

Sources and Distribution of Resistance to Crown Rust Within Perennial Ryegrass

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ABSTRACT

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Fifty-seven cultivars and selections of perennial ryegrass (*Lolium perenne*) and one selection of annual ryegrass (*L. multiflorum*) were screened under greenhouse conditions for resistance to the crown rust fungus (*Puccinia coronata* f. sp. *lolii*). Variation in resistance to crown rust among entries was dramatically evident, which indicated a potential for developing ryegrass cultivars with improved resistance to the crown rust fungus. Frequency distributions based on rust reaction of cultivars provided insight into the type of resistance and mode of inheritance. Bimodal distributions based on rust reaction were indicative of major genes undergoing segregation. The advantage of using frequency distributions when evaluating germ plasm for resistance to pathogens in cross-pollinated populations is discussed.

Crown rust (*Puccinia coronata* Corda f. sp. *lolii* Brown) is a cosmopolitan pathogen that infects annual (*Lolium multiflorum* L.) and perennial (*L. perenne* L.) ryegrasses, which are cross-pollinated species. The fungus parasitizes host plants and depletes their carbohydrate reserves (10). Crown-rust-infected ryegrass is less palatable to sheep and is of lower nutritional quality (2). Severe infestations render turf unsightly, detract from growth and recuperative potential, and may predispose plants to winter injury. Because chemical control is expensive, it is more desirable to control crown rust

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proposals would generally be more useful in annual as contrasted with perennial crop species.

The objective of this study was to identify sources of rust resistance and provide an assessment of relative resistance among ryegrass cultivars and selections. In addition, frequency distributions based on rust resistance may be indicative of the mode of inheritance within any given cultivar or selection in these cross-pollinated species.

MATERIALS AND METHODS

Sixteen individual plants from each of the 58 ryegrass cultivars or selections were included in each test. Each entry was observed in either three or six tests, during 1 and 2 yr, respectively. Plants were grown in plastic flats divided into 48 individual cells. Each 5-cm-square cell contained one plant. A 2:1:1 (v/v) ratio of sandy loam soil, peat, and vermiculite comprised a greenhouse mix with a pH of 6.5. The soil was fertilized periodically with either water-soluble 20-20-20 fertilizer or KNO₃. Individual plants were sheared with hand clippers to promote tillering. During periods of low light intensity, 2.43-m Grow-Lux tubes were used to provide 14-16 hr of daylight.

After 12 wk of growth, the plants were inoculated in a wood-framed chamber covered with clear polyethylene plastic. Moisture was provided by a humidifier controlled by a 60 pin per hour timeclock. A water bath maintained at 20 C provided a stable temperature throughout the 12-hr inoculation period. Crown-rust-infected ryegrass plants were collected from several locations throughout New Jersey, New York, and Pennsylvania and transferred to a

through genetic resistance. Traditionally, identification and incorporation of specific (8), major gene (4), or vertical resistance (11) in many cases has provided ephemeral control (9), especially when airborne pathogens are involved. Such resistance to rust is normally characterized by the host's hypersensitive reaction (6), and is usually oligogenic in nature. Current interest lies in the development of general (3) or horizontal (8) resistance where quantitative response is, most often, polygenically inherited. In theory, this multigene resistance offers increased stability because the host has an increased buffering capacity against the pathogen.

Stability is an important component of disease resistance, especially in perennial species. Resistance to disease, based on vertical or major genes, is usually subject to erosion and thus may be less stable over time (13). This has led to proposals to increase the stability of vertical resistance. The use of multilines (5) and strategic development of vertical resistance genes in space and time has been suggested (1). These "gene management"

greenhouse for use as a source of inoculum. Urediniospores from sporulating pustules on these plants were collected as needed by a cyclone vacuum pump. The pump was used to dust the plants with freshly harvested spores, diluted 1:10 with talc until the plants were uniformly covered with inoculum. Plants were misted immediately before inoculation. Humidity and temperature were

adjusted according to greenhouse conditions. After 12 hr, the chamber covers were raised a few inches for 2-4 hr to allow the leaves to dry slowly. The flats were then removed to greenhouse benches. Plants were rated on visual estimate based on the percentage of rust pustules on the foliage. A progressive scale of 0-9 was used, with 0 = no rust; 1 = trace; 3 = 10%; 5 = 30%; 7 = 50%; and 9 =

≥70% of the foliage covered with rust. The rating values represented a relative estimate of leaf area occupied by rust pustules, and not reaction type. For each seedling, the average rust reaction was computed from three individual ratings taken during periods of abundant rust development.

The experiment was analyzed as a completely randomized design with

Table 1. Population responses under greenhouse conditions of perennial ryegrass cultivars and selections to *Puccinia coronata*

Cultivar or selection	No. of tests ^a	Percent of plants in each class ^b										Rust rating ^c	Standard deviation ^d
		0	1	2	3	4	5	6	7	8	9		
Elka	6	99	1	0	0	0	0	0	0	0	0	0.0 a	0.0
H969	6	89	7	0	1	0	2	0	0	1	0	0.2 ab	0.9
Ruanui 909	3	70	25	5	0	0	0	0	0	0	0	0.3 abc	0.5
R-39 A	3	78	11	2	7	0	0	2	0	0	0	0.5 abc	1.0
Loretta	6	78	12	2	3	1	5	0	0	0	0	0.5 abc	1.1
U-103 ^e	3	77	7	7	5	2	2	0	0	0	0	0.6 abcd	0.9
Elliot	3	44	38	15	3	0	0	0	0	0	0	0.8 abcde	0.7
Donata	6	50	24	12	3	5	4	2	1	0	0	1.1 abcde	1.5
Prelude	3	54	15	13	6	2	6	2	2	0	0	1.3 abcde	1.6
Sprinter	6	51	24	6	4	7	4	1	3	0	0	1.3 abcdef	1.0
Cropper	6	50	18	6	12	4	5	6	0	0	0	1.4 abcdefg	1.6
Talbot	6	51	18	7	8	2	6	7	1	0	0	1.5 abcdefg	1.7
Premier	6	49	24	2	4	4	7	5	3	2	0	1.5 abcdefg	2.1
FRR-1	3	56	8	6	6	9	6	6	2	0	0	1.6 abcdefg	2.1
Fiesta	6	49	15	12	5	2	7	3	4	2	0	1.7 abcdefg	1.8
Delray	3	48	9	13	9	4	6	11	0	0	0	1.8 abcdefgh	2.1
R-35	6	46	15	3	13	4	6	9	3	1	0	2.0 bcdefgh	2.0
Capper	6	34	24	13	6	5	6	4	7	1	0	2.0 bcdefgh	1.7
Pennant	6	35	17	12	9	6	10	9	2	0	0	2.1 bcdefgh	2.0
Palmer	3	44	17	2	2	13	7	11	2	2	0	2.1 bcdefgh	2.5
Pelo	6	31	18	13	9	10	13	1	4	1	0	2.2 cdefgh	1.7
Goalie	3	27	26	7	7	9	11	7	4	2	0	2.4 defghi	2.3
Frances	6	23	24	14	10	6	11	4	8	0	0	2.4 defghij	1.9
Birdie	6	35	14	5	7	8	14	12	5	0	0	2.5 defghij	2.5
Dasher	6	31	20	5	7	7	6	15	7	2	0	2.5 efghij	2.4
S-321	6	13	15	22	13	16	13	7	1	0	0	2.7 fghijk	1.5
Grandstand	6	35	10	4	9	7	19	9	6	1	0	2.8 ghijk	2.2
Linn	6	17	20	12	10	7	20	9	5	0	0	2.9 ghijk	1.9
Acclaim	6	25	16	6	5	12	11	13	10	3	0	3.1 hijkl	2.5
Blazer	6	19	9	3	17	12	16	17	5	1	0	3.3 hijklm	2.2
Belle	6	18	10	5	13	16	20	7	11	1	0	3.4 hijklmn	2.2
Score	6	22	14	8	1	9	14	16	15	2	0	3.5 hijklmno	1.6
Pronto	3	19	8	4	15	13	13	12	15	2	0	3.6 hijklmno	2.4
Ranger	6	14	9	4	12	16	22	21	2	1	0	3.7 ijklmno	2.0
Rex	6	16	7	8	9	15	18	15	12	1	0	3.8 ijklmno	1.2
NK 100	6	11	12	10	6	16	14	18	10	3	0	3.9 jklmno	2.0
Ensporta	6	13	6	5	11	21	16	16	8	3	1	4.0 jklmnop	1.8
Pennfine	6	16	8	8	8	4	13	20	18	5	0	4.1 klmnop	2.2
Citation	6	13	14	6	5	10	11	19	13	7	2	4.2 klmnop	2.4
Barry	6	3	4	13	13	16	17	16	12	5	2	4.5 klmnopq	1.4
Yorktown II	6	1	3	4	13	23	30	22	4	1	0	4.5 klmnopq	1.2
Diplomat	3	0	0	0	25	27	23	17	8	0	0	4.6 klmnopqr	1.3
Regal	6	4	3	13	14	11	13	22	16	4	1	4.7 lmnopqr	1.8
Lp 20	3	6	2	4	2	21	23	29	8	5	0	4.8 lmnopqrs	1.8
Clipper	3	10	4	0	6	7	25	33	8	4	2	4.8 lmnopqrs	2.2
Bellatrix	3	0	2	6	4	35	15	13	19	6	0	5.0 mnopqrs	1.0
Arno	6	2	3	11	8	4	21	28	17	6	0	5.1 mnopqrs	1.5
Caravelle	6	2	1	2	7	15	27	33	12	2	0	5.1 mnopqrs	1.3
Derby	6	2	1	2	13	13	24	22	16	6	1	5.2 nopqrs	1.3
Eton	3	2	2	2	10	8	21	30	19	6	0	5.3 nopqrs	1.6
Yorktown	3	0	2	0	6	17	21	35	19	0	0	5.4 opqrs	1.1
Omega	6	2	1	0	10	11	20	26	23	8	0	5.5 pqrs	1.6
Exponent	6	2	3	2	4	10	17	29	22	10	1	5.6 pqrs	1.2
Barclay	3	0	0	0	6	10	30	13	33	9	0	5.8 pqrs	1.3
NK-200	6	1	1	2	2	2	18	37	25	7	4	6.0 qrs	0.9
Manhattan	6	1	1	1	4	3	16	33	29	13	0	6.1 rs	1.1
Barcelona	3	0	0	0	2	0	13	42	23	17	4	6.5 s	0.8

^aSixteen plants were used in each test.

^bScale based on visual estimates approximating area of foliage rusted: 0 = no rust, 1 = trace, 3 = 10%, 5 = 30%, 7 = 50%, 9 = ≥70%.

^cMeans followed by the same letter are not significantly different from each other (Duncan's multiple range test, $P = 0.05$).

^dStandard deviation represents pooled variances from all tests.

^eU-103 is a selection of annual ryegrass, *Lolium multiflorum*.

different test dates as replicates. The grand means of each entry were compared using Duncan's multiple range test. The standard deviation for each entry was derived from the pooled variance of each entry per test.

RESULTS AND DISCUSSION

There was a significant difference in mean crown rust scores among ryegrass cultivars and selections ($P < 0.0001$), while no significant differences were attributed to years or the cultivar \times year interaction ($P > 0.10$). Crown rust responses ranged from 0 infection on all plants of the cultivar Elka to a range of 5 to 9 on most plants within the cultivar Barcelona (Table 1). Elka was the most crown-rust-resistant perennial ryegrass cultivar in this test. Elka has also shown high levels of rust resistance when tested in the field at many locations (C. R. Funk, unpublished).

Frequency distributions are indicative of the mode of inheritance of crown rust resistance in ryegrass populations. These data provide a starting point for selection of germ plasm based either on vertical or horizontal resistance. A bimodal type of distribution is indicative of major genes for vertical resistance undergoing segregation in this cross-pollinated species. Selection and hybridization of rust-free plants from such entries as Citation, Dasher, Score, Rex, Palmer, Ranger, and Pennfine may serve as a source of genes when breeding for vertical resistance (Table 1). Hides and Wilkins (7) stated that skewed frequency distributions indicated the presence of one or two major genes controlling crown rust resistance in certain ryegrass populations (ecotypes), whereas populations normally distributed for rust reaction may have horizontal resistance, which is inherited quantitatively (8).

Selection and hybridization of plants with low levels of rust from cultivars like Loretta, Prelude, and Sprinter could be used in a phenotypic recurrent selection program when breeding for improved horizontal resistance (Table 1). In such instances, some level of infection is acceptable and, in theory, the rust resistance longer lived and more stable.

Yorktown II perennial ryegrass was originally selected for vertical resistance to crown rust. The rust resistance of this cultivar is reported as not uniform, but varying during different years and locations throughout the United States (C. R. Funk, unpublished). This inconsistent host response indicated the

presence of a race(s) of *P. coronata* able to overcome this vertical resistance. In the presence of this race, Yorktown II continues to exhibit a moderate level of horizontal resistance as indicated by the normal distribution of rust reaction (Table 1).

The inherent variability of crown rust resistance in ryegrass reported here suggests a potential for developing ryegrasses with improved crown rust resistance. The time and effort required to exploit rust resistance and develop rust-resistant ryegrass cultivars depends on the genetic control of crown rust resistance and the agronomic qualities of the donor parents. Selection for both turf quality and rust resistance is not difficult when both donor and receptor parents are of acceptable turf quality, regardless of the genetics of resistance. In such instances, if rust resistance is controlled qualitatively, minimal or no backcrossing is necessary to recover high turf quality. By performing the appropriate sib and testcrosses, plants with genes homozygous for rust resistance can be used as parents in cultivar development. If resistance is inherited quantitatively, then crown-rust-resistant, turf-type cultivars can be developed by phenotypic recurrent selection.

When the source of rust resistance is from plants of unacceptable turf quality, development of turf-type plants with crown rust resistance becomes more time consuming and difficult. If crown rust resistance is inherited qualitatively, several backcrosses are required to recover plants with both adequate rust resistance and the necessary turf quality. When quantitatively inherited crown rust resistance is transferred from a nonturf-type donor plant, either a complementary recombination scheme or modified backcrossing becomes necessary to recover plants with rust resistance and high turf quality. This requires screening large populations in each generation, because only a few plants will have the desired genotype for both rust resistance and high turf quality.

Screening plants from a heterogeneous cultivar or population and constructing a frequency distribution curve offers useful information for application in plant breeding programs. Quantitative information is provided that may be of value in developing ryegrass cultivars that maintain an acceptable level of horizontal resistance to crown rust (9).

Since *P. coronata* has the capacity to overcome host resistance by evolving new

virulent races (12), it is important that many genetically diverse rust resistance sources and types of resistance be identified, conserved, and used. These data identify sources of qualitative and quantitative resistance and offer insight on the inheritance of crown rust resistance. Such information is beneficial when investigating sources of rust resistance in a heterogeneous cross-pollinated species like ryegrass, where individuals in the populations are normally highly heterozygous.

Ryegrass cultivars and polystands having diverse sources of rust resistance should offer improved stability of rust resistance in long-lived turfs.

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