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GENETICAL STUDIES OF RESISTANCE TO DOWNY MILDEW (*PLASMOPARA HELIANTHI* NOVOT.) IN SUNFLOWERS

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SUMMARY

Studies were made to determine which downy mildew resistance genes (PI) are active against *Plasmopara helianthi* races present in France.

Crosses were made between either RHA266 (PI1) or RHA274 (PI2), resistant in France only to race 1 of downy mildew, and lines resistant to all known races (HA335, HA338 and HAR5). F2 and test-cross progenies were tested with race 1 and isolates A and B (close to races 4 and 3). No segregation was found with race 1. With isolates A and B, the progenies showed segregation agreeing with the presence of one gene active against these races.

Two hypotheses which could explain these results are compared : firstly that sunflower lines resistant to several races contain several resistant genes each active against only one race, secondly that resistance genes may be active against many races and are either allelic or closely linked.

INTRODUCTION

The resistance of sunflowers to downy mildew (*Plasmopara helianthi*) is conditioned by dominant, major genes noted "PI" These genes give functionally complete resistance, although some symptoms may occur during tests of young plants (VEAR, 1978).

Seven downy mildew races have been identified (GULYA et al., 1991). Three are present in France : race 1 (European race), isolate A and isolate B (TOURVIEILLE et al, 1991). At present, ten resistance genes, noted PI1 to PI10, have been proposed (GULYA et al, 1991). All these genes are considered as different, although certain have the same reactions to the different races : PI2 and PI4 are effective against races 1 and 2, PI6, PI7 and PI8 are effective against all known races.

This paper reports comparison of a number of different inbred lines resistant to one or more of the French downy mildew races, in order to determine the relations between the resistance genes they contain.

MATERIALS AND METHODS

Sunflower genotypes : The lines used are listed in Table 1, which also gives their reaction to the French downy mildew races. They are all maintained at INRA, Clermont-Ferrand by selfing under paper bags. Crosses were made between the lines and the resulting F1 hybrids were either selfed to obtain F2 progenies or crossed onto a susceptible line to obtain a test-cross progeny.

Table 1 : REACTION TO FRENCH DOWNY MILDEW RACES OF SUNFLOWER GENOTYPES USED FOR GENETIC STUDIES

Genotype	Pl genes	P.helianthi isolate 1	A	B
RHA266	Pl1	R	S	S
RHA274	Pl2	R	S	S
HIR34	Pl4	R	S	S
PUS2	Pl2	R	S	/
HA335	Pl6+?	R	R	R
HA338	Pl7+?	R	R	R
HAR5	Pl?	R	R	R

R : resistant S : susceptible / : not tested

Downy mildew races : Race 1, isolate A and isolate B. Sunflower differentials do not distinguish isolate A from race 4 and isolate B from race 3 (TOURVIEILLE et al., 1991). All 3 races are maintained in separate growth chambers, race 1 on a completely susceptible genotype, the other two on hybrids resistant only to race 1.

Resistance test : Young seedlings are infected following the technique described by COHEN and SACKSTON (1973).

Seeds are soaked in water for 5h. They are then put to germinate in Petri dishes containing wet filter paper for 48h in daylight. At this stage, they generally show radicles 5 to 20mm in length. Any non-germinated seeds are discarded. A suspension of downy mildew zoosporangia is obtained by washing the cotyledons of susceptible seedlings which show sporulation. A concentration of about 30-50 000 zoosporangia/ml is obtained. The germinated seeds are soaked twice in this suspension for 5h with a 24h interval, at 18°C. They are then planted in boxes of soilless compost and maintained at 18°C, 60-70% humidity and a 16h light period with an intensity of about 200μE/m^2/s. A row of a susceptible control is planted in each box to check that the infection is successful.

After 12 days, the boxes are covered with polythene bags to create a saturated atmosphere and induce downy mildew sporulation. After 48h, observations are made. A plant is considered as **susceptible** (noted S) if sporulation is evident on **cotyledons** and **true leaves**. When there is **no sporulation** on the shoot, or a **sparse sporulation on the cotyledons only and not on the true leaves**, the plant is noted as **resistant** (R).

RESULTS

The results of the downy mildew resistance tests on F2 and test-cross progenies are given in Table 2. In all cases, at least 2 tests were made, and after a chi-square homogeneity test the results were summed.

TABLE II : RESULTS OF DOWNY MILDEW TESTS ON SOME PROGENIES OF CROSSES BETWEEN RESISTANT GENOTYPES

CROSSES	Isolate*	R	S	Ratio tested	X^2
S x (HIR34 x RHA274)F1	1	168	0	3R : 1S	56.0
(HA335 x RHA266)F2	1	163	0	15R : 1S	10.9
S x (HA335 x RHA266)F1	1	84	0	3R : 1S	28.0
	B	36	28	1R : 1S	1.0
(HA338 x RHA266)F2	1	146	0	15R : 1S	9.7
S x (HA338 x RHA266)F1	1	47	0	3R : 1S	15.6
	B	37	43	1R : 1S	0.5
(HA338 x RHA274)F2	1	153	0	15R : 1S	10.2
	B	87	23	3R : 1S	1.0
S x (HA335 x RHA274)F1	1	92	0	3R : 1S	30.6
	A	221	107	1R : 1S	39.6
S x (HAR5 x PUS2)F1	1	99	0	3R : 1S	33.0
	A	41	38	1R : 1S	0.1

X^2 limits (1 df) - $P < 0.05$ $X^2 = 3.84$
- $P < 0.01$ $X^2 = 6.63$

S = Susceptible, R = Resistant

* : 1, A and B isolates detailed in Materials

No segregation appeared for any of the tests with race 1. The lines RHA274 and HIR34 having no resistance to isolates A and B, this combination was tested only with race 1. In all the other combinations of lines, one was resistant to isolates A and B and test-cross or F2 progenies were therefore tested with a second race. In all cases segregation appeared, confirming that the resistant lines, always females, had indeed been crossed by the second parent.

The test-crosses from the combinations (HA335 * RHA266) and (HA338 * RHA266), with isolate B, gave segregations which do not differ from 1R : 1S, indicating the presence of 1 gene (PI6 from HA335 and PI7 from HA338). The F2 progeny from the cross (HA338 * RHA274), with isolate B, gave a segregation of 3R : 1S, also indicating 1 gene (PI7 from HA338). In contrast, the test-cross of (HA335 * RHA274), tested with isolate A, showed an excess of resistant plants (221R : 107S), where theoretically, one would expect 50% R : 50% S. The testcross of (HAR5 * PUS2), tested with isolate A gave a segregation agreeing with the hypothesis of 1 gene (PI? in HAR5).

DISCUSSION

The absence of any segregation in the F2 or test-cross progenies tested with race 1 is particularly striking. The lines HA335 and HA338 had previously been shown to have only one PI gene active against race 1 (MOUZEYAR et al, 1991) and PI1 (RHA266) and PI2 (RHA274) (VRANCEANU et al., 1981) and PI6 and PI7 (MILLER and GULYA, 1991) have been shown to be independant.

RHA274 * HIR34 : RHA274 and HIR34 are resistant to the same races and the result could indicate the presence of the same gene in the 2 lines, as suggested by Sackston (1981). The absence of segregation could also be explained by PI2 and PI4 being alleles at the same locus or tightly linked.

HA335 * RHA266 and HA338 * RHA266 : Since HA335 and HA338 are resistant to all known races, they must have genes functionally different from PI1 in RHA266. The absence of segregation in the progenies tested with race 1 could suggest that they contain also PI1 and that PI6 and PI7 are not active against race 1. The alternative hypothesis is that HA335 and HA338 each contain only 1 gene, active against all races, and that PI1 and PI6 and PI1 and PI7 are allelic.

HA335 * RHA274 and HA338 * RHA274 : The unexpected segregation of the test-cross progeny of (HA335 * RHA274) may be compared with some other results. In some F2 progenies with susceptible lines , RHA274 has given segregations closer to 15 : 1 than 3 : 1 (data not shown) and G.M. GARCIA (personal communication) has indicated that he also found excess resistant plants in progenies with RHA274 and has postulated the presence of 2 complementary genes conferring resistance to races 2 and 7. This could be the explaination for unexpected segregation in this progeny.

With this exception, the results are the same as for the combinations with RHA266 discussed above. To explain the absence of segregation, HA335 and HA338 could contain PI2 in addition to PI6 or PI7. This would appear unlikely since it is suggested in the paragraph above that they contain PI1 and yet against race 1, they only have 1 active resistance gene. It may also be suggested that PI2 is allelic with PI6 and PI7, active against all races, and therefore also with PI1. This is entirely contrary to the results showing independance of PI1 and PI2 and of PI6 and PI7.

HAR5 * PUS2 : Since HAR5, containing an unknown PI gene, is resistant to all races of downy mildew, it is comparable with HA335 and HA338. PUS2, containing PI2 is similar to RHA274. The cross is thus comparable with that above and the results are exactly the same. The gene in HAR5 must either be allelic with PI2 or it must be inactive against race 1 and HAR5 contain PI2.

It is difficult to make any clear conclusion from these results. Two hypotheses remain possible : Either each resistance gene is active against only one race, and the sunflower lines resistant to several races contain several resistance genes; alternatively most of the resistance genes are alleles at a single locus. To test these ideas, it would be necessary to obtain sunflower lines that are resistant, for example only to isolate A or isolate B and not to race 1 or race 2, or to have genetic, perhaps molecular, markers whose distance from resistance genes can be measured by recombination frequencies.

CONCLUSION

In conclusion, the inheritance of resistance to downy mildew in sunflowers is not as simple as it first appears. Work remains neccessary to clarify the situation. It may be suggested that some of the discrepancies between results come from the fact that the resistance provided by different PI genes in different genetic backgrounds varies in its completeness. When the presence of a few zoosporangia are taken to indicate susceptibility, the result will certainly be different from that if plants with some sporulation on cotyledons are counted as resistant. It may not only be a question of interpretation. There may be conditions, or genotypes in which there is a tendancy for resistance genes to permit considerable sporulation on cotyledons, or for some minor genes to inhibit sporulation on seedlings containing no PI genes.

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