Inheritance of Reaction of Orchardgrass to *Puccinia graminis* f. sp. *dactylidis*

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ABSTRACT

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The genetics of reaction of autotetraploid *Dactylis glomerata* to *Puccinia graminis* f. sp. *dactylidis* was studied on S_1 populations of 29 F_1 plants (clones) from a cross of two nearly self-incompatible parents. While segregation for reaction in each selfed population was observed and there was similarity in the segregation of the progeny from most of the clones in each of 2 yr, distinct autotetraploid ratios were not found. The reasons for this were not clear though environmental effects, linkage of self-incompatibility and reaction, aneuploidy, allelism, chromotid versus chromosome segregation, preferential pairing, and the possibility that segregation for reaction occurred at more than one locus are suggested as possible causes.

Additional key words: stem rust of orchardgrass.

Stem rust caused in orchardgrass (*Dactylis glomerata* L.) by *Puccinia graminis* Pers. f. sp. *dactylidis* Guyot et Massinot reduces herbage yield (4) and quality (2). Tajimi (16) concluded that size of the uredium was determined by gene dosage (incomplete dominance). Large elongated pustules developed on clones thought to lack alleles for low reaction, small circular pustules developed on clones with one allele, no pustules developed on clones with two or more alleles. Schubert's (14) observations indicated more variability in infection type than did Tajimi's (16). Since *D. glomerata* is an autotetraploid ($2n = 4x = 28$) (8,9,11) four alleles for low reaction are possible at one locus. These may all be the same allele or various combinations of two to four different alleles. Thus gene dosage and various allelic combinations could result in considerable variability in infection type. The objective of our study was to develop a better understanding of the inheritance of reaction in orchardgrass to *P. graminis* f. sp. *dactylidis*.

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MATERIALS AND METHODS

The culture of *P. graminis* f. sp. *dactylidis* used in this study. C Pga 1 was collected from orchardgrass in the fall of 1972 at the University of Missouri, Southwest Research Center near Mt. Vernon, MO. Inoculum was produced on clone 11 of orchardgrass in the greenhouse and stored in liquid nitrogen (5) until needed.

The orchardgrass clones used in the study were from the F_1 of a cross of clones 37 and 41 that were part of an ongoing plant breeding project at the University of Missouri-Columbia. Twenty-nine self-fertile F_1 clones were selfed in the field in the summers of 1978 and 1979 by placing glassine bags over the inflorescences just before anthesis. After maturity, the bagged heads were harvested and threshed, and the seed were planted in 10-cm-diameter clay pots in the greenhouse. Approximately 80 seedlings were transplanted at the three-leaf stage to individual peat pots. Survival was never 100%, thus the actual population sizes were usually less than 80 individuals. Six to 8 wk later, the seedlings, along with ramets from the parent plant were inoculated with C Pga 1.

Inoculation was by injecting a water suspension of uredospores into the leaf-sheath bundle of the tillers until some of the suspension was forced out of the top. After inoculation, the plants

TABLE 1. Segregation of the S_1 of five orchardgrass clones based on infection types on seedlings inoculated with *Puccinia graminis* f. sp. *dactylidis*, culture Pga 1, in 1978 and 1979 illustrating the various segregation patterns

Clone number	Infection type of parent	Number of seedlings of infection type:[a]										Average infection type	No. of clones with similar segregation
		0	1	2	3	4	5	6	7	8	9		
12-204	2[b]	1	11	5	1	9	3	2	12	9	9	5.23	5
	7[c]	0	2	17	9	7	8	4	8	2	12	4.78	
12-10	1	19	39	10	3	0	2	1	2	1	4	1.75	19
	1	6	37	6	5	3	1	0	0	0	0	1.40	
14-180	9	0	0	0	6	7	3	6	7	4	37	7.30	1
	9	0	0	4	13	7	9	11	5	5	21	6.00	
12-252	6	0	0	1	2	5	8	1	11	0	46	7.69	3
	7	0	2	15	16	6	3	1	6	0	1	3.52	
12-272	1	1	3	24	0	0	0	0	1	0	6	3.26	1
	2	0	17	37	11	1	3	0	1	0	3	2.42	

[a] 0 = flecks without uredia to 9 = large elongate uredia.
[b] 1978 data.
[c] 1979 data.

were grown at approximately 22 ± 3 C in the greenhouse.

Notes on infection type were taken on the lower leaf surface 2 wk after inoculation. The 0–4 scale, used for stem rust of wheat (15), was inadequate to rate stem rust of orchardgrass, as pustules developed in a range of sizes and shapes different from those of stem rust of wheat. An adaptation of the 0–9 scale of Browder (1) was used for rating the parental clones and the S_1 plants. Zero indicated a fleck reaction with no uredial development, 1 and 2 indicated uredial development where the uredia were small and circular in form, 2 being slightly larger than 1. Three through 9 indicated the increasing size of elongated uredia and decreasing chlorosis and necrosis. We found that classifying the same 50–100 infected plants twice in 1 day almost never resulted in variation of more than one interval on the 0–9 scale.

In preliminary studies, it appeared that a lower infection type (IT) developed on ramets from established plants than when the same plants were at the 6–7 leaf stage. Thus, 52 random seedlings were inoculated at the 6–7 leaf stage, the IT was recorded, and the seedlings were transplanted to the field in the spring. In the fall, ramets from these plants were established in the greenhouse and reinoculated approximately 7.5 mo after the first inoculation.

RESULTS

Table 1 shows the distribution of the S_1 progeny of five representative clones. The variability in reaction of the plants in each population was very wide. In some cases, there was at least one plant in each of the ten reaction classes. Nevertheless the pattern of variation of the S_1 populations of each clone in the 2 yr was similar for 25 of the 29 clones, indicating a degree of repeatibility.

Nineteen of the 29 S_1 populations segregated with most of the progeny at the low end of the scale in both years, five had progeny spread out over the scale, and one had most of the progeny at the high end of the scale. The progeny of one clone showed a bimodal segregation in 1978, but not in 1979; and three clones had progeny at the high end of the scale in 1978, but at the low end in 1979. No clone was homozygous for reaction.

An attempt was made to fit the data to classical autotetraploid ratios with little success. The best fit was obtained when seedlings with IT 0 to 4 were placed in a low-reaction class and those with IT 5 to 9 were placed in a high-reaction class. This classification of IT was also the best from the point of view of classical concepts of resistance and susceptibility in stem rust studies. In 1978, segregation of the progeny of eight clones fitted a 3:1 ratio, six a 35:1 ratio, and 15 did not fit any known ratio ($P = 0.1$). In 1979, segregation for reaction of eight clones fitted a 3:1 ratio, twelve a 35:1 ratio, and nine did not fit either ($P = 0.1$). Only 10 (34%) of the 29 clones displayed the same ratio in both years, and four did not fit either ratio in either year (Table 2).

The average IT of each S_1 population was calculated from the sum of plants in each class multiplied by the class number divided by the total number of plants. In most cases, the average IT for 1978 and 1979 was similar for each clone although there were several exceptions (Table 1). The average IT for S_1 progenies was also compared with that of ramets of the parent clone. Usually the latter were lower by two or more scale intervals. This was probably due to relative age of the plants as shown in another study. Of 52 plants inoculated at 6-wk of age and again at 9-mo, three were rated higher at 9 mo than at 6 wk, nine were given the same IT rating, 10 were lower by one interval on the rating scale, and 30 (59%) were rated lower by at least two scale intervals. The average IT of the 52 plants was 7.1 at 6 wk and 5.3 at 9 mo.

TABLE 2. Relationship between segregation ratios of S_1 progeny from orchardgrass clones selfed in 1978 and 1979 based on infection types when inoculated with *Puccinia graminis* f. sp. *dactylidis*, culture Pga 1

1979	1978			
	3:1	35:1	Other	Totals
3:1	2	0	6	8
35:1	3	4	5	12
Other	3	2	4	9
Totals	8	6	15	29

DISCUSSION

Reports on the inheritance of reaction of orchardgrass to the stem rust fungus are very limited. The data reported here do not show clear segregation patterns characteristic of autotetraploid inheritance. The absence of such patterns could be due to many factors including: inadequacy of the rating scale, effect of environment on IT, self-incompatibility in orchardgrass, random chromatid versus chromosome segregation, aneuploidy, allelism, preferential pairing, and the possibility that more than one locus was involved.

Before the work reported here was done, it was evident that the 0–4 scale used for stem rust of wheat could not be used for stem rust of orchardgrass. This problem was studied for 2 yr using several trial scales. The 0–9 scale was decided upon because it appeared to be repeatable. Nevertheless, it could be inadequate for genetic studies.

The work was done in a greenhouse over a period of time and in 2 different years. The environment involving light intensity and day length as well as minor temperature variations may have affected the phenotypic expression of IT.

Orchardgrass is normally a cross-pollinated species in the field and many clones are highly self-incompatible. The clones used in our study were selected because they appeared to be self-fertile and sufficient seed could be obtained from selfed plants for the studies. Nevertheless, there may have been genes for self-incompatibility segregating which reduced the seed-set potential. If such genes were linked with a gene for reaction this would cause abnormal segregation for reaction.

Mather (6) pointed out that quadrivalent formation and the proportion of the equational separation of the chromatids could

influence segregation patterns. Quadrivalent formation has been reported in orchardgrass (3,7,9,10,13) to be 3.0–3.9 per cell. While this is low, it could result in an increase in the number of plants with the higher ITs indicated by many of the data.

Aneuploidy also could result in skewed segregation. In a cytological study, Myers and Hill (12) found that of 116 random orchardgrass clones 41% (47 plants) were aneuploid. If the aneuploidy involved a chromosome(s) carrying a gene(s) for reaction, abnormal segregation would be expected.

Allelism could account for much of the variability observed. In an autotetraploid there could be as many as four different alleles for reaction at one locus. Each of these could result in slightly different phenotypes and be additive. Thus, different combinations of alleles, or different numbers of the same allele, could result in different phenotypes.

Although orchardgrass is considered to be an autotetraploid, it is possible that there is some preferential chromosome pairing. If this did occur for chromosomes carrying the loci for reaction, it could affect segregations and expected ratios would not be observed.

We found that the IT produced on ramets of the parent plant was usually one to two scale intervals lower than the average of the S_1 seedling population from that plant, indicating a tendency for high reaction to be partially dominant. However, we found that the IT on 52 random seedlings at 6 wk of age averaged two scale intervals higher than that of the ramets taken 7.5 mo later from the same plants after maturing in the field during the growing season. This may account for the difference in reaction between the parent clone and the average of its S_1 population.

If two loci were involved and there were two doses of a single allele for low reaction at each locus, the segregating population could have 25 different combinations of alleles for low and high reaction. If the alleles for low reaction consisted of three or four kinds, the number of combinations increases geometrically. If we also assume some additive effects, the observed variability in the material would be expected.

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