

Vogel Foundation Progress Report

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Progress Report Year: 2008

Title: Discovering New Genes for Effective Resistance to Stripe Rust Using Highly Efficient Molecular Mapping Approach

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Results:

1. Testing parental genotypes and F₂ populations for 92 common X common wheat crosses for resistance to stripe rust in the field

In 2008, 92 spring wheat genotypes and the F₂ populations of their crosses with susceptible common wheat 'Avocet Susceptible' (AVS) were tested for parental resistance and progeny segregation at the Tukey Farm near Pullman in 2008. About 30 to 50 seeds for each parental line and 150 seeds for each cross F₂ population were planted. The F₂ seeds were space-planted for facilitating disease record and harvest of F₃ seeds from individual F₂ plants. Stripe rust infection types (ITs) and severities were recorded for each parental line and each F₂ plant at the flowering stage. Table 1 shows the stripe rust reactions and severities of the 92 wheat genotypes used as resistant parent and the susceptible parent AVS. Of the 92 previously resistant wheat genotypes, 13 became susceptible (IT 7-9), 10 became intermediate (IT 4-6), and 69 were still resistant (IT 0-3). The crosses of the 69 resistant genotypes will be used to identify new and effective resistance gene.

2. Genetic analysis for number of genes and inheritance of stripe rust resistance based on F₂ data of common X common wheat crosses obtained in 2008 field and greenhouse tests

In addition to segregation data obtained for 70 of the 92 crosses tested in the field, we tested F₂ populations for 35 common wheat X common wheat crosses with race PST-100 under controlled greenhouse conditions. As shown in Table 2, in the field tests, 21 crosses showed a single dominant gene, 43 showed two genes with various interactions, and 6 showed three genes for resistance. In the greenhouse tests, 10 crosses had a single dominant gene, 23 had two genes, and 2 had three genes for resistance. Of 26 crosses that were tested both in the field and greenhouse, 23 had the same results in both tests while 3 had different segregation ratios. The disease reaction data will be used to identify different new genes for effective resistance to stripe rust.

3. Testing previously resistant durum wheat parents, determining chromosome numbers for individual F₃ plants, and analyzing number of genes and inheritance of resistance in 23 common X durum wheat crosses

In 2008, resistances of 23 durum wheat genotypes that previously showed resistance to various early races of the stripe rust pathogen were determined with races PST-100 (currently predominant race) and PST-127 (a new race with the most virulence pattern) at the seedling stage under the controlled greenhouse conditions. These durum genotypes were still resistant; only 4 of the 23 genotypes had little bit higher infection types (IT 3 or 4) than the previously

recorded IT 2. The results showed that the resistances in these cultivars are still effective to the current races and should be useful in breeding programs to develop stripe rust resistant cultivars.

Because durum (28 chromosomes) and common (42 chromosomes) wheat genotypes have different numbers of chromosomes, progenies of common X durum wheat crosses do not segregate normally as in common X common crosses. A total of 23 common X durum crosses were selected based on the resistant reactions of the durum parents in all tests with early and new races and availability of F₃ seeds from previously made over 90 common X durum crosses. Chromosome numbers in 15 to 67 individual F₃ plants were determined for each of the 23 crosses using the Feulgen staining root-tip technique and examined under a microscope. The numbers of plants with different chromosomes for these crosses are shown in Table 3. F₃ plants with 28 chromosomes were obtained for all of the 23 crosses, from which the derived F_{3:4} populations can be used for identifying and mapping resistance genes in these crosses. F₃ plants with 42 chromosomes were obtained for 18 of the 23 crosses, from which the derived F_{3:4} populations can be used for developing common wheat germplasms with resistance genes from the durum wheat genotypes, as well as identifying and mapping resistance genes in these crosses.

The segregation for resistant to susceptible plants was determined with 13 to 41 F_{3:4} plants for each of the 23 common X durum crosses tested with race PST-127 in the seedling stage under controlled greenhouse conditions. The relatively small number of plants used in each test was for obtaining preliminary genetic data to select crosses for further phenotyping and molecular mapping studies. The F_{3:4} segregation ratios of the crosses are shown in Table 3. Of the 23 crosses, 4 showed a single dominant gene, 1 showed a single recessive gene, and 18 showed two genes for resistance with various modes of inheritance and intergenic interactions. These results are useful for further mapping the resistance gene(s) in each cross. However, we selected from the crosses for immediate studies to identify and map different new genes for effective resistance.

4. To determine common wheat genotypes with potential different genes for resistance to stripe rust through simultaneous bulk segregant analysis with RGAP markers for common X common wheat crosses with 38 resistant genotypes

Resistant and susceptible DNA bulks were constructed by DNA isolated from 10 resistant and 10 susceptible F₂ plants, respectively, for each of the 38 selected common X common wheat crosses. The resistance gene analog polymorphism (RGAP) technique was used to identify markers associated to at least one of the crosses using simultaneous bulk segregant analysis (sBSA). A total of 91 RGAP markers were identified with various resistance genes in these crosses. Markers shared by two or more crosses indicate that their resistant parents have common or linked genes. Cluster analysis of the 38 wheat genotypes with the molecular markers showed that none of the genotypes are genetically identical. These results show that a large number of unique genes are present in these wheat germplasms. When these unique genes are confirmed in our coming detailed mapping studies, they will be highly valuable for breeding wheat cultivars with new effective resistance.

5. Identification and molecular mapping of a new gene for resistance to stripe rust in common wheat genotype PI 181434

We have completed the study for identification and molecular mapping of a new gene for resistance to stripe rust in spring common wheat genotype PI 181434, originally from Afghanistan. The genotype was resistant to all nine tested races (PST-17, PST-37, PST-43, PST-45, PST-78, PST-100, and PST-127). It was previously crossed with AVS and the F₂ progenies were tested under the natural infection of the stripe rust in the field in 2007. The F₂ derived F₃ population (103 F_{2:3} lines) were tested in the seedling stage with both race PST-100 and PST-127 under controlled greenhouse conditions in 2008. DNA was extracted from stripe rust

phenotyped F_2 plants. Genetic analyses of the F_2 and F_3 data indicated a single dominant gene for resistance in PI 181434. The phenotype data of 103 F_2 plants and $F_{2,3}$ lines were used for identifying molecular markers linked to the resistance gene using the BSA technique. The RGAP technique was used to identify molecular markers linked to the resistance gene. We identified eight RGAP markers and were able to use one of them to locate the gene and marker to the long arm of wheat chromosome 3D using the Chinese Spring nulli-tetrasomic and ditelosomic lines. By screening chromosome 3D specific SSR markers, we identified two SSR markers polymorphic between the two parents and used them confirmed the chromosomal location of the resistance gene. Using the 8 RGAP and 2 SSR markers, we constructed a linkage group for the resistance gene as shown in Fig. 1. The two most closely linked flanking markers were within 4.8 and 5.8 cM distances, respectively. Based on the unique chromosomal location and resistance to a wide range of stripe rust races, we conclude that the gene is novel and confers effective resistance to stripe rust. We determined polymorphisms of the two flanking RGAP markers in 46 wheat cultivars. The markers were polymorphic in 36 (78%) of the wheat cultivars, indicating that these markers are directly useful for marker-assisted selection to incorporate the resistance genes into majority of currently grown cultivars.

6. Genetic analysis of stripe rust resistance in durum wheat genotype PI 480148 and identification of molecular markers

The cross AVS X PI 480148 was selected for detailed studying of the resistance. The durum wheat PI 480148, originally from Ethiopia, was resistant to all eight tested races (PST-21, PST-43, PST-45, PST-70, PST-78, PST-100, PST-127, and PST-130). The preliminary genetic analysis with 41 $F_{3,4}$ plants indicated that the genotype has a single dominant gene for stripe rust resistance (Table 3). Further testing with 150 $F_{3,4}$ plants derived from a single heterozygous F_3 plants with 42 chromosomes confirmed the genetic result. The RGAP technique was used to identify molecular markers for the resistance gene. Three RGAP markers were identified and the closet marker was within 3.0 cM. Because there is no stripe rust resistance genes that have been mapped to wheat chromosomes from durum wheat, this gene is likely a novel gene. We have incorporated this gene into the common wheat background, which makes it easier for breeding programs to use the gene in their breeding programs. The markers also should be useful for marker-assisted selection.

Accomplishments:

1. We phenotyped mapping populations for 92 common X common and 23 common X durum crosses. We further studied 38 common X common wheat crosses with various races of the stripe rust pathogen and molecular markers for determining the genetic diversities of the resistant parents. The results show that there are a large number of unique genes for effective resistance to stripe rust.
2. We completed genetic analyses for determining the number of genes and inheritance of the resistance in 38 common X common and 23 common X durum wheat cultivars. The results are being used for further mapping different and novel genes for resistance.
3. We harvested leaf samples of F_2 plants for DNA extraction and F_3 seeds for 92 common X common wheat crosses for further molecular mapping of the resistance genes.
4. We completed identification and molecular mapping of a dominant resistance gene each in spring wheat cultivars Zak and IDO377s. We mapped both genes on the long arm of chromosome 2B, but at different locations. These genes are different from all previously named stripe rust resistance genes. They can be useful in combination with other genes for developing

cultivars with effective resistance genes, and especially useful for understanding and monitoring virulence changes in the pathogen population.

5. We completed the study to identify and map a novel gene for high-temperature adult-plant (HTAP) resistance to stripe rust in wheat genotypes *Yr8* near-isogenic line and *Compair*. The gene was mapped to wheat chromosome 2D and molecular markers were developed for incorporate the gene for durable resistance in wheat cultivars.

6. We completed identification and mapping a novel gene for stripe rust resistance in common wheat genotype PI 181434. The gene was mapped to chromosome 3DL. Molecular markers were identified for the gene and should be useful in marker-assisted selection for using the new genes to develop wheat cultivars with the new effective resistance gene.

7. We identified a single dominant gene in durum wheat PI 480148, incorporated the gene into the common wheat background, and identified molecular markers for this gene. The gene, common wheat lines, and molecular markers should be useful in breeding for cultivars with the novel resistance gene.

The objectives of this project are to identify at least 10 new effective genes for resistance to stripe rust, map them to wheat chromosomes, and develop molecular markers for efficiently incorporating the new resistance genes into common wheat cultivars. This research will demonstrate the efficiency of the high-throughput molecular approach in discovery of new resistance genes. Based on the progress made so far, we expect to achieve or exceed the goal set for the project.

Publications:

Lin, F., and Chen, X. M. 2007. Genetics and molecular mapping of genes for race-specific all-stage resistance and non-race specific high-temperature adult-plant resistance to stripe rust in spring wheat cultivar *Alpowa*. *Theor. Appl. Genet.* 114:1277-1287.

Lin, F., and Chen, X. M. 2007. Molecular mapping of genes conferring non-race specific and race-specific resistances to stripe rust in spring wheat cultivar *Alpowa*. *Phytopathology* 97:S169.

Chen, X. M., and Zhao, J. 2007. Identification of molecular markers for *Yr8* and a gene for high-temperature, adult-plant resistance against stripe rust in the *AVS/6*Yr8* wheat line. *Phytopathology* 97:S21

Chen, X. M., and Lin, F. 2007. Identification and molecular mapping of genes for all-stage and high-temperature adult-plant resistance to stripe rust in 'Express' wheat. *Phytopathology* 97:S21.

Lin, F., and Chen, X. M. 2008. Molecular mapping of genes for race-specific overall resistance to stripe rust in wheat cultivar *Express*. *Theor. Appl. Genet.* 116:797-806.

Cheng, P., and Chen, X. M. 2008. Molecular mapping of a gene for resistance to stripe rust in spring wheat cultivar *IDO377s*. *Phytopathology* 98:S38.

Lin, F. and Chen, X. M. 2008. Quantitative trait loci for non-race-specific, high-temperature adult-plant resistance to stripe rust in wheat cultivar *Express*. *Theor. and Appl. Genet.* 00:000-000. On line: <http://dx.doi.org/10.1007/s00122-008-0894-0>

Presentations and Reports:

1. America Phytopathological Society Pacific Division meeting, presented “Molecular mapping of genes conferring non-race specific and race-specific resistances to stripe rust in spring wheat cultivar Alpowa”, June, 2006. Boise, Idaho.
2. America Phytopathological Society annual meeting, presented “Identification of molecular markers for *Yr8* and a gene for high-temperature, adult-plant resistance against stripe rust in the AVS/6**Yr8* wheat line” July, 2007. San Diego, California.
3. America Phytopathological Society centennial meeting, presented “Molecular mapping of a gene for resistance to stripe rust in spring wheat cultivar IDO377s”. July, 2008. Minneapolis, Minnesota.
4. Washington Wheat Commission Progress Report. Presented “Control of stripe rust, leaf rust, and stem rust of wheat”. February 14, 2008, Pullman, Washington.

Table 1. Stripe rust infection type (IT) and severity (%) of 92 previous resistant genotypes used to make crosses with susceptible ‘Avocet Susceptible’ (AVS) evaluated at Tukey Farm near Pullman under natural stripe rust infection in 2008.

Cross No.	Accession No.	Name	Origin	IT	%
1	PI 153779	HINDI 62	Egypt	0	0
2	PI 159918	PUNJAB 8A	India	0	0
3	PI 167835	4234	Turkey	7	5
4	PI 178759	Iragi	Iraq	2	2
5	PI 178760	Kirdish	Iraq	0	0
6	PI 180957	9D	India	0	0
7	PI 181410	180	Afghanistan	0	0
8	PI 181432	203	Afghanistan	9	40
9	PI 181434	205	Afghanistan	2	2
10	PI 182087	S-12	Pakistan	3	2
11	PI 182097	S-23	Pakistan	0	0
12	PI 182109	S-36	Pakistan	0	0
13	PI 182121	PUNJAB C217	Pakistan	0	0
14	PI 182124	Tatta	Pakistan	0	0
15	PI 182126	Moro of Sind	Pakistan	0	0
16	PI 183527	11192	India	0	0
17	PI 184597	William Som	Argentina	0	0
18	PI 185208	Temporao de Coruche	Portugal	0	0
19	PI 185220	3321	Portugal	0	0
20	PI 185285	H1037 SEL 47 651	Argentina	0	0
21	PI 185345	Ruivo Tardio A	Portugal	6-8	20
22	PI 185365	PUSA 12	India	0	0
23	PI 185392	3291	Portugal	8	20

24	PI	185614	HINDI 62	Egypt	0	0
25	PI	185862	II-56-8C-2C-26C	Mexico	9	20
26	PI	189743	49575	Pakistan	0	0
27	PI	189744	49576	Pakistan	0	0
28	PI	189747	C 591	India	0	0
29	PI	189757	1	Pakistan	0	0
30	PI	189758	2	Pakistan	2	5
			Zona Media de			
31	PI	191236	Guipuzcoa	Spain	0	0
32	PI	192108	3522	Mozambique	0	0
33	PI	192132	Addis Alem	Ethiopia	6	10
34	PI	192252	3709	Portugal	0	0
35	PI	192400	Hindi	Egypt	2	2
36	PI	192467	KAAL INDIE	South Africa	0	0
37	PI	192529	H 2 A 13324	Portugal	9	30
38	PI	192556	H 16 H 13373	Portugal	9	30
39	PI	192673	Kenya	Australia	9	30
40	PI	192696	Roemer	Germany	0	0
41	PI	192756	PIKA	Portugal	0	0
42	PI	192856	Tomarens	Portugal	0	0
43	PI	193107	DOCTEUR MAZET	France	0	
44	PI	194039	9006	Ethiopia	5	20
45	PI	195097	9353	Ethiopia	0	0
46	PI	197734	PROGRESS	Sweden	5	20
47	PI	199802	II-137-46	Peru	9	30
48	PI	205726		Peru	2	2
49	PI	217546	C 250	Pakistan	7	20
50	PI	220432	Line 950	Egypt	5-8	20
51	PI	220447	Line 1240-213	Egypt	5	10
52	PI	220463	Line 1262-26	Egypt	8	60
53	PI	263416	Bumaflor 22259	Algeria	8	30
54	PI	266148	LEONE	Italy	0	0
55	PI	266867	S1574	India	3	2
56	PI	268075	KOMPOLTI SZALKAS	Hungary	6	10
57	PI	282907	Helomuni	Argentina	6	10
58	PI	315836	GABY	Belgium	0	0
59	PI	323648	PANONIJA	Yugoslavia	0	0
60	PI	434692	NS 669	Yugoslavia	8	30
61	PI	436384	299	Chile	4	10
62	PI	436385	300	Chile	3	5
63	PI	479242	TIAN XUAN 15	China	0	0

64	PI	479812	Sindi	Ethiopia	0	0
65	PI	479841	MG 31381	Ethiopia	0	0
66	PI	519420	VI 8-2-2-6B-2T-3B-4T	Ecuador	0	0
67	PI	519576	T-2480-28T-4T-1V	Chile	0	0
68	PI	534286	Aggia	Ethiopia	0	0
69	PI	578208	Z2	Australia	0	0
70	PI	578210	Z4	Australia	0	0
71	PI	592029	KANTEGIRSKAYA 89	Russian Federation	5	15
72	PI	592030	IRGINA	Russian Federation	5	15
73	PI	592110	MARIS PINION	United Kingdom	0	0
74	PI	592117	DOLLAR	United Kingdom	0	0
75	PI	592127	ROTHWELL TRIDENT	United Kingdom	8	20
76	PI	600923	A99AR	United States	6	20
77	PI	606284	IGUACU	Brazil	0	0
78	PI	620708	3950730	United States	0	0
79	PI	620743	SEG951007	United States	0	0
80	PI	625264	IWA8610565	Iran	5-7	20
81	PI	625272	IWA8610586	Iran	0	0
82	PI	182111	S-39	Pakistan	0	0
83	PI	182116	S-47	Pakistan	0	0
84	PI	189739	S-518	Pakistan	0	0
85	PI	189749	C 518	India	0	0
86	PI	209797	NOS NORDGAU	Germany	0	0
87	PI	220451	Line 1241-336	Egypt	0	0
88	PI	220472	Line 1274-106	Egypt	3-5	5
89	PI	220474	Line 1274-111	Egypt	1	1
90	PI	220481	Line 1282-241	Egypt	0	0
91	PI	436382	295	Chile	0	0
92	PI	610755	CIGM90.483	Mexico	0	0
			Avocet S (Susceptible Parent)		8	80

Table 2. Genetic analyses showing number of resistance genes for each of 92 crosses tested in field under natural stripe rust infection at Tukey Farm near Pullman and in seedling stage inoculated with race PST-100 under controlled greenhouse conditions.

Cross no.	Resistant parent	Adult-plant test in field							Seedling test with PST-100 in greenhouse					
		No. of F ₂ plants	Obs. no.		Exp. ratio	Gene no.	P	No. of F ₂ plants	Obs. no.		Exp. ratio	Gene no.	P	
1	PI 153779	88	50	38	9:7	2	0.91	84	49	35	9:7	2	0.70	
2	PI 159918	100	90	10	55:9	3	0.24							
3	PI 167835	86	39	47	7:9	2	0.77	83	37	46	7:9	2	0.88	

54	PI	266148												
55	PI	266867	125	77	48	9:7	2	0.23						
56	PI	268075	113	67	46	9:7	2	0.51						
57	PI	282907	90	66	24	3:1	1	0.72	86	61	25	3:1	1	0.38
58	PI	315836	81	58	23	3:1	1	0.48						
59	PI	323648	116	104	12	15:1	2	0.07						
60	PI	434692												
61	PI	436384	116	94	22	13:3	2	0.95						
62	PI	436385	105	90	15	55:9	3	0.95						
63	PI	479242	103	61	42	9:7	2	0.54	103	49	54	7:9	2	0.43
64	PI	479812	88	83	5	15:1	2	0.83						
65	PI	479841	85	74	11	55:9	3	0.77						
66	PI	519420	102	68	34	9:7	2	0.03						
67	PI	519576												
68	PI	534286	31	19	12	9:7	2	0.57						
69	PI	578208	104	80	24	3:1	1	0.65						
70	PI	578210	101	68	33	3:1	1	0.07	97	65	32	3:1	1	0.07
71	PI	592029												
72	PI	592030	80	57	23	3:1	1	0.44						
73	PI	592110	83	80	3	15:1	2	0.32	85	81	4	15:1	2	0.56
74	PI	592117							45	32	13	3:1	1	0.55
75	PI	592127							46	37	9	13:3	2	0.89
76	PI	600923							40	17	23	7:9	2	0.87
77	PI	606284												
78	PI	620708	113	90	23	13:3	2	0.66	108	86	22	13:3	2	0.67
79	PI	620743	89	63	26	3:1	1	0.36						
80	PI	625264												
81	PI	625272	82	43	39	9:7	2	0.49	82	43	39	9:7	2	0.49
82	PI	182111	80	48	32	9:7	2	0.50	75	45	30	9:7	2	0.51
83	PI	182116	106	54	52	9:7	2	0.27	105	54	51	9:7	2	0.32
84	PI	189739	42	40	2	15:1	2	0.69	74	3	71	1:15	2	0.44
85	PI	189749	84	71	13	13:3	2	0.44						
86	PI	209797	100	73	27	3:1	1	0.64						
87	PI	220451	63	43	20	9:7	2	0.05	63	43	20	9:7	2	0.05
88	PI	220472	63	53	10	13:3	2	0.56	42	20	22	7:9	2	0.61
89	PI	220474	92	64	28	3:1	1	0.23						
90	PI	220481	59	29	30	7:9	2	0.40	59	29	30	7:9	2	0.40
91	PI	436382	91	63	28	3:1	1	0.20	89	61	28	3:1	1	0.16
92	PI	610755							98	66	32	3:1	1	0.08
93	PI	182103							94	11	83	9:55	3	0.51
94	PI	182119							39	18	21	7:9	2	0.76

Table 3. Infection types of durum wheat genotypes tested with stripe rust races PST-100 and PST-127, number of plants for F₃ plants with different number of chromosomes, and segregations of F₄ plants derived from heterozygous F₃ plants with 42 or 28 chromosomes tested with PST-127

Cross no.	Resistant parent	IT*	No. of F ₃ plants with different no. of chromosomes			Segregation of F _{3:4} plants** tested with PST-127				
			28	29-41	42	Total	R	S	Ratio	P
1	PI480016	2	12	5	3	13	8	5	9:7	0.70
2	PI470761	3	15	1	0	14	13	1	15:1	0.89
3	PI624904	3	10	12	6	26	19	7	3:1	0.82
4	PI519616	2	16	0	0	16	16	0	15:1	0.30
5	PI480013	0	24	5	1	19	16	3	13:3	0.74
6	PI624903	1	12	1	1	12	6	6	9:7	0.66
7	PI320113	1	8	7	6	13	7	6	9:7	0.86
8	PI387731	1	13	4	2	14	14	0	15:1	0.33
9	PI387681	0	0	3	13	17	12	5	3:1	0.67
10	PI282911	2	17	0	0	14	12	2	13:3	0.67
11	PI331260	1	5	7	16	23	17	6	3:1	0.90
12	PI387665	0	16	3	1	16	14	2	13:3	0.52
13	PI480294	1	15	2	1	14	14	0	15:1	0.33
14	PI480148	2	34	11	12	41	32	9	3:1	0.65
15	PI480101	0	28	14	14	32	13	19	7:9	0.72
16	PI387674	1	17	6	3	17	11	6	9:7	0.48
17	PI479996	3	13	13	12	19	6	13	1:3	0.51
18	PI480001	0	12	2	8	19	18	1	15:1	0.86
19	PI479989	0	18	3	0	14	14	0	15:1	0.33
20	PI320126	1	19	7	6	17	16	1	15:1	0.95
21	PI610760	4	7	19	7	20	19	1	15:1	0.82
22	PI480448	2	24	0	0	21	20	1	15:1	0.78
23	PI480004	2	5	8	3	16	9	7	9:7	1.00

* IT data were the same or similar in tests with races PST-100 and PST-127.

** F₃ plants used to develop F_{3:4} mapping populations had 28 chromosomes for crosses of resistant parents PI476701, PI519616, PI282911, PI479989, and PI480448 and had 42 chromosomes for the remaining 18 crosses.

- M1: Xa1NBS-F/Ptokin 3
- M2: Xa1NBS-F/Ptokin 3
- M3: Ptokin 2/AS1-INV
- M4: Ptokin4/XLRR_INV2
- M5: NLRR-INV2/Ptokin4
- M6: XLRR Rev/LM 637
- M7: RLRR Rev/Ptokin 2
- M8: RLRR Rev/Ptokin 1IN
- M9: WMC 656
- M10: Barc 6

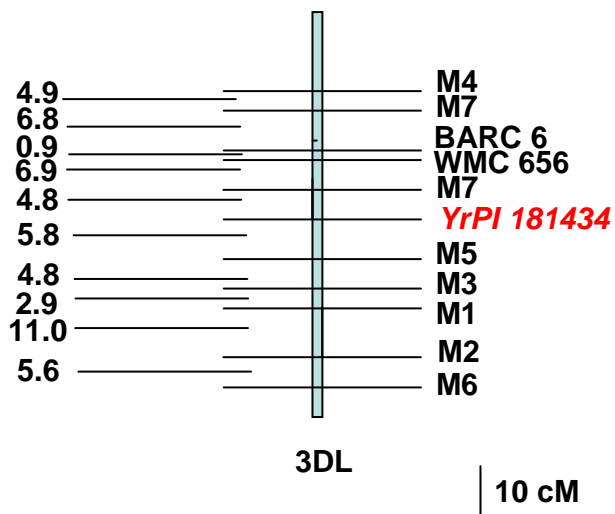


Fig. 1. Linkage group for a resistance gene located on the long arm of wheat chromosome 3D in common wheat genotype PI 181434. Markers M9 (BARC 6) and M10 (WMC 656) are SSR markers and M1 to M8 are RGAP markers.