IMPROVING THE SUNFLOWER DOWNY MILDEW RESISTANCE TEST

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Abstract

The resistance of new sunflower varieties to downy mildew races present in France is determined when they are registered in the official French variety catalogue. With the seedling test, it is difficult to define the resistance of some varieties that show sporulation on cotyledons. A two-year programme between eight public and private laboratories showed that inoculum concentration, radicle length and relative humidity of growth chambers during incubation cause differences in sporulation but that variations in soaking time and light quality have no effects on disease symptoms. However, no modification of methodology was found to simplify determination of resistance/susceptibility of genotypes with cotyledon-limited sporulation in seedling tests.

Résumé

Deux années de collaboration entre 8 laboratoires privés et publics ont permis de fiabilisé le protocole officiel d'évaluation de la résistance du tournesol au mildiou *(Plasmopara halstedii)* dans le cadre du Comité Technique Permanent de la Sélection (organisme d'inscription des nouvelles variétés sur le catalogue français). Cette étude a mis en évidence quelques facteurs influençant l'expression des symptômes ce qui a permis de proposer quelques modifications au protocole. Cependant, aucune modification, même

majeur, ne permet de faciliter de façon significative la lecture du comportement de génotypes possédant des gènes majeurs de résistance efficace en culture mais présentant des sporulations en test de laboratoire.

Introduction

Some of the sunflower varieties proposed for registration in the French catalogue carry downy mildew (*Plasmopara halstedii* [Farl.] Berl. and de Toni) resistance genes which show cotyledon-limited sporulation in seedling resistance tests (Vear, 1978; Sackston, 1990 and Mouzeyar et al., 1993). This makes definition of resistance more difficult than in the case of varieties carrying genes which show no sporulation at all. The amount of sporulation may vary and causes problems of differentiation from susceptible varieties which show sporulation on cotyledons and leaves. A two-year programme involving 8 laboratories aimed to determine the effects of different test conditions on seedling reactions and, if possible, to define an improved methodology for the downy mildew resistance test.

Materials and Methods

Parasite Isolate. The race 710 of *Plasmopara halstedii* was used.

Sunflower Genotypes. Eight genotypes (seven hybrids and one open-pollinated variety, Peredovik) were chosen from knowledge of their downy mildew reactions in the field and in seedling tests. G1: reaction difficult to define; G2 (Peredovik) and G6: susceptible checks; G3 and G8: resistant checks showing no sporulation; G4, G5 and G7: hybrids showing sporulation or hypersensitive reactions in seedling tests.

The field resistance of these genotypes was checked in the field in 2003 at INRA, Clermont-Ferrand, where race 710 was present, with irrigation to favour infection. Observations were made each week from 2 weeks after sowing. After 6 weeks (end of May), percentage infection was stabilised with: G6: 73%, G2: 60%, G1: 53%, G5; 9%, G4: 5%, G7: 4%, G3: 2%, G8: 0%. This confirmed the resistance reactions of G4, G5 and G7.

Seedling Tests. The trials were made by eight laboratories in 2002 (CETIOM, INRA, GEVES, Maïsadour, Monsanto, Panam, Pioneer, and RAGT) and seven in 2003. All these laboratories had official recognition for work on a quarantined parasite.

The basic test consisted of infecting germinated sunflower seed with a suspension of zoosporangia collected on seedlings which had sporulated for not more than 48 h in 100% relative humidity. The concentration was 100,000 zoosporangia/ml. The germinated seeds were soaked for 4h in the suspension before being pricked out in trays of soilless compost and placed in growth chambers. They were maintained for 12 days at 18C under 12 h lights (12 000 lux) per day followed by 48 h under 100% relative humidity to favour sporulation on the shoot.

In the first year of trials, biotic (inoculum) and abiotic factors (light, temperature, and relative humidity) were varied to determine those which gave the best differentiation of resistance and susceptibility. Each factor was studied by two laboratories. One seed production for each variety served for all the trials. In the second year, the factors of inoculum concentration, duration of soaking and compost type were studied in more detail. The seven laboratories studied the same factors on the same eight genotypes. Inoculum

concentrations were 100,000 sporangia/ml and 10,000 sporangia/ml. Soaking time was 1h and 4h. Three types of compost were used.

Symptom Classes. All the laboratories used the same classes of symptoms: (1) no sporulation on cotyledons or leaves, (2) weak sporulation on cotyledons and a hypersensitive reaction, (3) weak sporulation on cotyledons with no hypersensitivity, (4) strong sporulation on vigorous plants, (5) strong sporulation on weak plants, (6) sporulation on cotyledons and leaves (7) not observable (damping off). The results were calculated as a percentage of plants showing sporulation which was the sum of classes (2) to (6) divided by the sum of classes (1) to (6), multiplied by 100. The results presented are the means of the two (2002) or seven (2003) laboratories studying the factor.

Results

Seed Germination. The results (Table 1) show that the effect of radicle length on infection varied according to genotype The genotype G4 (resistant in the field) showed much less sporulation when infections were made on radicles at least 2cm long.

Table 1. Percentage of plants showing sporulation according to radicle length (2002).

Length(cm)	0.2	0.5	1.0	2.0
G1	94.5	95.3	91.3	80.0
G2	93.0	93.0	95.7	92.0
G3	0.0	0.0	0.0	0.0
G4	81.0	85.3	83.7	29.0

Water Used for Zoosporangia Suspensions. Tap water contains a low concentration of chloride but this had no negative effect on downy mildew zoosporangia. A pH of 8 had a negative effect on zoosporangia germination.

Inoculum Concentration. The results in 2002 (Table 2) showed that below 10,000 sporangia/ml the infection level dropped, but that for the susceptible control G2, there was no difference between 10,000 and 100,000 sporangia/ml. In 2003, the susceptible genotypes showed different levels of infection between laboratories, but G6 was more stable than G2. The highest and most stable infections were obtained (in 6 of 10 tests) with G2 and a concentration of 10,000 sporangia/ml. This did not agree with the 2002 results and the difficulties encountered in some of the trials made the concentration of 100,000 sporangia/ml appear preferable.

Table 2. Percentage of plants showing sporulation according to inoculum concentration in 2002.

Concentration sp/ml	1,000	10,000	100,000	1,000,000
G1	52.3	88.5	94.4	95.3
G2	33.1	63.6	83.0	77.4
G3	0.0	0.0	0.3	0.3
G4	16.9	46.6	57.7	71.3

Soaking Time. Table 3 shows that percentage of sporulation did not vary according to the duration of the period (from 1 to 8 h) of soaking of germinated seed in the zoosporangia suspension, in agreement with Meliala (2001) who reported that 95% of zoosporangia liberate their zoospores in the first 20 min after preparation of a suspension. However, in 2003, when 1h and 4h soaking were compared, the susceptible genotypes G2 and G6 showed the highest levels of infection after the 4h treatment (7 of 10 trials).

Table 3. Percentage of plants showing sporulation according to soaking time (in hours) (2002).

Time	1	2	3	4	5	6	7	8
G1	97.5	96.5	98.0	94.5	99.5	95.0	91.5	93.0
G2	99.0	99.5	99.5	100.0	100.0	97.0	99.0	98.5
G3	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
G4	81.0	79.0	71.0	76.5	78.0	81.5	82.0	85.0

Inoculum Conservation in a Refrigerator. Conservation of sporulated cotyledons for up to 72h in a refrigerator (4C = in a closed recipient between several sheets of dry blotting paper had no negative effect on infections at a concentration of 1,000,000 sporangia/ml (Table 4).

Table 4. Percentage of plants showing sporulation according to time of maintenance of inoculum in a refrigerator (in hours).

Hours	0	24	48	72
G1	99.7	99.2	99.8	99.2
G2	99.5	99.2	99.4	100.0
G3	0.0	0.0	0.0	0.0
G4	95.8	96.7	92.8	92.3

Effect of Compost Type. The compost generally used by each laboratory was tested by all the participants. The results showed that best results were obtained in each trial with the compost used by that laboratory. However, it was found that substrates such as vermiculite do not appear adapted to this resistance test. Some substrate types showed phytotoxic effects.

Light Quality. Light colour did not have any significant effect on sporulation, in particular for G4, the resistant genotype showing cotyledon-limited sporulation (Table 5).

Table 5. Percentage of plants showing sporulation according to light colour.

	OSRAML 58w/60	OSRAML 58w/62	OSRAML 58w/67	PHILIPS 58w/865	PHILIPS 58w/840
Light colour	Red	Yellow	Blue	White-Red	White-Blue
G1	96.3	100.0	96.4	98.0	97.9
G2	98.2	100.0	99.0	97.9	95.5
G3	0.0	0.0	0.0	0.0	0.0
G4	89.6	88.5	96.2	89.7	91.1

Photoperiod and Day/Night Changes in Temperature. A photoperiod of 15h instead of 12h and day/night changes in temperature (14/25C) instead of a constant 18C had no significant effect on the percentage of plants showing sporulation.

Relative Humidity. Reduction of relative humidity only during the light period had no effect on sporulation whereas a continuously dry atmosphere reduced the percentage of G4 plants showing sporulation from 95% to 27%. However, this modification cannot be recommended because the susceptible check G2 also showed only 59% of plants with sporulation.

Conditions Giving the Least Sporulation on the Percentage of G4 Plants Showing Downy Mildew Symptoms. Following the results presented above, a methodology with these conditions was experimented.

Table 6. Percentage of plants showing sporulation using a methodology combining conditions favouring absence of sporulation.

	Genotypes	
Factors	**G2 (susceptible)**	**G4 (resistant in the field)**
Radicle length 2cm	92%	29%
PH 8	100%	27%
Zoosporangia concentration 10,000 sporangia/ml	85%	60%
Substrate	With the best compost G2 shows much sporulation	With the best compost G4 shows little sporulation
Yellow light	100%	87%
Photoperiod 15h	99.5%	97.5%
Temperature alternation 14/25C	97.5%	94.0%
Constant 40% relative humidity	59%	27%

Table 7. General effects of the combination of factors giving least sporulation.

Genotypes	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
% sporulation	100.0	97.5	0.0	96.3	88.8	95.0	26.6	0.0

This methodology applied on the eight genotypes tested in 2003 did not make it possible to eliminate sporulation of the genotypes resistant in the field (G4, G5 and G7) in seedling tests.

Discussion

The two years of trials led to the establishment of a seedling test methodology which gives stable results concerning the reaction of sunflower hybrids to downy mildew. This method permits the definition, with certainty, of 90% of the hybrids tested in official trials but it has not resolved the problem of genotypes which present cotyledon-limited sporulation. In 2004, for official definition of resistance/susceptibility of the (at present) 10% of difficult varieties, a committee of experts will discuss the results to try to come to a satisfactory conclusion. In the longer term, it will be necessary to develop different tests (for example on older plants, or in the field) to permit full use in breeding of the diverse sources of resistance genes which are increasingly used in breeding programmes.

Conclusions

Downy Mildew Seedling Test Method Proposed. **Inoculum preparation:** Inoculum should be collected after 48h at 100% relative humidity from sporulating cotyledons and used directly or kept in a refrigerator for up to 72h (in a closed contained between dry paper). The zoosporangia suspension should have a concentration of 100,000 zoosporangia/ml. **Infection:** Germinated sunflower seed, with radicles of at least 0.5 cm should be soaked for 4 h in the zoosporangia suspension and then pricked out in trays containing compost, placed in a growth chamber. **Culture Conditions:** The plants should be maintained for 12 days at 18C, under 12 000 lux, with 12h light per day and 80% relative humidity. After this, 100% relative humidity should be maintained for 48h to encourage downy mildew sporulation on the shoot. **Observation Criteria:** Plants are considered as susceptible if sporulation is visible on both cotyledons and leaves, when the latter are well developed, or if there is heavy sporulation on the cotyledons and the leaves show little growth.

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