Indian J. Genet., **61**(1): 16-18 (2001)

Chromosome location of leaf rust and stem rust resistance genes in a wheat-rye recombinant line 'Selection 212'

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(Received: August 1999; Revised: September 2000; Accepted: October 2000)

Abstract

Monosomic analysis of a wheat-rye recombinant line 'Selection 212' using leaf rust pathotypes 77-1, 77-3, 77-4 and 77-5 and stem rust pathotype 40A located the linked genes for leaf rust and stem rust resistance on chromosome 2B. The leaf rust resistance gene was hemizygous ineffective against pathotypes 77- 4 and 77-5. Similarly the stem rust resistance gene was hemizygous ineffective against pathotype 40A.

Key Words: *Secale cereale*, leaf rust, stem rust, resistance, genes, chromosome location

Introduction

A wheat-rye recombinant line 'Selection 212' (Sel. 212) was developed through homoeologous recombination between wheat and rye chromosomes. This line was resistant in field to leaf rust (*Puccinia recondita* f. sp. *tritici*) and stem rust (*P. graminis* f. sp. *tritici*) [1]. Inheritance studies conducted earlier [2] revealed that leaf rust resistance of Sel. 212 to pathotypes 77-1, 77-3, 77-4 and 77-5 was controlled by a recessive gene. Similarly, the stem rust resistance in Sel. 212 against pathotypes 40A and 122 was controlled by a recessive gene [3]. Linkage analysis showed close association between these leaf rust and stem rust resistance genes [4]. The location of these leaf rust and stem rust resistance genes on specific chromosome of Sel. 212 is reported in the present study.

Materials and methods

All the 21 monosomics of wheat cultivar Chinese Spring, cultivar Chinese Spring (CS) and Sel. 212 were used. Pathotypes 77-1, 77- 3, 77-4 and 77-5 of *P. recondita* f. sp. *tritici* and pathotype 40A of *P. graminis* f. sp. *tritici* obtained from D.W.R. Regional Station, Flowerdale, Shimla were used. Avirulence/virulence formulae of these pathotypes are given in Table 1. These pathotypes were selected because of their virulence on Chinese Spring and avirulence on Sel. 212 Table 1. All the 21 monosomic lines of cultivar Chinese Spring were grown in open experimental field along with cultivar Chinese Spring. Monosomic plants were identified cytologically at first meiotic metaphase in all 21 monosomic lines and crossed with Sel. 212 the male parent. Cultivar Chinese Spring was also crossed with Sel. 212. Monosomic F_1 plants were cytologically identified at first meiotic metaphase. All monosomic F_1s were selfed and F_2 seeds were obtained. The F_1 and F_2 seedlings from all the crosses were tested against leaf rust pathotypes 77-1, 77-3, 77-4 and 77-5 and stem rust pathotype 40A. The segregation pattern of the monosomic derived F_2s was compared with that of the disomic F_2 for identifying the chromosome carrying resistance gene. It was presumed that the F_2 involving non-critical monosomic would exhibit normal segregation like disomic F_2 (Chinese Spring × Sel. 212) while the F_2 involving critical monosomic will deviate significantly from the disomic F_2 segregation.

Rust inoculum of pathotypes obtained from Shimla was multiplied following the procedure described by

Table 1. The avirulence/virulence formulae of leaf and stem rust pathotypes based on near-isogenic lines/stocks with known ***Lr/Sr*** genes

Pathotypes	Avirulence/virulence formula
Leaf rust	
77-1	P *Lr9, Lr17, Lr19, Lr23, Lr24, Lr25 ,Lr28, Lr29, Lr32/p Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr15, Lr16, Lr18, Lr20, Lr26*
77-3	P *Lr9, Lr19, Lr20, Lr23, Lr24, Lr25, Lr28, Lr29, Lr32/p Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr15, Lr16, Lr17, Lr18, Lr26*
77-4	P *Lr9, Lr19, Lr20, Lr24, Lr25, Lr26, Lr28, Lr29, Lr32/p Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr15, Lr16, Lr17, Lr18, Lr23*
77-5	P *Lr9, Lr18, Lr19, Lr24, Lr25, Lr28, Lr29, Lr32/p Lr1, Lr2a, Lr2b, Lr2c, Lr3, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr15, Lr16, Lr17, Lr20, Lr23, Lr26*
Stem rust	
40A	P *Sr13, Sr21, Sr24, Sr25, Sr26, Sr27, Sr30, Sr31, Sr32, Sr37/p Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9e, Sr11, Sr16, Sr18, Sr19, Sr20, Sr28*

Joshi *et al.* [5], and tested on differentials to ascertain the purity of pathotypes. Test material was grown in rectangular trays (11" × 4" × 3"). Ten seeds in each line and 10 lines in each tray were grown. One week old seedlings were inoculated by spraying urediospores suspension in water. Material was incubated for 24h in humid chambers and then shifted to glasshouse benches. Reactions were classified 12 days after inoculation according to Stakman *et al.* [6].

Results and discussion

The observations on chromosome location of genes for resistance to leaf and stem rust are given in Table 2 and discussed here. The F_2 seedlings from the cross Chinese Spring × Sel. 212 as well as the 20 crosses, excluding the cross of monosomic for chromosome 2B, segregated in a 1 resistant (R) : 3 susceptible(S) ratio (monogenic recessive gene ratio) against pathotype 77-1. Pooled data of above crosses showed a good fit to the ratio 1R:3S (χ^2 = 2.595, P > 0.10). The 47 F_2 seedlings obtained from the monosomic 2B F_1 hybrid contained only six susceptible seedlings thus, this F_2 ratio deviated significantly (χ^2 = 97.085, p < 0.001) from recessive monogenic ratio 1R:3S suggesting the location of recessive gene for resistance to pathotype 77-1 on chromosome 2B.

The F_2 seedlings from all the monosomic hybrids, except monosomic 2B, segregated in a ratio of 1R:3S (χ^2 = 0.013-2.178, P > 0.95-0.10) when tested with pathotype 77-3. The F_2 of disomic cross (CS × Sel. 212) also segregated in the ratio of 1R:3S. Analysis of pooled data of these 21 crosses has good agreement (χ^2 = 1.955, P > 0.10) with expected 1R:3S ratio. Critical cross involving chromosome 2B was identified by highly significant deviation (χ^2 = 143.47, P < 0.001) from monogenic ratio 1R:3S. Therefore, the recessive leaf rust resistance gene of Sel. 212 against pathotype 77-3 is also located on chromosome 2B.

The critical monosomic against pathotype 77-4 could not be determined because F_2's of crosses involving all the 21 monosomics segregated in similar pattern with non-significant deviation from the expected

Table 2. Seedling reaction of F_1s (CS monosomics × Sel. 212) and F_2s (derived from monosomic F_1s) against pathotypes 77-1, 77-3, 77-4 and 77-5 of leaf rust and 40A of stem rust at 14°C-35°C

Cross of Sel. 212 with monosomic	Leaf rust pathotypes																		Stem rust pathotype					
	77-1				77-3				77-4				77-5						40A					
	F_2				F_2				F_2				F1		F_2				F_1		F_2			
	R	S	Total	χ^2 1R:3S	R	S	Total	χ^2 1R:3S	R	S	Total	χ^2 1R:3S	R	S	R	S	Total	χ^2 1R:3S	R	S	R	S	Total	χ^2 1R:3S
-1A	09	21	30	0.400	12	34	46	0.029	06	15	21	0.143	0	06	31	73	104	1.282	0	08	12	39	51	0.059
-1B	12	26	38	0.877	14	40	54	0.025	07	24	31	0.097	0	06	30	75	105	0.714	0	07	14	44	58	0.023
-1D	06	22	28	0.190	07	14	21	0.777	07	16	23	0.362	0	07	29	87	116	0.000	0	08	11	30	41	0.073
-2A	05	20	25	0.333	12	29	41	0.398	05	16	21	0.016	0	05	24	54	78	1.385	0	08	10	31	41	0.008
-2B	41	06	47	97.085*	54	04	58	145.988*	07	18	25	0.120	0	10	30	94	124	0.053	0	07	11	40	51	0.105
-2D	09	20	29	0.563	12	42	54	0.222	03	14	17	0.490	0	09	22	86	108	1.235	0	07	17	40	57	0.708
-3A	11	27	38	0.316	11	24	35	0.771	03	15	18	0.667	0	08	32	94	126	0.011	0	04	11	37	48	0.111
-3B	12	22	34	1.922	11	19	30	2.178	05	17	22	0.061	0	08	33	106	139	0.118	0	04	12	37	49	0.006
-3D	11	25	36	0.532	18	41	59	0.141	14	40	54	0.025	0	09	35	107	142	0.009	0	09	10	39	49	0.551
-4A	10	38	48	0.444	16	33	49	1.531	07	18	25	0.120	0	04	31	109	140	0.609	0	05	12	35	47	0.007
-4B	08	30	38	0.351	16	35	51	1.105	09	19	28	0.762	0	06	37	94	131	0.735	0	04	12	31	43	0.194
-4D	15	27	42	2.571	13	30	43	0.628	16	33	49	1.531	0	08	36	115	151	0.106	0	08	16	35	51	0.105
-5A	06	26	32	0.667	17	38	55	1.024	12	29	41	0.398	0	07	41	129	170	0.071	0	04	14	37	51	0.163
-5B	11	29	40	0.133	11	42	53	0.509	16	30	46	0.348	0	04	44	126	170	0.071	0	03	15	38	53	0.308
-5D	09	24	33	0.091	05	21	26	0.461	07	20	27	0.012	0	04	11	40	51	0.320	0	03	12	27	39	0.692
-6A	08	18	26	0.462	06	19	25	0.013	13	39	52	0.000	0	08	40	125	165	0.051	0	03	10	23	33	0.495
-6B	12	29	41	0.398	18	36	54	2.000	12	42	54	0.222	0	05	41	122	163	0.002	0	05	14	42	56	0.000
-6D	10	27	37	0.081	16	43	59	0.141	15	32	47	1.198	0	08	42	149	191	0.923	0	03	19	38	57	2.111
-7A	07	25	32	0.167	09	33	42	0.286	14	29	43	1.310	0	07	29	85	114	0.011	0	03	09	25	34	0:039
-7B	10	18	28	1.714	14	44	58	0.023	21	45	66	1.636	0	08	29	122	151	2.704	0	03	12	40	52	0.103
-7D	13	26	39	1.444	09	38	47	0.858	22	54	76	0.632	0	04	20	65	85	0.098	0	05	11	36	47	0.064
CS	12	42	54	0.222	12	45	57	0.474	15	55	70	0.476	0	10	23	84	107	0.701	0	07	19	66	85	0.318
Total	206#	542#	748#	2.574	221#	738#	959#	1.955	236	620	856	3.016	0	151	690	2141	2831	0.594	0	118	283	810	1093	0.464

*Highly significant, #Excluding cross involving CS mono 2B; R = Resistant, S = Susceptible.

Table 3. Meiotic studies of pathotype 77-5 resistant F_2 plants, derived from monosomic F_1 hybrids involving Chinese Spring monosomics (Mono) and Sel. 212.

Cross	No. of plants analysed at metaphase-I		Total
	Disomic (21^{II})	Monosomic ($20^{II} + 1^{I}$)	
Mono- 1A × Sel. 212	3	7	10
Mono-1B × Sel. 212	2	8	10
Mono-1D × Sel. 212	4	6	10
Mono-2A × Sel. 212	3	7	10
Mono-2B* × Sel. 212	9	0	09
Mono-2D × Sel. 212	2	7	09
Mono-3A × Sel. 212	3	7	10
Mono-3B × Sel. 212	4	5	09
Mono-3D × Sel. 212	4	6	10
Mono-4A × Sel. 212	4	6	10
Mono-4B × Sel. 212	3	7	10
Mono-4D × Sel. 212	3	6	09
Mono-5A × Sel. 212	2	8	10
Mono-5B × Sel. 212	2	6	08
Mono-5D × Sel. 212	2	5	07
Mono-6A × Sel. 212	2	6	08
Mono-6B × Sel. 212	2	7	09
Mono-6D × Sel. 212	3	7	10
Mono-7A × Sel. 212	2	8	10
Mono-7B × Sel. 212	3	7	10
Mono-7D × Sel. 212	2	8	10
Total excluding 2B	57	142	199

* = Critical line

1R:3S ratio. This indicates that the gene for resistance against pathotype 77-4 is hemizygous ineffective. Under this situation only the 24% disomics will be resistant and 73% monosomic plus 3% nullisomic will be susceptible leading to 24R:76S ratio which is indistinguishable from 1R:3S ratio. These theoretical assumptions are based on the report of Morris and Sears [7].

The F_1 seedlings from crosses involving Chinese Spring monosomics and Sel. 212 when tested with pathotype 77-5 were susceptible inferring that the gene for resistance against pathotype 77-5 is also hemizygous ineffective. The F_2 genotypes from crosses of all the 21 monosomics with Sel. 212 segregated in a 1R:3S ratio against pathotype 77-5. For identification of critical chromosome carrying resistance gene effective against pathotype 77-5, 10 resistant F_2 seedlings from each cross involving all the 21 monosomics and Sel. 212 were transplanted in the field. All the plants which survived in the field were subjected to meiotic chromosome analysis at metaphase-I (Table 3). Except the cross involving chromosome 2B, in all other crosses resistant plants were both types, the disomic (21") and the monosomic (20" + 1') types while all the nine plants from the cross involving chromosome 2B were disomic (21") leading to the conclusion that the hemizygous ineffective gene imparting resistance to pathotype 77-5 is located on chromosome 2B.

Same set of crosses when tested against stem rust pathotype 40A, all the 21 F_1's were susceptible and all 21 F_2's (derived from monosomic F_1's) segregated in 1R:3S ratio suggesting hemizygous ineffective nature of the stem rust resistance gene. Although the chromosome location of this stem rust resistance gene was not confirmed on the basis of observations reported here, this gene is also proposed to be located on chromosome 2B based on its close linkage with leaf rust resistance gene [4] located on chromosome 2B.

The present monosomic analysis revealed the location of a leaf rust resistance gene imparting resistance to pathotypes 77-1, 77-3, 77-4 and 77-5 of leaf rust and a stem rust resistance gene providing resistance to pathotype 40A on chromosome 2B. The leaf rust resistance gene was hemizygous effective against pathotypes 77-1 and 77-3 and it was hemizygous ineffective against pathotypes 77-4 and 77-5. Knott [8]also observed that recessive resistance genes are often ineffective in hemizygous state. The possible explanation to these observations may lie in pathogenic variability i.e. the genotype of the avirulence locus in the pathogen. With pathotypes 77-1 and 77-3 recessive gene is able to exhibit resistance reaction in single dose while with 77-4 and 77-5 it requires two doses (homozygous condition) to be able to express resistance phenotype which means gene could be hemizygous ineffective if the avirulence locus is heterozygous.

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