	26.09	0.00	9.30	0.00	0.00	0.00
QYrww.wgp.7B-3	13.30	19.39	4.90	3.78	13.04	68.75	27.91	22.22	16.67	11.63
Mean freq. (%)	14.76	13.24	12.86	17.29	12.47	24.88	17.42	15.72	14.65	13.87
No. of genes/QTL	51	45	43	48	37	35	43	33	32	31
Rate of gene/entry	0.06	0.27	0.21	0.56	0.40	2.10	0.50	0.91	1.07	0.72