Modification of Tolerance of Oats to Crown Rust by Mutation Induced with Ethyl Methanesulfonate

M. D. Simons

Research Plant Pathologist, Plant Science Research Division, ARS, USDA, Ames, Iowa 50010.

Cooperative Investigation of the Plant Science Research Division, ARS, USDA, and the Iowa Agriculture and Home Economics Experiment Station, Project No. 1752, Journal Paper No. J-6782.

Accepted for publication 2 April 1971.

ABSTRACT

Oat (*Avena sativa*) kernels were treated with ethyl methanesulfonate (EMS), and the crown rust (*Puccinia coronata* var. *avenae*) tolerance (as measured by yield and kernel weight) of lines derived from this material was compared with untreated controls. Variance of the tolerance of treated lines was significantly greater than the variance of the control. Heritability of tolerance of treated lines, estimated from analysis of 2 years' data, was relatively high. The over-all mean tolerance of treated lines was significantly lower than that of the control and, individually, many treated lines were less tolerant than the control. A few lines, however, were superior to the control in both yield and kernel weight response to infection. In the absence of crown rust, however, all "superior" lines yielded significantly less than the control. Phytopathology 61:1064-1067.

Additional key words: disease resistance.

The theoretical advantages of tolerance as opposed to resistance in oats (*Avena sativa* L.) to the crown rust fungus (*Puccinia coronata* Cda. var. *avenae* Fraser & Led.), were reviewed recently (13). The study reported herein was undertaken to determine whether tolerance to crown rust could be modified by treatment of the host with the mutagenic chemical, ethyl methanesulfonate (EMS).

There are many studies on the effects of mutagenic agents on quantitative characters of plants other than disease reaction (6, 8, 9), and the literature now contains numerous reports on the more or less successful induction of mutations for disease resistance (4). Several reports (1, 2) are concerned with increased resistance of oats to crown rust. Recently published reports include those of Murray (12), who irradiated stolons of peppermint (*Mentha piperita*) with neutrons and X-rays and obtained progenies resistant to *Verticillium albo-atrum*; of Tanaka (15), who irradiated rice (*Oryza sativa*) seed with gamma rays and obtained lines resistant to the rice blast fungus, and of Edwards et al. (3), who treated wheat (*Triticum aestivum*) with EMS and obtained seedling resistance to stem rust (*Puccinia graminis*).

Tolerance to crown rust is inherited as a quantitative trait with reasonably high heritability (13). Most studies on the artificial induction of disease resistance in plants have dealt with resistance that is inherited in simple Mendelian fashion. Some, however, might apply to resistance that is controlled polygenically, and one paper (5) briefly mentions increased field tolerance to crown rust found among oat populations derived from X-rayed material.

I chose the chemical EMS over X-rays and other forms of radiation for use in this study because it is generally believed that chemical mutagens are more effective than radiation in inducing point mutations. Chemicals also cause less gross chromosome damage than do any of the available forms of radiation. EMS seems superior (16, 17) to any of the other chemicals investigated intensively for inducing mutations in plants.

Materials and Methods.—About 2,000 kernels of the oat cultivar Richland, which is highly susceptible to damage from crown rust, were soaked 4 hr in 0.06 M EMS. The kernels then were rinsed thoroughly in running tap water, dried, and planted in a greenhouse isolated from other oats. The treated seed germinated and grew slowly, and showed a high degree of sterility in comparison with nontreated controls. The M_2 generation was grown in an isolated field where vigor and fertility of most plants were seemingly normal. Plants visibly different from the controls, with particular regard to maturity (14), were discarded in the M_2, M_3, M_4, and M_5 generations. The M_3 and M_4 were grown as single plants in the greenhouse. The M_5 was grown in the field in headrows. One hundred thirty M_6 and M_7 lines, each tracing to a separate single treated kernel and 20 "dummy" control lines, were then planted in replicated hill-plot tests in 1968 and 1969. Six M_8 lines selected for high crown rust tolerance and six for low were planted for confirmatory field testing in 1970.

Epiphytotics of crown rust were initiated artificially in the nurseries in 1968, '69, and '70 by hypodermic injection of one plant in each hill with a spore suspension of race 326. Data on yield, kernel weight (based on a sample of 200 kernels), and heading date were recorded.

Duplicate plantings were maintained free of rust with a fungicide to provide an estimate of differences among lines in the absence of rust. Responses to infection were expressed as ratios obtained by dividing the values from rusted plots by corresponding values from unrusted plots. This eliminated differences attributable to most causes other than infection (13).

In a parallel investigation, the oat cultivar Clintland-60 was handled in a manner essentially similar to Richland, except that the 130 M_7 lines were grown for only a single year.

Results.—*Richland.*—1) *Bulked data.*—Crown rust developed uniformly and heavily in all the Richland lines in 1968 and 1969. The severity of rust infection was such that the 20 nontreated Richland controls averaged only 57% in yield and 64% in kernel weight of their fungicide-protected counterparts.

The untreated Richland controls furnished an estimate of background variability that might be expected to exist in the treated lines. With this in mind, variances of the yield and kernel-weight ratios of the control lines were compared with corresponding variances of the treated lines. Variances of the treated lines for both characters were significantly greater (.01 probability level) than variances of the controls.

Values for treated lines seemed normally distributed about their mean for yield response (Fig. 1-A), with 67 lines below and 63 above the mean. In kernel-weight response (Fig. 1-B), however, 73 lines were below the mean, and 57 above. This skewness was significant at the .01 level.

The over-all means of treated lines for both yield and kernel weight responses were significantly lower (.001 probability level) than corresponding means of the controls. There were, however, appreciable numbers of treated lines that were above the control means. Of the 130 treated lines, 34 and 38 were above the control means for yield and kernel weight response, respectively, while 96 and 92 were below.

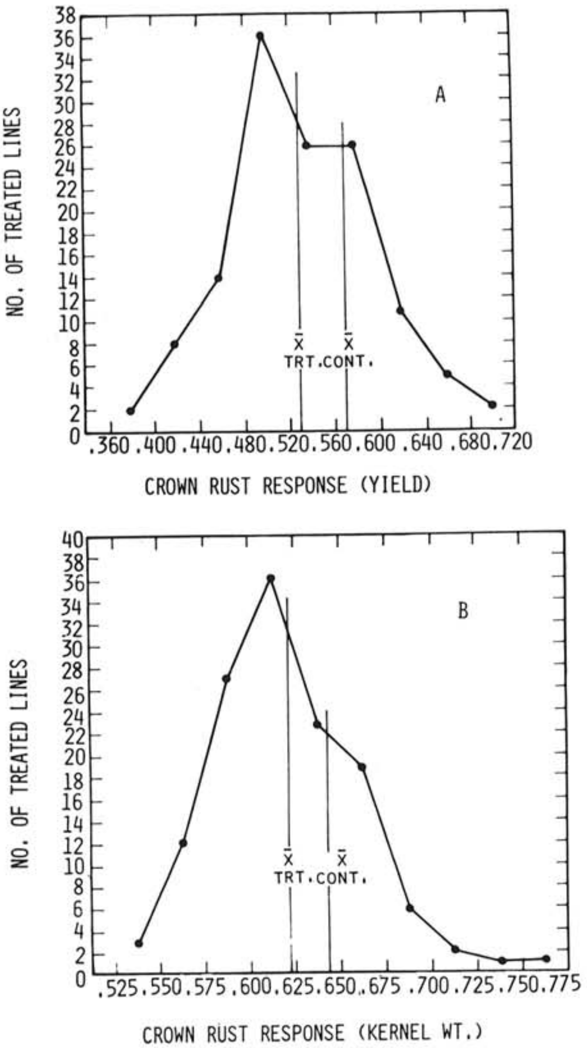

Fig. 1. Distribution of lines of EMS-derived Richland oats for **A)** yield response (ratio of rusted to unrusted yield); and **B)** kernel weight response to crown rust infection.

The year-to-year relationships of treated and control lines reflect the effect of EMS on crown rust tolerance. Correlation coefficients for yield and kernel weight indexes of treated lines between years were +.438 and +.495, respectively. These coefficients were both significant at the .01 level. Corresponding coefficients calculated for the 20 control lines were low (+.111 and −.041, respectively), and were not statistically significant.

Heritability values for yield and kernel weight indexes were calculated by the components-of-variance method (13) from analysis of variance combined over the 2 years. This calculation gave heritability estimates of 59 and 56% for yield and kernel weight indexes, respectively.

2) *Individual lines.*—Lines derived from EMS-treated kernels compared individually with the nontreated control, responded differentially to crown rust infection (Table 1). Ten treated lines were significantly less (.05 or .01, LSD test) affected by crown rust in yield, and 15 lines were similarly less affected in kernel weight. A much larger number of lines showed greater damage from rust infection than did the control. Thus 60 of the 130 treated lines were significantly less tolerant than the control as measured by yield response, and 72 were less tolerant in kernel-weight response.

3) *Performance of EMS-derived lines in the absence of rust.*—Yield of the Richland lines, in the absence of crown rust, was adversely affected by EMS treatment. Only eight of the 130 treated lines equaled the control; the rest were inferior. None of the eight highest-yielding lines showed significant improvement in rust tolerance over the control. On the other hand, six of the eight were rated as less tolerant than the control on the basis of either yield or kernel-weight response.

Thirteen EMS-treated lines significantly exceeded (.01 level) the control in kernel size in the absence of crown rust. One of these was superior to the control in crown rust tolerance as measured by kernel weight response, whereas six were inferior in yield and/or kernel-weight response. Fifty-five treated lines equaled the control in kernel size, and 62 had smaller kernels. Kernel size did not seem strongly related to crown rust tolerance.

Clintland-60.—Crown rust infection in the 130 EMS-treated lines of Clintland-60 was extremely heavy in 1968. Untreated controls in the rusted part of the nursery yielded only about one-fourth as much as their unrusted counterparts, and their kernel weight was only about half that of the controls. Experimental error was high, and, although the variance of the treated lines for yield response was larger than for the controls, it failed to reach significance at the .05 level. Variance for kernel-weight response of the

TABLE 1. Yield, kernel-weight, and heading-date[a] data for EMS-treated Richland lines that differed significantly (.01 level) from the control in both yield and kernel-weight response to crown rust infection

Line no.	Response (ratio)[b]		Unrusted (g)		Heading date (June)
	Yield	Kernel wt	Yield	Kernel wt	
51	.645**	.687**	23.2**	.0192**	19.0
53	.662**	.772**	16.7**	.0208	20.0
54	.638**	.673**	22.9**	.0204	20.0
89	.683**	.678**	20.1**	.0196**	19.7
144	.675**	.696**	21.8**	.0200**	19.5
Control	.573	.644	30.6	.0204	19.5
8	.436**	.558**	21.8**	.0186**	20.3
11	.507**	.598**	19.3**	.0134**	20.2
18	.429**	.558**	22.7**	.0186**	20.2
20	.503**	.550**	21.1**	.0191**	19.5
28	.406**	.568**	24.3**	.0203	20.5
32	.440**	.594**	26.2**	.0204	20.3
47	.443**	.584**	24.6**	.0206	20.2
69	.461**	.593**	20.9**	.0204	20.2
77	.475**	.611**	24.9**	.0196**	19.3

[a] Heading dates are within one standard deviation of the control mean.
[b] Ratios obtained by dividing values from rusted plots by corresponding values from unrusted plots.
** Indicates a difference from the control significant at the .01 level (LSD test).

treated lines, however, exceeded that of the controls beyond the .01 level.

As with the Richland material, mean yield and kernel weight ratios of the EMS-treated lines were significantly lower than corresponding ratios of the control. Individually, there were many lines significantly inferior to the control in crown rust response, and a few that were significantly better.

Confirmatory testing.—The 12 EMS-treated Richland lines selected for confirmatory field testing in 1970 had to be discarded because of adverse field conditions. The 12 treated Clintland-60 lines also suffered from the same conditions, but to a lesser extent, and, while experimental error was high, the data are of interest. Only one of the six lines that had been selected for high tolerance was significantly superior to the control in yield response. The mean yield response of these six lines did not differ significantly from that of the control. Only one of the six low lines was significantly inferior in yield response to the control, but the mean of the six was significantly below the control.

Data for seed-weight response, which were less affected by poor field conditions than were yield response data, showed a better correlation with previous year's testing. The mean of the six high lines was significantly higher, and the mean of the six low lines was significantly lower, than the control (.001 level). Individually, three of the high lines were significantly more tolerant than the control, and four of the low lines were less tolerant.

DISCUSSION.—Several lines of evidence from this study indicate that tolerance of oats to crown rust can be modified by EMS treatment. There was significantly greater variability in response to infection (as measured by variance) among the EMS-treated lines than among "dummy" lines of the nontreated control. The treated lines as a group were significantly more damaged by crown rust than was the control, indicating a real difference between the two. The treated lines showed a significant year-to-year correlation for rust response, and heritability values calculated on the basis of 2 year's data were relatively high. Certain individual, treated lines differed significantly from the control in response to crown rust infection.

This study poses certain questions regarding the use of EMS for creating cultivars of oats with a commercially valuable degree of rust tolerance. One is that all treated lines with improved rust tolerance had very low inherent yielding ability, suggesting a genetic linkage or other relationship between tolerance and low yield. However, only a very few of the treated lines were not low-yielding, and only a few had relatively high tolerance. Consequently, it seems quite possible that my failure to isolate lines combining these characters was simply due to my relatively small populations. Larger populations would presumably provide a larger number of high-yielding lines from which to select for tolerance.

Another question concerns the magnitude of the observed improvement in tolerance. Although this improvement was statistically significant, it was not as large, in absolute terms, as one might have hoped. Conceivably, use of larger populations or less severe epiphytotics might reveal lines with a greater degree of tolerance. Another approach would be to carry out recurrent cycles of EMS (or other mutagen) treatment and selection (7, 10, 11).

The preponderance of lines showing reduced tolerance has interesting theoretical implications. It is well known that mutagenic agents in general tend to reduce the vigor of plants; e.g., yield in the present study. However, damage due to crown rust ordinarily does not increase with reduced host vigor. On the contrary, a susceptible oat cultivar growing vigorously under optimum environmental conditions will suffer more damage from crown rust attack than it will under some environmental stress situation such as inadequate soil nutrients. Obviously, something other than the usual relationship of host vigor to rust damage is involved. This might be explained by assuming that there are numerous genes conditioning whatever degree of tolerance any given cultivar may have. Natural selection over a long period of time, and possibly artificial selection, has resulted in the accumulation of genes conditioning a degree of tolerance that is higher than would have been the case had there been no selection. Mutations of these genes, whether toward recessive or dominant alleles, would, on the average, be expected to be in the direction of lower tolerance, resulting in a majority of the lines with modified tolerance having less, rather than more, tolerance than the original cultivar.

LITERATURE CITED

1. Atkins, I. M., M. C. Futrell, Q. J. Raab, W. E. Lyles, P. E. Paw Lisch, G. W. Rivers, & M. J. Norris. 1964. Studies of the progenies of irradiated oats. Texas Agr. Exp. Sta. MP-742:1-12.
2. Chapman, W. H., H. H. Luke, A. T. Wallace, & A. O. Lunden. 1959. Multiple variation including crown rust resistance in irradiated Floriland oats. Agron. J. 51:163-165.
3. Edwards, L. H., N. D. Williams, F. G. Gough, & K. L. Lebsock. 1969. A chemically induced mutation for stem rust resistance in 'Little Club' wheat. Crop Sci. 9:838-839.
4. Favret, E. A. 1965. Induced mutations in breeding for disease resistance, p. 521-536. *In* The use of induced mutations in plant breeding. Suppl. to Radiat. Bot. Vol. 5.
5. Frey, K. J. 1954. Artificially induced mutations in oats. Agron. J. 46:49.
6. Frey, K. J. 1968. Induced variability in diploid and polyploid Avena sp., p. 41-56. *In* The present state of mutation breeding, Gamma Field Symp. No. 7, Ohmiya, Japan.
7. Gaul, H. P. K., E. Ulonska, C. Zum Winkel, & G. Braker. 1969. Micro-mutations influencing yield in barley. Studies over nine generations, p. 375-398. *In* Induced mutations in plants; Symp. Int. Atomic Energy Agency and Food Agr. Organ. U.N., Proc.
8. Gregory, W. C. 1966. Mutation breeding, p. 189-218. *In* K. J. Frey [ed.]. Plant breeding. Iowa State University Press, Ames, Iowa.
9. Gustafsson, A., & I. Gadd. 1965. Mutations and crop improvement. VI. The genus Avena L. (Graminae) Hereditas 53:327-373.
10. Joshi, S. N., & K. J. Frey. 1967. Genetic variability in oats from recurrent and alternate treatment with physical and chemical mutagens. Radiat. Bot. 7: 513-520.
11. Khadr, F. H., & K. J. Frey. 1965. Recurrent irradiation for oat breeding. Radiat. Bot. 5:391-402.
12. Murray, M. J. 1969. Successful use of irradiation breeding to obtain Verticillium-resistant strains of peppermint, Mentha piperita L., p. 345-371. *In* Induced mutations in plants; Symp. Int. Atomic Energy Agency and Food Agr. Organ. U.N., Proc.
13. Simons, M. D. 1969. Heritability of crown rust tolerance in oats. Phytopathology 59:1329-1333.
14. Simons, M. D., & L. J. Michel. 1968. Oat maturity and crown rust response. Crop Sci. 8:254-256.
15. Tanaka, S. 1969. Some useful mutations induced by gamma irradiation in rice, p. 517-527. *In* Induced mutations in plants; Symp. Int. Atomic Energy Agency and Food Agr. Organ. U.N., Proc.
16. Wellensiek, S. J. 1965. Comparison of the effects of EMS, neutrons, gamma- and X-rays on peas, p. 227-235. *In* The use of induced mutations in plant breeding, Suppl. to Radiat. Bot. Vol. 5.
17. Zannone, L. 1965. Effect of mutagenic agents in Vicia sativa L. Comparison between effects of ethyl methane sulphonate, ethylene imine and X-rays on induction of chlorophyll mutations, p. 205-213. *In* The use of induced mutations in plant breeding. Suppl. to Radiat. Bot. Vol. 5.