INHERITANCE OF RESISTANCE TO BLACKLEG (Leptosphaeria maculans) OF CANOLA

D.W. Sippell (1), W. McNabb (1), Robert Hall (2), J.Patel (1)

(1) Allelix Crop Technologies, 6850 Goreway Drive, Mississauga, Ontario, CANADA L4V 1P1

(2) Department of Environmental Biology, University of Guelph, Guelph, Ontario, CANADA N1G 2W1

INTRODUCTION

Blackleg or stem canker of canola (Brassica napus L.) occurs in most canola production regions of the world. The disease is potentially destructive where susceptible cultivars are grown in close rotation. Several sources of germplasm with resistance to Leptosphaeria maculans (Desm) Ces. et de Not. (Phoma lingam (Fr.) Tode) are available for the development of new resistant cultivars of spring canola.

To facilitate the breeding process, an understanding of the inheritance of disease resistance is important. Delwiche (1975) studying inheritance of resistance in seedlings, concluded that resistance was controlled by a single gene. Cargeeg and Thurling (1980) suggested that differences in resistance between host genotypes and in virulence between isolates were polygenically determined.

This study determines the inheritance of resistance to blackleg in 13 canola crosses involving parents of diverse origin with varying levels of disease resistance using generation mean analysis.

MATERIALS AND METHODS

Crosses were made between double haploids from eight B. napus spring canola lines used in breeding programs in Canada and Northern Europe. Crosses representing combinations of susceptibility and resistance were crossed to obtain F_1 hybrids. Appropriate selfs and crosses were made to obtain the F_2 and the back crosses (BC_1, BC_2). These six generations were grown in the greenhouse at Allelix Research Farm, Georgetown, Ontario. Because of the large number of crosses and the number of growing seasons necessary to obtain seed of all crosses, and because of limited availability of greenhouse space, families were split and grown in two experiments. All crosses are referred to by their code number throughout this paper (Table 1).

In the greenhouse, families were planted in 18 cm. peat pots. Eight to 60 plants depending on the generation, were grown in a completely randomized design with three replicates.

Table 1. Canola families used to study the inheritance of resistance to Leptosphaeria maculans in 1989 and 1990 in the greenhouse at Allelix Research Farm, Georgetown, Ontario.

Code	1989	Code	1990
89/1	Karat(MR)* X Regent(S)	90/1	Global(MR) X Marnoo(MR)
89/2	AO087(MS) X Karat(MR)	90/2	Global(MR) X Karat(MR)
89/3	Topas(MS) X Karat(MR)	90/3	Marnoo(MR) X AO087(MS)
89/4	AO087(MS) X Westar(VS)	90/4	Global(MR) X AO087(MS)
89/5	Line(MR) X Topas(MS	90/5	Marnoo(MR) X Karat(MR)
89/6	Westar(VS) X Topas(MS)	90/6	AO087(MS) X Karat (MR)
89/7	Regent(S) X Westar(VS)		

*VS-very susceptible, S-susceptible, MS-moderately susceptible MR-moderately resistant.

An isolate of the aggressive strain of L. maculans (LM26) collected from canola stubble at Souris, Manitoba was selected because it caused the most severe disease symptoms on a number of cultivars in previous tests. Furthermore, it produced pycnidia abundantly in culture and it reproduced consistently on artificial media. The isolate was maintained and increased on potato dextrose agar for inoculation purposes.

Five week old plants were inoculated by injecting 10 ul of pycnidiospores (10^7 spores/ml) in crowns with a sterile disposable hypodermic syringe attached to a Eppendorf repeater pipette. Plants were incubated at 18-20° C for 7 weeks. Length of lesions was measured with a Manostat Caliper. This method has previously been shown to be correlated to field disease ratings. (Sippell, personal communication).

Analysis of variance was performed on log transformed data to identify differences among and within crosses. F-tests were used to determine presence of statistical significance. Individual T-tests were computed to compare generation means within crosses.

A generation mean analysis was done for each of the crosses to estimate additive, dominance and epistatic effects, following Mather & Jinks (1971) model:

$$Y = m + a_1d + a_2h + a_3i + a_4j + a_5l$$

in which Y is the mean of the generation, m is the mean of all possible homozygous lines at F_∞, [d] is the pooled additive gene effects, [h] is the pooled dominance gene effects, [i] is the pooled additive x additive epistatic gene effects, [j] is the pooled additive x dominance epistatic gene effects, [l] is the pooled dominance x dominance epistatic gene effects and a_1 - a_5 are the respective coefficients of the equation (Table 2).

Table 2: Coefficient of terms in the equation of estimation for generation means in terms of additive [d], dominance [h], additive x additive [i], additive X dominance [j], and dominance x dominance [l] genetic effects in the model $Y= m + a_1d + a_2h + a_3i + a_4j + a_5l$

Y	m	[d]	[h]	[i]	[j]	[l]
P_1	1	1	0	1	0	0
P_2	1	-1	0	1	0	0
F_1	1	0	1	0	0	1
F_2	1	0	1/2	0	0	1/4
B_1	1	1/2	1/2	1/4	1/4	1/4
B_2	1	-1/2	1/2	1/4	-1/4	1/4

To determine the fit of the additive dominance model, a scaling test (Mather, 1946) and a joint scaling test (Cavalli, 1952) were performed. Perfect fit solutions (Jinks & Jones, 1958) for estimates of [i], [j] and [l] were calculated not only to detect the effects of the non-allelic interaction, but also to calculate their magnitude.

Broad-sense heritability was calculated:

$$h_b^2 = (V_A^2 + V_B^2)/ V_{F2} \times 100$$

where V_A^2 represents the additive genetic variance, V_B^2 represents the dominance genetic variances and V_{F2} represents the variance of F_2.

The number of effective factors (K_1) was calculated as K_1 = (phenotypic range in the F_2)^2/8(V_{F2} - V_{F2}) in which the V_{F2} is the environmental variance obtained by pooling the within plot variance of the P_1, P_2 and F_1 generations.

RESULTS

Stem lesion lengths between families in 1989 and 1990 were significantly different. Within family generation means were also significant except in 89/1, 89/2 and 89/5. These families were not analysed.

The generation means for stem lesion length in the 10 families are presented in Table 3. In all cases, disease ranking of the more resistant double haploid parent was lower than that the corresponding susceptible parent. In seven of these crosses (89/4, 89/7, 90/2, 90/3, 90/4, 90/5 and 90/6) parental means were significantly different.

The results of the scaling tests A, B, C are represented in Table 4. The statistical significance of these scaling tests indicated failure of the simple additive-dominance model in the progeny genotype in all families except 89/6, 90/3 and 90/6.

Table 4. Scaling test for inheritance of blackleg resistance based on generation means for the detection of epistasis.

	89/1	89/4	89/6	89/7	90/1	90/2	90/3	90/4	90/5	90/6
A	**	NS	NS	**	NS	NS	NS	NS	**	NS
B	NS	**	NS	NS	NS	NS	NS	NS	**	NS
C	NS	NS	NS	NS	**	**	NS	**	NS	NS

** - Significant at the 0.01 level of probability
NS - Not significantly different

The joint scaling test confirmed that a simple additive dominance model was generally inadequate to explain inheritance. The only family that fit the additive dominance model was 90/6. (X^2 = 4.758 P = 0.20 - 0.10)

Results of the perfect fit solution are found in Table 5. Degrees of freedom are not available to test significance of fit of this model.

Table 5. Estimates of the additive, dominance and interaction parameters for inheritance of blackleg resistance to canola fitted to six generation means.

	89/1	89/4	89/6	89/7
m	96.4±15.1**	8.8±22.4**	27.0±17.7**	91.46±20.1**
[d]	1.90±4.0	13.5±2.9**	5.8±4.1	9.6±3.6**
[h]	-95.3±38.8**	219.7±58.2**	123.9±48.7**	-39.5±53.1
[i]	-44.9±14.6**	50.3±22.2**	39.8±17.2**	-28.4±19.8
[j]	-20.5±12.5	-52.1±16.9	21.0±16.2**	-43.4±16.4**
[l]	69.9±27.3**	-146.1±39.7	-65.3±34.8**	-48.7±36.1
X^2	-	-	-	-
P	-	-	-	-

Table 5. (continued)

	90/1	90/2	90/3	90/4
m	73.5±8.7**	95.5±8.9**	50.8±9.6**	3.2±7.1**
[d]	0.7±2.5	-5.8±1.2**	-9.9±2.2**	-10.1±3.2**
[h]	-96.8±22.0**	-141.6±21.6**	-55.6±24.7**	46.6±20.5**
[i]	-45.6±8.3**	-65.9±8.8**	-22.0±9.3**	28.1±6.3**
[j]	-8.3±7.3	-0.0±5.5	11.3±6.6**	7.2±8.6
[l]	48.9±15.0**	81.9±15.3**	48.3±23.8**	-34.5±14.**
X^2	-	-	-	-
P	-	-	-	-

Table 5. (continued)

	90/5	90/6
m	100.8±16.3**	86.3±20.3**
[d]	-4.8±1.79**	11.9±5.7**
[h]	-192.9±36.5**	-87.9±52.4
[i]	-73.7 2**	-
[j]	-4.7±6.8	-
[l]	128.2±26.6	-
X^2	-	4.75
P	-	0.1 - 0.2

** Significant at the 0.01 level of probability.

Secondary statistics were calculated (Table 6).

Table 6. Estimates of broad-base heritability (percentages) and number of effective factors for blackleg reaction on five canola populations.

Family	Broad-sense heritability	Effective factors
89/4	56.8	4.0
89/7	46.2	6.6
90/1	34.8	9.3
90/2	57.8	2.9
90/5	55.7	5.2

In all families but one, a positive deviation of the F1 means from the midparent value was found. In five families (89/1, 89/4, 89/6, 89/7, and 90/6) there was a statistically significant difference. Significant negative deviation was found in one family (90/4).

DISCUSSION

Susceptibility to L. maculans was predominantly due to dominance genetic effects in all but two families studied. Resistance was primarily recessive. Additive genetic effects, while significant in all but three families studied, accounted for a smaller portion of the total genetic variation. However, inheritance does not follow the simple additive-dominance model except in one family. Epistatic effects play an important role in blackleg resistance. The estimates of dominance and of dominance x dominance interaction were predominantly of opposite signs (in all but three families) so interactions are mainly of the duplicate, dominant

epistatic or recessive suppressor kind.

In three crosses that included Westar as a parent, susceptibility was always dominant. All crosses are not predictable however. On crosses with Global, resistance was primarily dominant in one case, but not in another.

Secondary statistics must be interpreted with caution. Epistatic effects will affect estimates. Broad-sense heritability is not high in the greenhouse. It is expected that this would be lower in the field. The number of effective factors confirms that disease resistance is polygenic.

Canola breeders must be cautious when breeding for disease resistance. When duplicate epistasis occurs, prediction of resistance in the cross can not be made based on inbred line resistance. As a population moves towards homozygosity, care must be taken not to lose resistance in the population. If heterozygous parents are used in hybrid production, the progeny will segregate for susceptibility and resistance. Selection for disease resistance in both sides of the heterotic blocks, especially in later generations increases the chances of developing hybrids with high blackleg resistance. Specific combining ability trials for improved blackleg resistance may be required because of epistatic effects.

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