Gene K^1 of Raja Flax: A New Factor for Resistance to Rust

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ABSTRACT

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The reactions to selected races of *Melampsora lini* of F_1, F_2, F_3, and testcross progenies of crosses between the flax cultivar Raja with the universal suscept Bison and five single-gene differentials (K, L^6, M^3, N^1, and P^3) indicated that the resistance of Raja is monogenic, dominant, and is an allele at the K locus. The gene, designated K^1, is new and conditions resistance to all races of flax rust in North America. F_2 segregation data indicate that genes K^1 and N^1 are linked, with an estimated recombination percentage of 25%. Indications are that the K, N, and P loci, in the order indicated, are carried in the same chromosome. Gene K^1 appears to be derived from Argentine Selection 1025.

Additional key words: *Linum usitatissimum*.

The flax (*Linum usitatissimum* L.) cultivar Raja (C.I. 1483), developed from the cross Argentine Selection 1025 × J.W.S. 153B9, was released in 1953 by the Agriculture Canada Research Station, Ottawa. Advantages offered by the new cultivar were earliness and resistance to all North American races of rust, *Melampsora lini* (Ehrenb.) Lév. Rust resistance was particularly important at the time because of severe yield losses from rust epidemics during the 1940s and early 1950s on the cultivars Koto and Dakota (4). Raja's rust resistance was effective also against the rust threat of 1962 posed by new races attacking resistance gene L (7), as well as against another rust threat in 1973, when races appeared that attacked resistance gene N^1 (8,16). Raja's rust resistance has persisted for more than three decades, but the identity of the conditioning gene has remained unknown. Zimmer (14), in studies on reaction to 19 exotic and indigenous rust races, concluded that the resistance in Raja was conditioned by an undescribed gene. This paper identifies a gene for rust resistance in the cultivar Raja.

MATERIALS AND METHODS

The methodology for identification of genes for rust resistance in flax is well documented (3–5,11,14,15) and was followed in this study. Accordingly, in order to determine the number of resistance genes, Raja was crossed with Bison (C.I. 389), a cultivar that is universally susceptible to all races of flax rust in North America (4). To determine the allelic relationship of the unidentified resistance gene(s), Raja was further crossed with differential cultivars or lines monogenic for rust resistance, and containing a dominant allele at one of the five known rust-conditioning loci (3,15). Each of 12, 11, 5, 7, 2, and 9 plants of, respectively, Bison and the lines containing genes K, L^6, M^3, N^1, and P^3 were crossed with a different Raja plant. Except for one instance, Raja was used as the pollen parent in producing the initial F_1s. Off-type plants in Raja and the M^3 line were recognized by their susceptibility to race 300, and were discarded before crossing; Bison and the four other lines were found to be uncontaminated. Seventeen F_2 populations were generated from Bison × Raja F_1s. In testcrossing, seven randomly sampled F_1s were backcrossed to Bison to produce the BC_1 generation. BC_1 seed of the testcrosses was sampled randomly,

and 62 plants were grown to maturity to obtain testcross F_2 progenies. From crosses with Raja and the single-gene lines, from 4 to 11 F_2 populations per line were produced, although from K × Raja the F_3 generation was produced as well.

To determine the rust reactions of the various generations, 12- to 14-day-old seedlings were either dusted with a 1:20 (v/v) mixture of urediospores and bentonite, or the seedlings were atomized with 2 ml of urediospores suspended in 500 ml of Soltrol, a light mineral oil (6). Plants dusted, and plants atomized and allowed to dry, were misted with tap water and incubated for 18–20 hr in a moist chamber. Twelve to 14 days after inoculation, plants with reaction types 0 to 2 were classified as resistant, and those with types 3 and 4 as susceptible (2).

The tester race most often used during the course of the study was race 300, which is avirulent to Raja and the five single-gene lines. Race 371, also avirulent to Raja and gene K, replaced race 300 in one experiment, while tester race 312 attacks gene K but not Raja. F_2 populations of Raja × Bison were all expected to segregate for reaction to race 300. F_2 populations involving Raja and the differentials were expected to segregate for reaction to race 300 if the resistance genes of the two parents differed, whereas nonsegregation would indicate an allelic relationship between two genes. Ultimate identification of the unknown gene would then require differentiation by a race virulent to the single-gene line but avirulent to the Raja gene. Subsequent results thus necessitated the use of race 312.

Pathogenicity studies of material involving gene K were conducted during the summer, either in a refrigerated walk-in growth room under conditions of fluorescent and incandescent light (about 250 $\mu E \cdot m^{-2} s^{-1}$), an 18-hr photoperiod, and a temperature of 17–25 C, or in a cooled greenhouse with temperatures ranging from about 16 to 30 C. Studies on populations involving Bison and the other four differentials were carried out in a growth cabinet with an 18-hr photoperiod (about 270 $\mu E \cdot m^{-2} s^{-1}$) and at 22 ± 2 C. Chi-square analysis was used to determine the probability of segregation ratios fitting hypothetical ratios.

RESULTS

Tested for reaction to race 300 in a growth cabinet, all plants of Raja and of the F_1s of Bison × Raja and of testcrosses were either immune (type 0) with or without some flecking, or produced a type 1 reaction where leaves showed both flecking and a few tiny pustules with or without some necrosis. However, of F_2 populations and testcross F_2 populations that were tested at a later occasion, plants classified as resistant included not only types 0 and 1, but also others where pustules were more frequent and where

TABLE 1. Seedling reaction to rust race 300 of F_2 populations of Bison × Raja flax and testcross F_2 progenies of Bison (Bison × Raja)

Raja parent and (cross)		F_2 populations			Testcrosses (no. populations)		Testcross F_2 populations (no. plants in segregating populations)		
		Res.	Susc.	P-value[a]	Susc.	Segr.	Res.	Susc.	P-value[a]
1	(a)	156	38	0.05–0.10	1	4	75	32	0.20–0.30
1	(b)	133	15	<0.01	4	4	67	26	0.50–0.70
2	(a)	59	17	0.50–0.70	7	8	144	50	0.30–0.50
3	(a)	154	37	0.05–0.10	4	3	53	18	0.90–0.95
3	(b)	156	37	0.05–0.10	4	4	65	22	0.90–0.95
4	(a)	102	31	0.50–0.70	6	6	102	42	0.20–0.30
4	(b)	112	24	0.02–0.05	4	3	42	25	0.02–0.05
5	(a)	151	48	0.30–0.50	...	...	...	...	...
5	(b)	113	29	0.20–0.30	...	...	...	...	...
6	(a)	119	36	0.50–0.70	...	...	...	...	...
6	(b)	166	28	<0.01	...	...	...	...	...
7	(a)	153	53	0.30–0.50	...	...	...	...	...
8	(a)	113	27	0.10–0.20	...	...	...	...	...
9	(a)	138	42	0.50–0.70	...	...	...	...	...
10	(a)	158	33	0.01–0.02	...	...	...	...	...
11	(a)	84	28	0.99	...	...	...	...	...
12	(a)	145	27	<0.01	...	...	...	...	...

[a] Based on an expected 3 resistant: 1 susceptible ratio; resistant: reaction types 0 to 2; susceptible: reaction types 3 and 4.

TABLE 2. Seedling reaction to rust of F_2 populations of crosses between five single-gene differentials and Raja flax

Cross[a]	Populations (no.)	Test race	P-value for homogeneity	Plants (no.) Observed[b] Res.:Susc.	Plants (no.) Expected[b] Res.:Susc.	P-value[c]
L^6 × Raja	6	300	0.10–0.25	669:50	674:45	0.30–0.50
M^3 × Raja	7	300	0.05–0.10	677:49	681:45	0.50–0.70
P^3 × Raja	9	300	0.50–0.70	854:47	845:56	0.20–0.30
Raja × P^3	1	300	...	221:22	228:15	0.05–0.10
N^1 × Raja	4	300	0.05–0.10	801:127	870:58	<0.01
K × Raja	11	300	0.99	2,268:0	2,268:0	...
K × Raja	5	312	0.70–0.80	285:103	291:97	0.80–0.90

[a] Crosses involve differential lines that contain a single rust resistance gene occurring at one of five known loci.
[b] Resistant: reaction types 0–2; susceptible: reaction types 3 and 4.
[c] Based on an expected 15:1 ratio for reaction to race 300, and a 3:1 ratio for reaction to race 312.

pustule size varied from very small to medium, approaching a type 3 reaction. Twelve F_2 populations, relating to 10 Raja parents, all fitted (0.05 < P < 0.99) a 3 resistant:1 susceptible ratio, indicating the presence of a single resistance gene in Raja. The fit to the postulated ratio was poor in case of five F_2 populations that were deficient in susceptible plants. Of the 62 Bison (Bison × Raja) testcross progenies, 30 were susceptible, whereas 32 populations segregated. Single-gene inheritance was indicated by the near perfect fit to a 1:1 ratio of susceptible and segregating progenies. The 32 segregating testcross F_2 progenies were derived from seven crosses and formed seven populations. Six populations produced segregation ratios that fitted (0.20 < P < 0.95) the 3 resistant:1 susceptible expectation, whereas that of one population did not. The data reaffirmed the presence of a single gene for resistance in Raja (Table 1).

All plants belonging to the 23 F_1s of Raja with the lines containing genes L^6, M^3, N^1, and P^3 were immune to race 300. The F_2 populations of these crosses all segregated, indicating that the resistance gene of Raja occupies a different locus and is thus different. The number of immune and susceptible F_2 plants fitted (0.05 < P < 0.70) a 15 resistant:1 susceptible ratio in crosses with the L^6, M^3, and P^3 lines; the results confirmed the presence of a single resistance gene in Raja (Table 2). The F_2 segregation ratio of the N^1 × Raja cross, however, deviated conspicuously from a 15 resistant:1 susceptible expectation, and showed an excess of susceptibles. The N^1 × Raja data (Table 2) are totals of two tests, a

repeat being carried out when the first test showed an unexpected ratio of 452 resistant:74 susceptible. The second test showed a similar ratio of 349 resistant:53 susceptible. The two populations are homogeneous (P > 0.70). Assuming linkage in the repulsion phase between the Raja and the N^1 resistance genes, a deficiency of susceptible plants would be expected to have resulted from the cross. In contrast, the excess of susceptibles indicated the reverse, i.e., the coupling phase. In studies on the inheritance of flower color in flax, Plonka (10) noted abnormal disjunction resulting in a deficiency of a male or female gamete, and crossing over would change a deficit to an excess and vice versa. This mechanism might have been operative in the present study. The same mechanism was suggested to have been also responsible for causing significant deviations from theoretical dihybrid ratios in other studies on identification of rust resistance genes of flax (15). Accepting the validity of Plonka's thesis, it is concluded that the Raja and N^1 resistance genes are linked. Theoretical considerations allowed speculation on the recombination value of the two genes. In F_2 segregation of the dihybrid AaBb, where A is gene N^1 and B is the Raja gene, phenotypes AB, Ab, aB, and ab occur in respective frequencies of $p^2 + 0.50$, $0.25 - p^2$, $0.25 - p^2$, and p^2, where p is a fraction. Because only two classes (AB, Ab, and aB are all resistant) of phenotypes were distinguished, the frequency of the susceptible class, i.e., the double homozygous recessive, equals $p^2 = 127/928 = 0.1369$. The ratio of the four phenotypes is thus postulated as 0.6369 AB:0.1131 Ab:0.1131 aB:0.1369 ab. The z value computed for the coupling phase is 0.146, and indicates a recombination value of 25.5% (12). Following Plonka's thesis, it is suggested that due to disjunction and crossing over, the repulsion phase turned into the coupling phase, converting an initial deficit of gametes AB and ab into an excess.

Nonsegregation occurred in two experiments in the K × Raja cross, where 2,268 and another 661 plants of 11 F_2 populations were all found to be immune or highly resistant (type 1) to races 300 and 371, respectively. Nonsegregation indicates that the resistance gene of Raja is an allele at the K locus. Gene K, the only allele thus far known here, is different from the allele in Raja, because race 312 is virulent to gene K but avirulent to Raja. Raja's resistance factor is new, and is designated K^1. This designation was verified when F_2 populations considered to consist of genotypes KK, KK^1, and K^1K^1 in a 1:2:1 proportion, were tested for reaction to race 312 under growth room conditions. Five F_2 populations showed segregation ratios, each fitting (0.10 < P < 0.95) the 3 resistant:1 susceptible expectation. The populations were homogeneous and the pooled data are recorded (Table 2). The experiment was a partial success only, however, because 13 of 53 plants of susceptible

checks—the cultivar Bison and the line containing gene K—failed to develop infections. A deficiency of susceptible plants was also evident in six F_2 populations that were segregating but where the ratios did not conform to expectation. Failure to develop infections was clearly due to lack of condensation, visually judged to vary from poor to good in the transparent plastic bags in which plants had been incubated.

The reactions of F_3 families to race 312, determined under summer conditions in a greenhouse, verified the monogenic segregation of the F_2 populations. F_3 progenies were generated from F_2 plants resistant to race 371, and per F_2 11 or 12 progenies were inoculated with race 312. Of the total of 131 progenies, 21 were resistant, 72 segregating, whereas 38 were susceptible. The data fitted ($P > 0.05$) the postulated 1:2:1 ratio.

DISCUSSION

The rust resistance of the cultivar Raja was shown to be conditioned by a single, dominant gene that occurs at the K locus, and is designated as K^1. Monogenic control of Raja's rust resistance was clearly evident from the Bison $\times$ Raja F_2, testcross, and testcross F_2 data, and was confirmed by the bigenic segregation for rust reaction in F_2 populations of Raja with the single-gene lines L^6, M^3, and P^3. Susceptible plants were, however, lacking in case of several Bison $\times$ Raja F_2 populations and in one testcross F_2 whose fit to the postulated ratio was poor. Under conditions in the growth cabinet where the experiment was conducted, direct exposure of some plants to rapid and continuous air movement causes excessive transpiration, a factor that reduces vigor. Vigorous plant growth is required for expression of typical host-rust interactions (2). The deficiency of susceptible plants in some F_2 populations is ascribed to atypical rust reactions of otherwise susceptible plants that were of suboptimal vigor.

Although nonsegregation to race 300 of F_2 populations of $K \times$ Raja indicated an allelic relationship, reactions to race 312 of F_2 and F_3 populations reconfirmed Raja's monogenic rust control and further substantiated the identity of the new gene.

Linkage between the K and N loci was indicated from segregation data of the $N^1 \times K^1$ F_2 populations, where the recombination value was high and estimated at about 25%. Flor (2) suggested that the rust resistance gene of the differential Clay, which carries gene K (3), occurred either outside the chromosomes carrying genes L, M, or N, or in the chromosome carrying gene N with a high percentage of crossing over. Our studies where genes N^1 and K^1 appear to be linked confirm the validity of the latter of Flor's suggestions. Flor and Comstock (5) indicated that the N and P loci are linked at an approximate crossover value of 20%. In the present study, however, linkage was not evident from the crosses between the K^1 and P^3 genes where the F_2 data closely adhered to the theoretical 15:1 ratio (1,075 resistant:69 susceptible). It is

concluded that the K, N, and P loci occur on the same chromosome and that the K and P loci are bilateral to the N locus.

The reservoir of resistance genes effective against North American races earlier included the genes L^6, L^{11}, L^{12}, M^3, M^6, P^3, P^4, and the so-called Kugler gene (11,13). The addition of gene K^1 enlarges the number of possible combinations of resistance genes. Gene K^1 conditions resistance in the cultivar Dufferin (14) and also occurs, combined with gene L^6, in the cultivar McGregor (9). The cultivar J.W.S. (C.I. 708-1) is the source of both Raja's male parent and of the single-gene differential Stewart (C.I. 1072), which contains resistance gene L^2 (2). Because there is no indication that J. W. S. contains more than one resistance gene (1,2), and because race 312 is virulent to gene L^2 but avirulent to Raja, it is suggested that the source of gene K^1 is Raja's female parent, Argentine Selection 1025.

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