

Inheritance of Stem Rust Resistance in *Avena sativa* L.¹

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Five genes (A, B, D, E and F) for resistance to stem rust of oats caused by *Puccinia graminis* Pers. f. sp. *avenae* Erikss. and Henn. are widely recognized. McKenzie and Green (7) recently summarized the races and sub-races to which each gene confers resistance. Genes A, B and F appear to be closely linked or pseudo-alleles (1, 5, 6). A and D have been recombined with very little crossing over between them (5). Apparent recombinations between genes F and A have been found but true breeding lines have not been isolated (6). Genes B and E are inherited independently of each other and of the A-D-F complex. Unfortunately in most breeding materials genes A, D and F are linked in repulsion.

Irradiation has been utilized in the search for additional sources of resistance to stem rust. Frey and Browning (3) obtained 61 mutant strains after treating the Huron oat variety with X-ray. Konzak (4) reported a dominant mutant gene conferring resistance to race 7A of stem rust from treatment of the Mohawk oat variety with thermal neutrons. The danger of outcross contamination following irradiation has been pointed out (2).

The inheritance of two variant resistances obtained following treatment of the Clintland variety of oats with the chemical mutagen diepoxybutane are presented.

Materials and Methods

Clintland spring oats, with D resistance, were treated with 1:2, 3:4-diepoxybutane. One ml of aqueous solution of the mutagen was hypodermically injected into the culms near the developing panicle primordia at the premeiotic to early post-meiotic stages after the method of Murphy and Patterson (8). Four concentrations of the chemical, .005, .010, .050 and .100%, in water were used. All panicles were bagged to exclude contaminating pollen and the treated plants were isolated in a screen house on the Purdue campus with no other oat plants in the area.

The first and second selfed generations were grown in isolated disease nurseries with Clintland serving as the susceptible spreader for race 7 of stem rust to avoid pollen contamination. A heavy epidemic of barley yellow dwarf virus occurred in the first generation so all panicles producing seed were saved and utilized for growing the second generation. Four lines, tracing back to 2 of 239 original treated plants, were found to be homozygous for resistance to race 7 in the field in the second generation. For genetic studies the variants are referred to as 1 and 2a, 2b and 2c or when data are combined, as variant 1 and variant 2 based on origin.

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The variants were compared to varieties of known genetic resistance for seedling reactions in the greenhouse to races 6, 7, 7A and 8 of stem rust. Inheritance of resistance of the variants were studied following crosses of the variants with varieties with resistance genes A, B, D, and F. Seedling reactions of progenies were studied in the F_2 and F_3 in the greenhouse at an average temperature of about 75°F. Spores were transferred directly from stock pots by shaking. Seedlings were incubated overnight and reactions were determined on a 0 to 4 scale about 14 to 16 days after inoculation. For genetic studies reactions 0, 1 and 2 were considered resistant and 3 and 4 susceptible.

Results

Resistance of Variants

The variant lines were compared with differential varieties and parent lines for breadth of race protection as the first step in classifying the resistance in the variants (Table 1). The variants were similar to each other and were similar to Newton (gene A) across the 4 cultures

TABLE 1
Seedling reactions to stem rust of induced variants and varieties of oats.

Variety	Resistance type	Race			
		6	7	7A	8
Variant 1	?	4	1	1	4
Variant 2a	?	4	1	1	4
Variant 2b	?	4	1	1	4
Variant 2c	?	4	1	1	4
Newton	A	4	1+	1+	4
White Tartar	D	4	4	4	1+
Canuck	B	1	1	4	1
Clintland	D	4	4	4	
Clintland 60	BD		1—	4	
Cartier	None		4	4	
Mo. 0-205	A		1	1	
Goodfield	AB		1—		
Purdue 5650	ABD		1—		
Kherson 27	F		1		

which identify genes A, B and D. The variants exhibited a greater necrosis around the pustules than Newton, indicating a possible genetic difference, but not much more than Mo. 0-205 also carrying gene A.

Inheritance of Resistance in F_2

The F_2 seedling reactions were studied following crosses of the variants with varieties of known genotypes to determine the inheritance

of resistance in the variants (Table 2). In the crosses of variant 2 to the Clintland (D) variety from which it was derived, the resistance of variant 2 behaved as a single factor dominant to gene D in tests to races 7 and 7A.

Clintland 60 (BD) is resistant to race 7 and susceptible to race 7A. In the cross of variant 2 with Clintland 60, resistance of variant 2 to race 7 behaved as a simple dominant factor and the B resistance as a recessive, common at higher temperatures, suggesting a 13:3 ratio. In tests to race 7A, resistance behaved as a single dominant factor.

In crosses of variant 1 and variant 2 with Cartier (susceptible), resistance to race 7A appeared to be governed by a single dominant factor. No segregation occurred in crosses of variant 1 or variant 2 with varieties containing resistance A.

In crosses of variant 2 with Kherson 27 (F) all F_2 plants were resistant.

TABLE 2

Stem rust reactions of F_2 seedlings in crosses of variants 1 and 2.

Cross	Test race	Number of F_2 plants		P value for 3:1 ratio	
		Resistant	Susceptible		
Clintland (D)	}	7	68	24	.80-.90
X Variant 2		7A	63	17	.30-.50
Clintland 60 (BD)	}	7	86	15	.30-.50*
X Variant 2		7A	55	19	.80-.90
Cartier (Susc.)	}	7A	28	10	.80-.90
X Variant 1					
Cartier (Susc.)	}	7A	13	5	.70-.80
X Variant 2					
Mo. 0-205 (A)	}	7	14	0	
X Variant 1					
Goodfield (AB)	}	7	42	0	
X Variant 1					
Purdue 5650 (ABD)	}	7	54	0	
X Variant 2					
Kherson 27 (F)	}	7	51	0	
X Variant 2					

* P value for 13:3 ratio. Resistance of BB is recessive at higher temperatures.

These limited F_2 data suggested that variant 1 and variant 2 possess a single dominant factor for resistance to races 7 and 7A of stem rust. The resistance may be the same as gene A, an allele to A, or a pseudo-allele to genes A and F from changes at the D locus. Studies were carried to the F_3 families to examine these possibilities. The variants did not possess the D resistance of the parental variety from which they were derived, so some type of change at this locus is involved.

Inheritance of Resistance in F_3

Because of the small number of F_2 families available in crosses of variants 1 and 2 with Clintland (D) and Cartier (susceptible), within family analyses were used in F_3 (Table 3). In all cases the resistance of variant 1 and variant 2 behaved as a simple dominant in tests to race 7, supporting the F_2 analyses.

In the cross of Clintland 60 (BD) with variant 2, 65 families were resistant, 51 were segregating and 13 were susceptible suggesting a 7:8:1 ratio (P value of .02-.05). This interpretation is based on the independent inheritance of the single factor resistance in variant 2 from factor B and that the heterozygote Bb is susceptible at the higher greenhouse temperatures as has been previously observed.

In crosses of variant 1 and variant 2 with Purdue 5650 (ABD) no segregation for susceptibility was detected in 141 F_2 families with a total of 3578 F_2 seedlings when tested to race 7. This supported the interpretation of F_3 data that no segregation occurred when the variants were crossed to varieties possessing resistance type A.

TABLE 3
Segregation of F_3 oat seedlings within F_2 families for resistance to race 7 of stem rust.

Cross	No. of F_2 families	No. of F_3 seedlings		P value for 3:1 ratio
		Resistant	Susceptible	
Clintland (D) X Variant 1	8	206	68	.90-.95
Clintland (D) X Variant 2	23	325	110	.80-.90
Cartier (susc.) X Variant 1	1	6	3	.50-.70
Cartier (susc.) X Variant 2	13	258	79	.50-.70

The relation between gene F and the single factor resistance in the variants was studied in crosses of Kherson 27 (F) with variant 1 and variant 2 with F_3 seedling tests to race 7 (Table 4). Rare cross-overs apparently occurred between gene F and the single factor resistance of the variants. The crossover events resulting in susceptible plants are those which would be recovered in this test. One must interpret these type recombinants with caution since cytological abnormalities resulting in the loss of a critical chromosome segment would result in susceptible plants. A more critical test would be the isolation of recombinants in segregating families carrying both resistance factors. At the time of this research sub-races were not yet available to detect these recombinants.

Assuming that susceptible plants are true recombinants, the crossover event occurred in 3 of 334 gametes leading to the 167 F_2 plants for about 0.90% crossing over.

Assuming the resistance factor in the variants 0.9 crossover units from gene F, one can examine its relationship with genes A and D. Gene order of A, D and F has not been established. Koo *et al.* (5) estimated that A and D were closely linked in the coupling phase in C.I. 7098. McKenzie and Green (6) in an analysis in the repulsion phase

TABLE 4
Tests for allelism between resistances of variant 1 and variant 2 with gene F.

Cross	No. of F_2 families			No. of F_3 seedlings
	Res.	Seg.	Susc.	
Kherson 27 (F) X Variant 1	22	0	0	330
Kherson 27 (F) X Variant 2a	44	1*	0	1039
Kherson 27 (F) X Variant 2b	27	2**	0	616
Kherson 27 (F) X Variant 2c	71	0	0	1599

* One susceptible plant in 40.

** One susceptible plant in 15 in one family and 3 susceptible plants in 15 in the other.

estimated that A and D were about .15 units apart, in essential agreement with the previous report. A and F were estimated .06 units apart in the repulsion phase (6). These data on the linkage of the variant resistance with gene F indirectly support the data of McKenzie and Green (6) that F is closely linked with A and D.

Since the function of gene D was lost in the variants, the variant resistance logically is a mutant at the D locus. The alternative explanation is that mutations occurred at 2 loci, D to d and a to A'. In the F_3 tests of Purdue 5650 (ABD) X variants no crossovers were detected. The presence of gene B reduces the precision in detecting crossovers between A and A'. The detection of susceptible recombinants would have established A' at locus D.

The possibility that the resistance of the variants is type A without mutation cannot be completely examined because progeny of the original treated plants are not available. Other treated tillers of the two original plants producing variants did not produce seed in the first generation isolation nursery. The original plants would have needed to contain resistances AD or AB to be resistant to race 8 and to have mutated for D or B. Since the resistance of the variants was true breeding, outcrossing in the field disease nurseries can be excluded as a possibility.

The original treated plants were isolated and bagged excluding outcrossing as a possibility at this stage.

Intercrosses of variant 1 and variant 2 have not been made to establish the assumption that they are similar based on identical reaction to races of stem rust and similar breeding behavior.

Discussion

A major consideration is whether irradiation has produced a new source of resistance. Additional tests of variant 1 and variant 2 have been made by B. J. Roberts of the Cooperative Rust Laboratory, University of Minnesota. They were tested by numerous isolates of races 2, 5, 6A, 6F, 6AF, 7A, 8A, 8AF, 10A and 13A of oat stem rust in 1964-65 and found to react identically to Richland (A) and Andrew (A).

With genes A, D and F closely linked or pseudo-alleles genetic tests to prove that the variant resistance is a mutant at locus D are impractical. Analyses must be based on the occurrence of susceptible plants and cytological irregularities may confuse the interpretation.

With the resistance in the variants linked in repulsion with genes A, D and F, its utility in plant improvement is greatly reduced.

Summary

Two variant resistances were obtained following treatment of Clintland oats with the chemical mutagen diepoxybutane. The 2 variants appeared identical to gene A in resistance to various races. Genetic tests indicated that the resistance was closely linked to gene F in the complex A-D-F region.

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