TSY1*02

Expression of resistance in the *Plasmopara halstedii* - sunflower pathosystem

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Abstract

In greenhouse experiments, involving six *Plasmopara halstedii* (PH) races and ten sunflower cultivars/inbred lines, a number of compatible and incompatible host-pathogen interactions have been compared and the disease reaction assessed based on any visible symptoms both above and below ground.

With susceptibility, the host reaction was uniform regardless of the pathogen race and sunflower cultivar involved. In incompatible interactions, however, the host appeared to react differently to different PH races, the reaction-type depending primarily on host genotype rather than on pathogen virulence. Besides total resistance (immune reaction), two additional, incomplete resistances did occur whereby the extent of fungal colonization within affected host tissues was the most characteristic feature often associated with reduced plant height and/or chlorotic/necrotic symptoms of cotyledon leaves.

Key words: downy mildew, sunflower, incomplete resistance.

Introduction

Downy mildew of sunflower, caused by *Plasmopara halstedii* (Farlow) Berlese et de Toni (PH) is a destructive disease of world-wide distribution. The fungal pathogen is soil-born with long-lived oospores. Attacked sunflowers become systemically infected resulting in various symptoms as severe stunting, leaf chlorosis, and no or poor seed-set. Mildewed sunflower will not be able to recover so that control measures such as the use of resistant

PH has long been thought to be uniform in its pathogenicity. However, it was in the early 1970s that a new variant of the fungus with altered virulence appeared in the USA and subsequently additional strains each with distinct virulence pattern have arised. By now nine pathogenic races have been identified and others are being examined in America and in Europe (Gulya et al., 1991; Virányi and Gulya, 1995). The co-evolution of PH and sunflower is shown in Table 1. With such changes in virulence of PH, a re-evaluation of the categories susceptibility/resistance appeared to be become essential with more knowledge on resistance expression related to pathogenic races and host cultivars.

Resistance to PH in sunflower is generally considered to be controlled by one or several dominant gene(s), designated as Pl genes, and expressed by the lack of fungal sporulation in inoculated sunflower seedlings. However, French (Vear, 1978) and Hungarian (Virányi, 1978) researchers showed that some sunflower genotypes carrying effective Pl genes supported fungal sporulation on the cotyledons of a number of sunflower seedlings in glasshouse tests. This was re-examined and confirmed by scientists in America (Ljubich and Gulya, 1988; Ljubich, 1989) and the phenomenon called cotyledon-limited infection (CLI) indicating that PH could colonize resistant sunflower seedlings to a limited extent. In addition, resistance to PH in sunflower was also found to be expressed in hypersensitive reactions of host tissues accompanied with (Virányi, 1980) or without (Wehtje et al., 1979) fungal colonization. Recently, Mouzeyar et al. (1993) reported that both susceptible and resistant sunflowers examined were invaded by the downy mildew pathogen but in the latter case fungal growth was strongly inhibited (PH mycelium did not reach the cotyledonary node) and was in any case associated with hypersensitive necrosis of affected host tissues.

Aim of our study was (i) to investigate resistance in detail by comparing as many pathogen race-host genotype combinations as possible, (ii) to characterize pathogen development and host response in these interactions, and (iii) to distinguish between resistance responses under our standardized experimental conditions.

Materials and methods

The pathogen. PH isolates of various geographical origin, representing known pathogenic races designated as races 1, 2, 3, 4, 8 and 9, each with distinct virulence character have been used throughout this study. The isolates (at least two of each race) were preserved at

ultra low temperature (Virányi, 1985) and increased on a universal suscept (sunflower cultivar with no known Pl gene) prior to test.

The host. Ten sunflower genotypes including an open pollinated cultivar (GK-70), two hybrids (IS-003 and IS-2000) and seven public lines (RHA-265, RHA-274, RHA-340, HA-335, HIR-34, DM-2, and 803-1) with or without Pl gene(s) were selected so that both compatible and incompatible interactions were available in reasonable numbers (Table 2).

Experimental conditions. Pre-germinated sunflower seeds (a total of 20 to 25 of each cultivar) were WSI-inoculated (Cohen and Sackston, 1973), i.e. immersed in a suspension of 20 000-30 000 PH sporangia/ml for 3-4 hrs at 16-18°C, planted either in pots or in flats (propagating vessels) filled with a standard soil mixture and grown in the greenhouse at 22 ±4°C for 12-14 days with a 16 hr photoperiod.

Evaluation. Disease assessment was based on records for the appearance of visual symptoms as fungal sporulation, leaf chlorosis (and/or necrosis) and stunting, or for internal development of the fungus in otherwise symptomless plants. For sporulation, the seedlings were transferred overnight in a humid chamber at 18°C. Pathogen development was checked by using microscopical observation of either intact plants or tissue sections.

Results

Experiments carried out in 1993 and in 1994 showed that in compatible pathogen-host combinations (a universal suscept cultivar inoculated with any pathogenic race or a resistant cultivar inoculated with a race virulent to that cultivar) host reaction was uniform regardless of the pathogen race and sunflower cultivar involved (Table 3). In incompatible interactions, however, the host was found to react differently to different PH races and the reaction-type obtained depended primarily on cultivar rather than on pathogen virulence. For example, inoculations with either race 1, race 2 or race 3 of PH resulted in 100 % incompatibility (immune reaction) on the cultivars DM-2, IS-2000, 803-1 and HA-335, whereas cvs. RHA-265, RHA-274, HIR-34 and RHA-340 with race 1, cvs. RHA-274, HIR-34 and RHA-340 with race 2, and cv. RHA-340 with race 3 did exhibit intermediate reactions: hypocotyl-limited hyphal growth (HLI) with some reduction in plant height and root development. Similar results have been obtained with races 4, 8 and 9 when inoculated onto their respective incompatible partners. except for cvs. IS-2000 and 803-1 inoculated with race 8 where cotyledon-limited

infection (CLI) occurred, or for HA-335 that was found to be totally free from any mycelium of any race (Table 3).

Experiments in 1995, involving inoculations of seven sunflower cultivars with isolates of PH races 1, 3 and 9, respectively, have been focussed on the occurrence of HLI in incompatible combinations, such as in the cvs. RHA-265, RHA-274, and RHA-340. The results obtained were in a good accordance with those of previous experiments except that in some cases sunflower seedlings of the cultivars RHA-265 or RHA-340 showed CLI reaction to race 1.

Discussion

Based on our findings explained here it can be concluded that resistance to PH in sunflower is rather complex and its expression diverse. Apart from the pure susceptibility, three distinct forms of resistance do exist in this pathosystem: there is a higly resistant or immune reaction with no symptoms and no fungal invasion at all into the host. In addition, there are two intermediate forms each characterized by the extension of fungal colonization within sunflower plants, either as hypocotyl-limited or as cotyledon-limited infection, and by the associated host response as reduced plant height and/or chlorosis/necrosis of affected cotyledon leaves.

Our results are in accordance with those of earlier investigations (Vear, 1978; Virányi, 1978; Virányi and Bartha, 1981; Ljubich and Gulya, 1988), whereby incomplete resistance to race 1 (Vear, 1978; Virányi, 1978; Virányi and Bartha, 1981) or to races 2, 3 and 4 (Ljubich and Gulya, 1988) of PH were equally found in some sunflower genotypes. Recently, Mouzeyard et al. (1993) reported in detail of the histology of downy mildew resistance in sunflower. Their observations are similar to ours except that they did not find any immune reaction among the pathogen - host combinations examined. They concluded that resistance was associated with hypersensitive reaction (HR) in resistant sunflowers and that the extent of fungal development in such plants depended on the time and intensity of HR appearance. The possible reason of contradiction between the results of the French group and of ours might be the limited number of sunflower genotypes (one susceptible and two resistant) and the only race 1 used by the French as compared to 10 cultivars and 6 races in our case.

Although sunflowers with incomplete resistance are considered as resistant in the

these lines will conserve the risk of downy mildew infection for years by keeping and/or increasing soil inoculum level, or by inducing the appearance of new pathogenic forms of PH. Therefore, unless providing multiple-race resistance, successful breeding programmes we believe should involve sunflower lines with immune-type reaction to the majority if not all of the relevant pathogen races.

Acknowledgement

The authors are grateful to the U.S. - Hungarian Joint Fund for financial support of this study under JFNo. 188/91.

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Table 1. *Plasmopara halstedii* race evolution over the years 1972-1993 (Virányi and Gulya, 1995)

Race	First appearance		Distribution
	Year	Location	
1	?	?	worldwide
2	1972	USA	North America
3	1980	USA	Americas, Europe
4	1985	USA	North America, Europe
5	1988	USA	North America
6	1990	Canada	North America, France
7	1990	Argentina	Americas
8	1990	USA	USA, Hungary
9	1993	USA, Hungary	USA, Hungary

Table 2. Known reactions of sunflower cultivars to *Plasmopara halstedii* races used in this study

Sunflower cultivar	Reaction to race					
	1	2	3	4	8	9
GK-70	+	+	+	+	+	+
IS-003	+	+	+	+	+	+
RHA-265	-	+	+	+	+	+
RHA-274	-	-	+	+	+	-
HIR-34	-	-	+	+	+	-
DM-2	-	-	-	+	+	+
IS-2000	-	-	-	+	-	+
803-1	-	-	-	-	-	-

Table 3. Infection-type found to occur as predominant with different compatible/incomaptible interactions between sunflower and *Plasmopara halstedii*.

Sunflower	*P. halstedii* race					
	1	2	3	4	8	9
GK-70	S	S	S	S	S	S
IS-003	S	S	S	S	S	S
RHA-265	HLI	S	S	S	S	S
RHA-274	HLI	HLI	S	S	S	HLI
HIR-34	HLI	HLI	S	S	S	HLI
DM-2	R	R	R	S	S	S
IS-2000	R	R	R	S	CLI	S
803-1	R	R	R	HLI	CLI	HLI
RHA-340	HLI	HLI	HLI	HLI	HLI	HLI
HA-335	R	R	R	R	R	R

Explanation: S = susceptible, R = totally resistant (immune), HLI = hypocotyl-limited, and CLI = cotyledon-limited infection.