Inheritance of Resistance to Phomopsis (*Diaporthe helianthi*) in Sunflowers

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Abstract

The inheritance of resistance of sunflowers to phomopsis (*Diaporthe helianthi*) was studied on 10 inbred lines representing a range of resistance and susceptibility levels, and 25 hybrids from a factorial cross of these lines. Observations of natural attack (presence of stem lesions greater than 5cm) over 3 years indicated that additive gene control was predominant, with few interactions between parental effects. Correlations between attack on parental lines and on the mean of their hybrids were not significant, certain inbreds which appeared quite susceptible giving hybrids with good levels of resistance. It appears necessary to determine the general combining abilities of parental lines to predict hybrid values. Some inbreds gave very high levels of resistance, others gave very high levels of susceptibility. A test measuring the rate of extension of *D.helianthi* mycelium on leaves was significantly correlated with results of natural attacks. In particular, it gave good distinction of the most susceptible genotypes, and thus may be used in first generations of breeding to eliminate the most susceptible plants.

Introduction

Diaporthe helianthi, causing the disease known as phomopsis, was first identified on sunflowers in ex-Yugoslavia in 1981 (Mihaljcevic *et al.*, 1982) and in France in 1984 (Lamarque and Perny, 1985). Genotypes showing resistance were first identified in breeding nurseries subjected to high levels of infection (Skoric, 1985). In the first studies of the heredity of resistance, this author indicated that a small number of genes were involved, whereas Vranceanu *et al.* (1992) reported that resistance was under additive control.

In France, first observations indicated that all the commercialised hybrids could be classed as "susceptible", compared with Yugoslav or Rumanian hybrids classed as resistant (Regnault and Perny, 1987). However, in following years, when a greater number of observations were available, it became evident that a continuous range of resistance of reaction exists among cultivated sunflowers, from extremely susceptible to highly resistant (Carre, 1993).

Thus, in order to develop efficient resistance breeding programmes and to be able to predict the level of resistance of hybrids, studies of the genetics of phomopsis resistance appeared necessary. A first study was made on hybrids from a factorial cross between five female and five male lines showing a range of phomopsis reactions. Resistance was determined by observations of natural or semi-natural attack, which have be shown to give satisfactory distinction between genotypes (Tourvieille, 1994). In addition, a test measuring resistance to mycelium progression on leaves was studied since this could permit selection either in areas without natural phomopsis attack or of individual plants.

Materials and methods

Sunflower genotypes : Five inbred lines, maintainers of CMS and five restorers (Table 1) and the 25 hybrids from a factorial cross between these lines. PRS9 and DPS3 came from the same cross. Select

is a Rumanian hybrid, Condor and Helios are Novi-Sad hybrids. Controls for the semi-natural attacks were Viki (susceptible) and Agrisol (resistant) and for the mycelium test, Agrisol and and a susceptible experimental hybrid 74F.

Table 1. Origins of the inbred sunflower lines used in a factorial cross to study reaction to phomopsis.

Code	Origin	Breeder
Females		
2603	Moroccan population	INRA
XK	Peredovik*_H.tuberosus_* hyb.	INRA
QL	VNIIMK 8931	INRA
XRQ	HA89*Progress	INRA
IF2	provided by Prograin Génétique	
Males		
RHA274	wild _H.annuus_*Peredovik	USDA
PRS9	INRA line (PRS7)*Select	INRA
DPC2	INRA line (PW3)*Condor	INRA
DPS3	INRA line (PRS7)*Select	INRA
DPH1	Helios	INRA

Fungal isolate : In the field infection was by the natural *D.helianthi* population. For the mycelium tests, the isolate PH1, isolated in 1990 in west France, was used in 1993 and PH2, isolated in 1993 in central France, in 1995.

Semi-natural attack : The hybrids and inbreds were sown in randomized block trials with 2 replications and plots of about 50 plants, at INRA, Toulouse, the inbreds in 1992, 1993 and 1994, the hybrids in 1993, 1994 and 1995. The methodology used was that of Tourvieille (1994), based on the provision of infected sunflower stems carrying *D.helianthi* perithecia, placed regularly in the trial plots, and irrigation by sprinklers during the susceptible phase, to homogenize conditions of attack as far as possible, whatever the earliness of the material. The two controls were sown at 3 dates in order to judge attack levels on plants with different flowering dates. Observations were made, 2 to 4 weeks after flowering, of the numbers of plants showing disease lesions at least 5cm long on the main stem. Results are given as percentages or as a disease index calculated by dividing the percentage attack of a plot by the mean attack on the two controls with the same flowering date.

Mycelium test on leaves : The method of Bertrand and Tourvieille (1987) was used, with infection by mycelial explants placed, at the star bud stage, on the tips of two fully grown leaves of ten plants per plot, with 2 replications. The plants were irrigated to avoid drying of the explants . Observations of the length of lesions along the main vein were made 18 to 21 days after infection. Several infection dates were used to cover the range of earliness and infect all plants at the same stage. Resistant and susceptible controls, with staggered sowing dates were infected on each occasion and results are presented as an index, i.e. tho ratio of the mean length of lesion of a plant to the mean length of controls infected on the same day.

Statistical analyses : One-way and factorial analyses of variance were made to determine genotype, parental and interaction effects.

Results

Semi-natural attack :

Hybrids : Since the observations for the 3 years were significantly correlated (1993-1994 r = 0.667**, 1994-1995 r = 0.740**, 1993-1995 r = 0.766**), the 3 years mean percentage attack and disease index for each hybrid are given in Table 2. These results show a continuous range of attack from 5.7% to 90.5%. Analyses of variance on the disease index showed significant genotype and parental effects. The distinction between male parents was greater than that between female parents (Fm = 41.34, Ff = 9.45). The inbred line DPH1 gave the most resistant hybrids, whereas RHA274 and PRS9 were parents of the most susceptible. The other two restorers, DPC2 and DPS3 were significantly different from the extremes. Of the females, IF2 gave the highest levels of resistance and 2603 the highest levels of susceptibility among the intermediates, XRQ was not different from IF2 and XK and QL could be grouped with 2603.

There were no significant female*male interactions, indicating that general combining ability effects were large and that specific combining ability was not important.

Table 2. Reaction of sunflower hybrids to semi-natural attack by phomopsis : mean percentages (in italics) and disease indices over 3 years (1993-1995).

M / F	2603	XK	QL	XRQ	IF2	x
RHA274	*90.5*	*85.9*	*84.6*	*74.9*	*67.1*	*80.8*
	1.48	1.48	1.32	1.17	1.02	1.29
PRS9	*91.3*	*76.6*	*78.1*	*61.5*	*58.4*	*73.2*
	1.48	1.40	1.23	1.06	1.06	1.25
DPC2	*74.3*	*72.4*	*65.9*	*55.2*	*35.8*	*60.7*
	1.23	1.22	1.10	0.87	0.47	0.98
DPS3	*63.7*	*47.8*	*50.5*	*31.8*	*26.8*	*44.1*
	1.04	0.86	0.76	0.57	0.69	0.78
DPH1	*37.4*	*21.4*	*5.7*	*12.0*	*6.2*	*16.5*
	0.57	0.36	0.08	0.19	0.10	0.26
x	*71.4*	*60.8*	*57.0*	*47.1*	*38.9*	*55.0*
	1.16	1.06	0.90	0.77	0.67	0.91

Analysis of variance on indices

F genotype : 8.85**
F females : 9.45**
F males : 41.34**
F interaction : 0.57 ns

lsd(5%) hybrids : 0.40
lsd(5%) F or M : 0.18

c.v. : 26.92

Inbred lines : Observations were made in 1992, 1993 and 1994, but while results for 1993 and 1994 were significantly correlated, the first year's observations were not correlated with the others. This was because DPH1 was noted as 90% infected whereas in 1993 and 1994, this line showed 0% infection, but with considerable drying of the stem which may have been confused with phomopsis attack the first year. The mean percentage attacks and disease indices given in Table 3 are therefore means of only 1993 and 1994. XRQ was the female line least attacked while 2603 and IF2 showed the most symptoms. Other than DPH1, DPC2 was the most resistant restorer and RHA274 the most susceptible.

Table 3. Reaction of sunflower inbred lines to phomopsis attack : mean of 2 years observation of semi-natural attack (percentage in italics and disease index) and results of mycelium test (lesion length indices).

	Semi-natural attack		Mycelium test (index)		
	%	Index	1993	1995	Mean
Females					
2603	*87.0*	1.33	1.87	1.55	1.71
XK	*57.3*	1.04	0.96	1.34	1.15
QL	*74.7*	1.15	0.99	1.40	1.20
XRQ	*22.7*	0.40	1.15	1.25	1.20
IF2	*81.2*	1.22	0.68	1.04	0.86
Males					
RHA274	*100.0*	1.58	1.38	2.08	1.73
PRS9	*96.0*	1.46	2.20	1.58	1.89
DPC2	*53.2*	0.63	1.03	0.73	0.88
DPS3	*51.2*	0.76	0.92	0.77	0.85
DPH1	*15.8*	0.08	2.11	1.16	1.64

Correlations between inbred lines and hybrids : The correlation between results of female inbred lines and the means of their hybrids was r = 0.399, not significant, in particular because of the behaviour of IF2, susceptible as an inbred, but giving resistant hybrids. Between male parents and their hybrids, the correlation was r = 0.942, highly significant, although the line DPC2 showed relatively better resistance as an inbred compared with the values for its hybrids.

Tests of resistance to mycelium growth on leaves :

The control varieties showed mean lengths of lesion of 2.4cm (Agrisol, resistant) and 4.6cm (74F, susceptible). For the hybrids, the results of the two years of tests, 1993 and 1995 were significantly correlated (r = 0.493*), so the values presented in Table 4 are means of the two years. Although genotype and parental effects are significant, only the effects of the most susceptible parents, RHA274, PRS9 and 2603 could be distinguished.

In contrast with the hybrids, the 1993 and 1995 values for the inbred lines were not correlated (r = 0.423), so the results of each year are presented in addition to the mean (Table 3). As for the observations of natural attack, the variation came mainly from the apparent behaviour of DPH1, which was observed to have large lesions only in 1993. RHA274, PRS9 and 2603 always gave large lesions.

Table 4. Reaction of sunflower hybrids to a phomopsis mycelium test on leaves : mean lesion length indices over two years (1993,1995).

M / F	2603	XK	QL	XRQ	IF2	x
RHA274	1.85	1.41	1.34	1.29	1.15	**1.41**
PRS9	2.36	1.93	2.07	1.61	1.56	**1.91**
DPC2	1.68	0.95	1.14	0.94	0.91	**1.12**
DPS3	1.30	1.26	1.09	1.28	0.97	**1.18**
DPH1	1.41	0.88	0.78	0.80	1.07	**0.99**
x	**1.72**	**1.29**	**1.28**	**1.18**	**1.13**	**1.32**

Analysis of variance

F genotype	: 2.54*	lsd(5%) hybrids	: 0.74
F females	: 4.03*	F or M	: 0.33
F males	: 9.65*		
F interaction	: 0.39 ns	c.v.	: 27.31

The correlation between the results of the females and the mean of their hybrids was r = 0.944** and for the males it was r = 0.880* (excluding DPH1).

Correlation between semi-natural attack and the mycelium leaf test:

For the hybrids this was r = 0.731 (HS), for the male inbreds excluding DPH1, it was r = 0.967** but for the females the correlation was not significant (r = 0.196). It may be noted that the correlations between the mycelium test on inbred lines and the means of their hybrids under semi-natural attack were significant (females : r = 0.816*, males (except DPH1) : r : 0.931*).

Discussion

Observations of natural attack by *D.helianthi* are repeatable and can therefore be used to determine genotype reaction to this pathogen. The lines used here showed resistance under additive control, with no significant interactions, indicating that the behaviour of particular hybrids is predictable from the general combining abilities of the parents obtained from a few test crosses. This material, although showing a range of reactions to phomopsis, does not cover all the known sources of resistance, so further studies are necessary to determine whether the present conclusions can be generalized for cultivated sunflowers. It is still possible that major genes for resistance do exist (for example, introduced from wild species), but, in this case, the additive genes effects will need to be

taken into consideration in studies of segregation of crosses with moderately susceptible cultivated sunflower lines.

The lack of significant correlation between the reaction of female inbred lines and the means of their hybrids resulted from the reactions of some of them, such as IF2, which appears susceptible *per se*, but gives resistant hybrids, and XRQ, which showed better resistance as an inbred than that transmitted to its hybrids. Thus, although observations of inbred lines (or early generations of breeding programmes) will generally be valid in the selection of resistant hybrids, some measures of general combining ability are necessary to forecast the behaviour of hybrids with security.

The difficulty of correctly observing DPH1 as an inbred line, both under semi-natural attack and for the mycelium test, because of its tendency to drying symptoms similar to those of phomopsis, whereas its hybrids all showed easily observable resistance, may be linked to its type of resistance, but is a further reason for observing experimental hybrids.

The mycelium test on leaves was significantly correlated with observations of semi-natural attack for the hybrids and the male inbreds (excluding DPH1), so it can be used to test for resistance. It may also be noted that this observation of a part of the overall phomopsis reaction of a genotype gave as good or better correlations between inbred line results and the means of their hybrids under semi-natural attack reaction as those of observations of inbred lines themselves under semi-natural attack. However, with this test, intermediate lines cannot be significanlty distinguished from more resistant genotypes, so its best use would be to eliminate highly susceptible plants in early breeding generations, before observations of F3 or F4 families, or of experimental hybrids under semi-natural attack.

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