ATTEMPTED INDUCTION OF CROSS PROTECTION AGAINST VIRULENT RACES OF PLASMOPARA HALSTEDII

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SUMMARY

Sunflower lines carrying no known genes for resistance to downy mildew (DM) (Plasmopara halstedii (Farl.) Berl. and de Toni), or carrying the Pl_1 or Pl_2 gene, were inoculated by the whole seedling immersion method (WSI) with DM race 1 (the "inciting" inoculation) or in water (the controls). Zoosporangial concentrations used were 1,000, 30,000, or 100,000 zoosporangia per mL. Inoculated seedlings were placed between moist filter papers in petri dishes at 15C for 4 to 5 hours. Part of the seedlings of each host line : pathogen concentration combination were then immersed in suspensions of 1,000 or 30,000 zoosporangia of DM race 2 or 3, or in water, at 15C for 5 hours (the "challenge" inoculation). They were then planted in a synthetic soil mix (Promix) in 10 cm pots. In other experiments seedlings were planted directly in Promix after the inciting inoculation with DM 1 with 30,000 zoosporangia per mL or with water. Five days later they were given a challenge inoculation by soil drench with DM 2 or 3 with 30,000 zoosporangia per mL. After planting all pots were kept in controlled environment cabinets maintained at 22/20C with a light/dark period of 16/8 hours. After 12 to 17 days the plants were covered with plastic bags for 24 hours to provide a saturated atmosphere to induce sporulation. No sporulation developed on control plants immersed only in water, or on plants with gene Pl_2 inoculated only with DM 1 or DM 2. Sporulation was profuse on plants with Pl_1 inoculated with DM 2 or DM 3 and on plants with Pl_2 inoculated with DM 3. There was no apparent protection against a challenge inoculation in a compatible combination by a prior inciting inoculation in an incompatible combination, regardless of the spore concentration or method of inoculation used.

RESUME

Des lignées de tournesol ne possédant aucun gène connu de résistance au mildiou (Plasmopara halstedii) et des lignées possédant les gènes Pl_1 ou Pl_2 ont été inoculées à l'aide de la méthode d'immersion (WSI) des plantules avec la race 1 (DM 1) du pathogène comme inoculation d'incitation. Des concentrations de 1,000, 30,000 et 100,000 zoosporanges par ml ont été utilisées. Les plantules inoculées ont été incubées entre des papiers filtres humectés, dans des plats de petri, à 15C pendant 4 à 5 heures. Des plantules de chacune des combinaisons d'hôtes et de concentrations de pathogène ont alors été immergées dans des suspensions de 1,000 ou 30,000 zoosporanges par ml des races DM 2 ou DM 3, ou dans l'eau, à 15C pendant 5 heures, recevant ainsi une inoculation dite de défi. Les plantules ont ensuite été plantées dans un mélange de sol synthétique (Promix) dans des pots de 10 cm de diamètre. Une autre série de plantules ont été plantées directement dans le Promix après l'inoculation d'incitation composée d'eau ou de la race DM 1 à un taux de 30,000 zoosporanges par ml. Cinq jours plus tard, ces plantules ont reçu une inoculation de défi de 30,000 zoosporanges de race DM 2 ou DM3 par ml, par arrosage du sol. Après les semis, tous les pots ont été gardés dans des chambres de croissance maintenues à 22/20C avec une période de clarté de 16 heures et une période d'obscurité de 8 heures. Après 12 à 17 jours, les

plants ont été couverts avec des sacs de plastique pendant 24 heures pour induire la sporulation. La sporulation n'a pas eu lieu sur les plants de contrôle immergés dans l'eau seulement, et sur les plants possédant le gène Pl_2 inoculés avec la race DM 1 ou DM 2. La sporulation a été abondante sur les plants possédant le gène Pl_1 inoculés avec les races DM 2 ou DM 3 et sur les plants possédant le gène Pl_2 inoculés avec la race DM 3. L'inoculation d'incitation préalable, dans une combinaison incompatible, n'a offert aucune protection apparente contre une inoculation de défi dans une combinaison compatible, peu importe la concentration de zoosporanges ou la méthode d'inoculation utilisées.

RESUMEN

Variedades de girasol susceptibles a mildiu (Plasmopara halstedii) o resistentes (con los genes Pl_1 o Pl_2) fueron inoculadas siguiendo el metodo de inmersion total de las plantulas (WSI) en suspensiones de 1,000, 30,000, o 30,000 zoosporangios/ml de la raza 1 (DM 1) del patogeno . Despues de esta inoculacion de "estimulo", las plantulas se colocaron entre hojas de papel filtro en cajas de petri y se incubaron a 15C durante 24 horas.Una porcion de las plantulas provenientes de cada tratamiento fue inoculada de la misma manera con suspensiones de zoosporangios de las razas DM 2 o DM 3 conteniendo 0 (testigo), 1,000, o 30,000 zoosporangios/ml (inoculacion de "reto"), e incubada a 15C durante 5 horas. Posteriormente las plantulas se sembraron en macetas de 10 cm que contenian una mezcla de suelo artificial (Promix). En otra serie de ensayos, las plantulas fueron sembradas inmediatamente despues de haber llevado a cabo la inoculacion de estimulo con agua destilada , o con DM 1 en una concentracion de 30,000 zoosporangios/ml. Al cabo de cinco dias se les sometio a la inoculacion reto, administrando 75 ml/maceta de una suspencion de 30,000 zoosporangios/ml de las razas DM 2 o DM 3. Todas las macetas sembradas se mantuvieron en camaras de ambiente controlado a 22/20C con 16/8 horas de luz/oscuridad. Despues de 12 a 17 dias las plantulas fueron cubiertas con bolsas de plastico para inducir la esporulacion. No se observo la formacion de zoosporangios en las plantulas tratadas con agua ni en las plantulas de genotipo Pl_1 tratadas con zoosporangios de las razas DM 1 o DM 2. La esporulacion fue abundante en las plantas de genotipo Pl_1 inoculadas con las razas DM 2 o DM 3 y en las plantas Pl_2 inoculadas con DM 3. Estos resultados muestran que no hubo ningun efecto de la inoculacion de estimulo con una raza avirulenta que ayudara a proteger a las plantas contra una raza virulenta, sin importar la concentracion de esporas o el metodo de inoculacion empleado.

INTRODUCTION

Downy mildew (DM) (Plasmopara halstedii (Farl.) Berl. and de Toni) is one of the major diseases of sunflowers in North and South America and in most sunflower producing countries of Europe (Sackston 1981). The discovery and incorporation of genes for resistance into varieties and hybrids provided excellent control of DM for about a decade. Gene Pl_1 was effective against the race found in Europe (DM 1); Pl_2 was effective against both DM 1 and the race found in the Red River Valley of north central North America (DM 2) (Zimmer and Kinman 1972).

A third race (DM 3) able to overcome both resistance genes Pl_1 and Pl_2 was found in the Red River Valley in 1980 and spread rapidly throughout the area (Gulya and Carson 1982), leaving seed treatment with metalaxyl as the only effective control measure. A new gene, Pl_5, is resistant to race 3

seedlings were just beginning to emerge they were given a challenge inoculation by drenching with 75 mL per pot of water or a suspension containing 30,000 zoosporangia per mL of DM 2 or DM3. They were covered with plastic bags 12 to 17 days later to induce sporulation.

RESULTS AND DISCUSSION

No sporulation developed on control seedlings immersed only in water, or on seedlings inoculated only in incompatible combinations of host genotype and pathogen race. Sporulation was profuse on seedlings inoculated only in compatible host:pathogen combinations.

Most of the seedlings exposed to an inciting inoculation in an incompatible combination followed by a challenge inoculation in a compatible host genotype:pathogen race combination developed symptoms and sporulation of downy mildew. Those seedlings in such combinations which remained apparently healthy were considered to be escapes, which can occur in even the most compatible combinations.

These results may indicate that cross protection against infection by a virulent race of downy mildew cannot be induced in sunflowers by prior inoculation with an avirulent race. Although resistance has been induced in a number of host:pathogen systems, the only instances reported for downy mildew were with blue mold of tobacco (Cohen and Kuc 1981, Kuc 1982a, 1982b,Tuzun and Kuc 1987, Tuzun et al 1986). The resistance induced in tobacco was attributed to compounds formed in the basal stem region receiving the inducing inoculation and transported systemically in the plant. Leaves on the upper portion of the stem were protected against local infection by spores of the pathogen sprayed onto them.

The time interval between inducing and challenge inoculations was found to affect the induction of resistance in some host:pathogen combinations. With blue mold of tobacco it took from three to four weeks to develop (Cohen and Kuc 1981, Kuc 1982a, 1982b). It is difficult to manipulate the time interval between inducing and challeng inoculations with P.halstedii because the period during which seedlings are susceptible to infection through the roots is usually very short, from 5 to 15 days (Cohen and Sackston 1973, Zimmer 1975).

Although induced resistance was not apparent when the challenge inoculation was made five days after the inducing inoculation, it is possible that systemic resistance may be induced over a longer period. It would be difficult to distinguish between such induced resistance and the apparent natural resistance of older tissues to infection by P. halstedii using the current methods of inoculation (Cohen and Sackston 1973, Zimmer 1975). It may prove possible to make such a distinction by using the leaf disk immersion (LDI) method of inoculation (Sackston and Vimard 1988). Phytoalexins have been reported in sunflowers (Tai and Robeson 1985). If they are formed and transported to the leaves in response to root infection by an avirulent race of downy mildew, their effect might be easier to demonstrate in a challenge inoculation by LDI than by root inoculation.

Relative concentrations of inducing and challenge inocula also may affect the induction of cross protection (Kuc 1982a, 1982b). The range of concentrations of zoosporangia used in these experiments was limited, from 1,000 to 100,000 zoosporangia per mL. Although infection has been shown to be possible with as few as eight or nine zoosporangia per mL (Cohen and

Sackston 1973), 1,000 to 30,000 zoosporangia per mL are used routinely in laboratory inoculations to increase the probability of successful infection. Very high concentrations of zoosporangia tend to increase the incidence of damping off, and may cause stunting or other reactions in resistant hosts in which typical symptoms of systemic infection and sporulation may not occur.

Despite such difficulties, further work should be done using a wider range of intervals between inoculations, a wider range of concentrations of inoculum, and also employing the LDI method. Even if induced resistance can be shown to occur in the sunflower:downy mildew system, it will probably be impossible to employ it as a practical method to control the disease in the field. It might prove valuable, however, in studies on the nature of susceptibilty and resistance of sunflowers to downy mildew.

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