Inheritance of Resistance to Stem Rust (*Puccinia graminis* subsp. *graminicola*) in Six Perennial Ryegrass (*Lolium perenne*) Crosses

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ABSTRACT

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Differences were found among four perennial ryegrass parental sources for stem rust reactions. Frequency distributions based on reaction patterns among segregating populations suggested resistance was predominately quantitatively inherited with minor and possibly some major genes. There was considerable genetic variability for resistance between parental lines as evidenced by different reaction patterns among progeny. Transgressive segregation toward susceptibility and slow-rusting types were evident. Selection for resistance would be more effective in the boot stage before anthesis (when higher heritability estimates were observed). Seedling reactions differed from adult plant responses, indicating that selection for resistance may be more successful if carried out in later growth stages. Results indicated that cultivars with greater and more durable resistance to stem rust could be obtained from these parental sources.

Perennial ryegrass (*Lolium perenne* L.) is a cross-pollinated species, with cultivars for turf or forage being used throughout the United States. Stem rust (*Puccinia graminis* Pers. subsp. *graminicola* Urban) (10) is a serious seed production problem of perennial ryegrass in western Oregon, requiring fungicide application from April through July to prevent seed yield losses as great as 93% (6).

Stem rust is prevalent on perennial ryegrass in many areas of the United States including Oregon, California, Washington, Pennsylvania, Nebraska, and New Jersey. France (A. J. P. van Wijk, *personal communication*) and New Zealand (2) have also reported stem rust on perennial ryegrass. Races of stem rust that infect perennial ryegrass have not yet been identified. Occurrences of new races of stem rust are common in other crop species and are expected to be found in perennial ryegrass (7). Before 1983, all commercial turf-type perennial ryegrass cultivars were susceptible to stem rust. Subsequently, several stem rust-resistant cultivars have been developed from three sources of resistance and tested in Oregon, New Jersey, and France (7).

In studies with perennnial and Italian ryegrass, resistance to crown rust (*Puccinia coronata*) was polygenically inherited in some populations, whereas in others, it was controlled by a few major genes (4,12,13). In theory, multigene resistance offers increased stability because the host has an increased buffering capacity against the pathogen (4). Forage and turf perennial ryegrasses are generally self-sterile, cross-pollinated species. Synthetic cultivars are restricted populations of elite clones, hence cultivars may contain large numbers of resistance genes and gene combinations (13).

The objectives of this study involving stem rust in perennial ryegrass were 1) to determine the nature of inheritance by analyzing frequency distributions of heterogeneous F_1 populations; 2) to study slow rusting types over time in relation to parents, F_1s, and the association between F_1s and parents; and 3) to determine if seedling reactions could be used to predict adult plant resistance.

MATERIALS AND METHODS

Four perennial ryegrass clones were crossed in a diallel manner resulting in six crosses excluding reciprocals. Three parents were stem rust-resistant on the basis of 9 yr of evaluation. Resistant sources 4A and 48A were collected in 1975 from old turf areas in St. Louis, MO; 77A was collected from old turf in Washington, DC. The susceptible parent, MP-2, was obtained from turf of Belle perennial ryegrass in Washington, DC.

Seed from each cross was planted and seedlings were inoculated with stem rust spores 2 wk after emergence. Inoculum consisted of rust-infected perennial ryegrass leaves taken from several sources adjacent to the experimental site that were chopped and mixed in water to make a spore suspension of about 1,000 spores per milliliter as determined by microscopic count. This solution was misted onto the seedlings, which were then kept in a moist chamber for 72 hr. Records were kept of which plants were susceptible as seedlings.

Sixty F_1 seedlings randomly selected from each cross were transplanted to the field in 5.5-cm peat pots on 19 October 1983. F_1 progeny were spaced 60 cm apart with 60 cm between rows in a sandy silt loam soil of the Willamette series near Hubbard, OR. A randomized block design was used with four blocks. Each block contained 15 progeny from each of the six crosses. Twenty-seven vegetative clones were taken from each parent plant and randomly planted among the first three replicates of the progeny. Standard fertilization and weed control practices for commercial ryegrass production were used.

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On 17 May and 12 June 1984, 10–15 chopped blades of stem rust-infected perennial ryegrass foliage obtained from the surrounding area were placed in the middle of each plant. From May through July, the F_1 progeny from each cross and the cloned parents were rated five times using a visual estimate based on the percentage of rust pustules on the foliage of individual plants. Degree of infection was rated on a scale of 1–9, where 1 = whole plant dead; 2 = stem and seed heads dead, a few green leaves; 3 = 70–85% of the stems, leaves, and seed heads infected; 4 = 50–70% of the stems, leaves, and seed heads infected; 5 = 30–50% of the stems, leaves, and seed heads infected; 6 = 10–30% of the stems, leaves, and seed heads infected; 7 = up to 10% of the stems, leaves, and seed heads infected; 8 = trace of foliar infection, one to three stems infected; and 9 = no rust.

A two-way analysis of variance was performed on mean stem rust ratings of the parents and F_1 progeny for five dates. Broad-sense heritability estimates were obtained on a family basis using the variances of the cloned parental and segregating F_1 populations in the field (5).

RESULTS AND DISCUSSION

A uniform disease pressure was created through artificial inoculations; 98% of the plants were infected with stem rust by 10 July.

Table 1 represents frequency distributions, totals, means, and standard deviations of the parent and F_1 population stem rust reactions taken on 10 July. This date was selected because the largest differences were observed in reactions between the resistant and susceptible parents.

Clear differences in stem rust reactions were found between the parents. Parent 48A had the highest mean value of 7.37 followed by parents 4A and 77A with mean values of 5.8 and 5.7, respectively. MP-2, with a mean value of 3.67, was the most susceptible parent.

Transgressive segregation toward susceptibility was evident between crosses involving resistant parents, suggesting the presence of different alleles for disease reaction between the resistant parents. The presence of moderately susceptible and susceptible plants in these crosses indicated genetic differences between the resistant sources. These different genetic sources of resistance among parents could be combined through breeding to produce a more durable resistance to stem rust.

Crosses 48A × 4A, 48A × MP2, and 48A × 77A produced segregating progeny with near-normal distributions, indicating polygenic inheritance or the presence of several races occurring in low frequencies. Parent 48A is common to all three crosses and appears to be contributing a polygenic-type resistance. Wilkins (13) found continuous variation with a large number of genes exhibiting an additive type of genetic variance for resistance to crown rust in ryegrass. Progeny that were bimodally distributed, indicative of major genes undergoing segregation, were observed in crosses 4A × 77A, 4A × MP-2, and 77A × MP-2. Thus, it appears that both parents 4A and 77A may be contributing major genes for resistance.

Similar growth stages of the parents and F_1 populations indicated that none of the plants escaped infection of stem rust because of differences in maturity. Differences in rust reaction patterns were then considered to be under genetic control.

Stem rust reactions over time were studied by plotting mean values for each parent and its respective F_1 population at each rating date. Crosses with parent 4A were not shown because the results were similar to those with parent 77A. F_1 plants of the cross 48A and 77A were more susceptible than either resistant parent because of transgressive segregation toward susceptibility. When compared with other F_1 means, the 48A × 77A F_1 progeny (Fig. 1) were more susceptible at the later rating dates. This could be due to parent 77A contributing different genes for resistance. Resistant × susceptible F_1 progeny of the 48A × MP-2 cross (Fig. 2) were more resistant than parent MP-2 at all dates, showing greater stem rust resistance in the first generation contributed by parent 48A.

The 77A × MP-2 progeny were less infected during the last 3 wk of the season than their susceptible parent (Fig. 3). This depicts a slow-rusting type of resistance, because early in the season, the plants had limited stem rust infection. As the stem rust epidemic progressed, the rate of pustule development on the resistant plants was reduced compared with susceptible plants. This resulted in resistant plants producing more healthy seed at harvest. In some wheat cultivars,

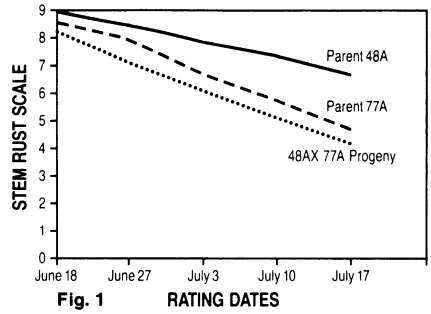

Fig. 1

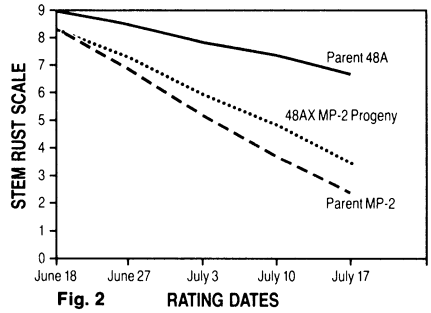

Fig. 2

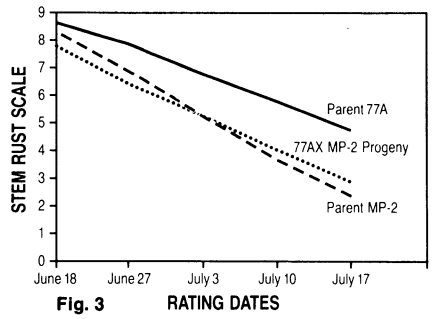

Fig. 3

Figs. 1–3. Mean stem rust reactions of parent and F_1 populations plotted over time. Degree of infection was rated on a scale of 1–9, where 1 = whole plant dead; 2 = stem and seed heads dead, a few green leaves; 3 = 70–85% of the stems, leaves, and seed heads infected; 4 = 50–70% of the stems, leaves, and seed heads infected; 5 = 30–50% of the stems, leaves, and seed heads infected; 6 = 10–30% of the stems, leaves, and seed heads infected; 7 = up to 10% of the stems, leaves, and seed heads infected; 8 = trace of foliar infection, one to three stems infected; and 9 = no rust.

Table 1. Two-way frequency distributions, means, and standard deviations of stem rust ratings of parent populations and F_1s taken on 10 July

Parents and F_1s	Stem rust rating scale[a]									Total	Mean	SD
	1	2	3	4	5	6	7	8	9			
48A	0	0	0	0	3	6	3	8	7	27	7.37	1.39
4A	0	0	0	2	9	3	4	1	1	20	5.80	1.32
77A	0	0	3	2	7	8	3	3	1	27	5.70	1.56
MP-2	0	11	0	2	2	5	1	0	0	21	3.67	1.91
48A × 4A	0	2	8	12	13	15	4	6	0	60	5.12	1.57
4A × 77A	3	11	5	4	8	10	12	6	13	60	4.95	2.25
4A × MP-2	4	13	6	7	10	13	2	3	2	60	4.33	2.12
48A × 77A	4	9	3	4	12	11	7	8	2	60	5.07	2.27
77A × MP-2	6	13	7	8	14	5	3	4	0	60	3.97	2.01
48A × MP-2	2	2	5	15	16	14	4	1	1	60	4.83	1.53
Total	19	61	37	56	94	90	43	40	15	455	4.93	1.90

[a] Stem rust scale based on visual estimates of area of foliage infected, where 1 = whole plant dead; 2 = stem and seed heads dead, a few green leaves; 3 = 70–85% of the stems, leaves, and seed heads infected; 4 = 50–70% of the stems, leaves, and seed heads infected; 5 = 30–50% of the stems, leaves, and seed heads infected; 6 = 10–30% of the stems, leaves, and seed heads infected; 7 = up to 10% of the stems, leaves, and seed heads infected; 8 = trace of foliar infection, one to three stems infected; and 9 = no rust.

slow rusting reactions have been reported to be under polygenic control (9).

Quantitatively inherited resistance found in this study should provide the needed genetic diversity to avoid erosion of resistance resulting from the development of new races of stem rust. The presence of many minor resistance genes provides a buffering effect, increasing the chances for the cultivar to remain resistant over a longer period.

To provide additional information regarding the amount of genetic variability for stem rust resistance for each of the crosses, broad sense heritability estimates were calculated for stem rust reactions of F_1 progeny on 27 June and 3 and 10 July (Table 2). Different heritability estimates among F_1 populations indicate that some parental combinations resulted in more genetic variability for stem rust resistance. The highest heritability estimates for stem rust reactions were assessed for 48A × 77A and 4A × 77A progeny, with values of 70 and 65%, respectively. These results suggest that selection within the cross 48A × 77A, followed by 4A × 77A and 77A × MP-2, would be the most promising. Heritability estimates indicate that there is considerable genetic variability for stem rust resistance based on variations between clones and their progenies.

Perhaps the most significant feature of these results is the consistent decrease in heritability estimates over time. Broad-sense heritability estimates were larger early in the growing season, which suggests a greater portion of the variance was genetic in the earlier stages of the epidemic. With the increase in inoculum over time, it appears that the rust reaction was dependent on the spore load. Thus, effective selection for resistance must be made during early anthesis. This will vary with the year; however, epidemics of stem rust generally coincide with higher temperatures, which usually occur during late summer in the Willamette Valley.

Table 2. Broad-sense heritability estimates for mature plant stem rust reactions involving four cloned parental lines and six resulting F_1 populations

F_1s	27 June	3 July	10 July
48A × 4A	0.54	0.46	0.33
4A × 77A	0.65	0.60	0.58
4A × MP-2	0.52	0.47	0.45
48A × 77A	0.70	0.64	0.61
77A × MP-2	0.55	0.41	0.27
48A × MP-2	0.49	0.13	−0.21

In an effort to determine if seedling reactions could be used to identify mature plant resistance, F_1 seedlings were inoculated and both susceptible and resistant seedlings were transplanted into the field. Susceptible F_1 seedlings, rating a 3 or 4 (1), were monitored throughout the growing season, and at harvest, their stem rust ratings were recorded (Table 3). The stem rust reactions of mature F_1 plants ranged from 1 to 8, whereas in the seedling stage, those same plants were all susceptible. Therefore, seedling reactions were not related to adult plant stem rust reactions in F_1s derived from the four parents used in this study. Three of the 4A × MP-2-susceptible seedlings had a resistant rating of 7 and a rating of 8 as adults. Likewise, many of the plants that were resistant as seedlings broke down to susceptible ratings of 1–4 as adult plants. Similar host responses of maize (8) and wheat (1) were reported where plants were susceptible as seedlings but resistant as adults.

Different stem rust reactions in the seedling and adult stages suggest two separate genetic systems are involved, one influencing the seedling reaction and a second contributing to the mature plant resistance. Flor (3) found that plants with horizontal or polygenic resistance to rust are relatively susceptible in the seedling stage. Observations of susceptible seedlings being resistant as mature plants indicate that a seedling screening process may not be warranted in a perennial ryegrass breeding program for stem rust resistance.

In summary, genetic differences for stem rust reactions were present among the three resistant sources. These differences were quantitatively inherited in the F_1 progeny involving both minor and major genes. Studies of stem rust reactions over time displayed differences between parents, F_1s, and the relationship between F_1s and parents. Slow-rusting characteristics were demonstrated by F_1 progeny from parents 4A and 77A. Selection for stem rust resistance would be most effective during early anthesis in late June, when a higher proportion of the resistant reaction is under genetic control. Seedling stem rust reactions differed from adult plant reactions in the F_1 generation. There may be separate gene systems responsible for stem resistance in seedlings and mature plants in perennial ryegrass. Therefore, a breeding program for stem rust resistance that uses a seedling screening procedure may be discarding genetic factors for adult plant resistance.

A strategy for breeding quantitatively inherited durable resistance to stem rust based on the results of this study for perennial ryegrass would be as follows. After making crosses between moderately resistant parental lines (rating of 6 or 7) or resistant × susceptible crosses with different sources for resistance, F_1s with ratings of 6 or 7 before anthesis could be selected and interpollinated in a polycross nursery. Only moderately resistant plants should be selected to avoid any masking effect of major genes. Polycross seed could then be used to start several cycles of phenotypic recurrent selection for stem rust resistance and other traits.

Large numbers of plants should be used for selection and maintenance of family size during recurrent selection to avoid inbreeding depression. If the stem rust-resistant parent is an undesirable plant type, modified backcrossing could be used with several desirable plants as the recurrent parents. Backcrossing could also be used to incorporate major gene resistance by selecting for low rust reaction throughout an epidemic. Seed yield tests could also be included after a few cycles of selection to estimate inbreeding depression. The reaction patterns and the apparent diversity of different genetic sources of resistance observed in the parents used in this study indicate that this strategy would be effective in developing stem rust-resistant cultivars of perennial ryegrass.

Table 3. Contingency table comparing seven populations of susceptible F_1 seedlings and the subsequent mature plant response to stem rust[a]

	Adult plant stem rust ratings on 17 July[b]									
F_1s	1	2	3	4	5	6	7	8	9	Total
4A × 48A	1	0	0	3	0	2	0	0	0	6
4A × MP-2	7	4	4	3	1	1	2	1	0	23
4A × 77A	5	3	0	1	1	2	0	0	0	12
48A × 77A	3	0	0	1	0	3	0	0	0	7
77A × MP-2	3	0	1	1	0	1	0	0	0	6
Total	19	7	5	9	2	9	2	1	0	54

[a] $\chi^2 = 25.72$ (no significant differences at $P = 0.05$). Four 48A × 4A seedlings died before maturity, and none of the 48A × MP-2 seedlings were inoculated.

[b] Stem rust ratings at harvest on a scale of 1–9, where 1 = whole plant dead; 2 = stem and seed heads dead, a few green leaves; 3 = 70–85% of the stems, leaves, and seed heads infected; 4 = 50–70% of the stems, leaves, and seed heads infected; 5 = 30–50% of the stems, leaves, and seed heads infected; 6 = 10–30% of the stems, leaves, and seed heads infected; 7 = up to 10% of the stems, leaves, and seed heads infected; 8 = trace of foliar infection, one to three stems infected; and 9 = no rust.

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