Eur J Plant Pathol (2007) 119:53–58
DOI 10.1007/s10658-007-9146-4

FULL RESEARCH PAPER

Inheritance of resistance to *Mycosphaerella pinodes* in two wild accessions of *Pisum*

Sara Fondevilla · José I. Cubero · Diego Rubiales

Received: 10 October 2006 / Accepted: 19 April 2007 / Published online: 25 May 2007

Abstract *Mycosphaerella pinodes* is one of the most devastating pea pathogens. Pea cultivars with adequate levels of resistance to control the disease are not so far available. However, promising levels of resistance have been identified in wild accessions of pea. In the present investigation the inheritance of resistance to *M. pinodes* was studied in two crosses between the susceptible pea cv. 'Ballet' and the partially wild resistant accessions P665 (*Pisum sativum* subsp. *syriacum*) and P42 (*P. sativum* subsp. *sativum* var. *arvense*). Both additive and dominant effects were important in control of resistance and susceptibility dominated over resistance.

Keywords Ascochyta blight · Pea · Genetic

Introduction

Pea is the most commonly produced grain legume in Europe and second-most in the world (FAOSTAT data, 2005; http://faostat.fao.org/). Ascochyta blight, caused by *Mycosphaerella pinodes*, the teleomorph of *Ascochyta pinodes*, is one of the most important pea pathogens (Moussart et al. 1998). It is widespread throughout the major pea-growing areas, especially in temperate regions of Europe, North America, Australia and New Zealand (Wallen 1965; Lawyer 1984; Bretag et al. 1995). Average yield losses in commercial pea fields have been estimated at 10%, and losses of >50% have been measured in some trials (Xue et al. 1997). The disease reduces number of seeds per stem and seed size (Tivoli et al. 1996).

Management of the disease by fungicide seed treatment, crop rotation and sanitation is possible, but each has deficiencies. Resistance appears to be the more practical way to reduce its effects (Zimmer and Sabourin 1986). Although extensive screening of pea germplasm has been conducted, only partial resistance has been identified that by itself, is inadequate to control the disease. Good levels of partial resistance have been reported in wild pea accessions (Zimmer and Sabourin 1986; Clulow et al. 1991a, Wroth 1998; Fondevilla et al. 2005). Knowledge of the genetic factors controlling resistance to *M. pinodes* in these wild accessions would facilitate gene transfer to pea cultivars. With this aim, the present work examines the inheritance of resistance to *M. pinodes* in two partially resistant wild accessions of pea.

S. Fondevilla
Centro-Alameda del Obispo, IFAPA, Junta de Andalucía, Apdo. 3092, Cordoba 14080, Spain

J. I. Cubero · D. Rubiales
Instituto de Agricultura Sostenible, CSIC, Apdo. 4084, Cordoba 14080, Spain

Materials and methods

Plant material

Two partially resistant accessions P42 (*Pisum sativum* subsp. *sativum* var. *arvense*) and P665 (*P. sativum* subsp. *syriacum*) (Fondevilla et al. 2005) were crossed with the susceptible commercial cultivar 'Ballet' (*P. sativum* subsp. *sativum*). The derived F_1 plants of both crosses were evaluated for resistance to *M. pinodes* and selfed to obtain the F_2 generation. In addition, the reaction to *M. pinodes* was examined in backcrosses obtained by crossing F_1 plants derived from the cross P665 × 'Ballet', with 'Ballet' (BC_1) and P665 (BC_2). The testa of seeds was pierced to aid inbibition before seeds were germinated and sown, one seed per pot, containing 440 cm^3 of 1:1 sand–peat mixture. Plants were grown in a glasshouse to the 3–4 leaf stage (approximately 14 days after planting). They were then transferred to a growth chamber (21 ± 2°C with a 12 h dark/12 h light photoperiod, at 106 µmol m^{-2} s^{-1}) and arranged according to a complete randomised design for inoculation.

Inoculation and incubation

Plants were inoculated using the monoconidial isolate CO-99 obtained from infected pea plants collected in commercial fields at Córdoba (Spain). The isolate was multiplied in Petri dishes of V8 medium (200 ml of V8 vegetable juice + 40 g of technical agar + 800 ml of sterile water $litre^{-1}$) at 23°C, subjected to a 16 h photoperiod of fluorescent illumination at 27 µmol m^{-2} s^{-1}. A spore suspension was prepared by flooding the surface of 12–14 day-old cultures with sterile water, scraping the colony with a needle and filtering the suspension through two layers of sterile cheesecloth. The concentration of spores was determined with a haemocytometer and adjusted to 5×10^5 spores ml^{-1}. Tween-20 (120 µl 100 ml^{-1} of spore suspension) was added as a wetting agent and the spore suspension was applied with a sprayer at a rate of 1 ml per plant. After inoculation, plants were incubated in a growth chamber at 21 ± 2°C with a 12 h dark/12 h light photoperiod, the first dark period commencing immediately after inoculation. During the first 24 h, plants were covered with a polyethylene sheet and high humidity was ensured by ultrasonic humidifiers operating for 15 min every 2 h. The polyethylene cover was then removed.

Disease assessment

Disease was visually assessed 14 days after inoculation using a 0–5 scale defined by Roger and Tivoli (1996) as follows:

0 = no lesions
1 = a few scattered flecks
2 = numerous flecks
3 = 10–15% of the leaf area necrotic and appearance of coalescent necrosis
4 = 50% of the leaf area dehydrated or necrotic
5 = 75–100% of the leaf area dehydrated or necrotic.

Average disease rating (DR) for each plant was defined as the mean disease score over its first, second and third leaves.

Data analysis

The joint scaling test proposed by Cavalli (Mather and Jinks 1971, pp 71–76) was used to analyse data. The test checks the conformity with the additive-dominance model and gives additional information about the weight of dominance and additive effects in the control of the trait. Cavalli's test estimates the parameters 'm', 'd' and 'h', from means of the available types of generations; with 'm' defined as the mid-parental value, 'd' as the half of the difference between parental values and 'h' as the deviation of F_1 generation from their respective mid-parental values. Subsequently, the observed generation means were compared with expected values derived from the estimates of the three parameters assuming that the cross fitted the additive-dominance model. In this study this comparison was performed by using the chi-square (χ^2) test and linear regression.

The requirements of the additive-dominance model are (I) normal diploid segregation of chromosomes, (II) homozygous parents, (III) no genotype by environment interaction, (IV) no reciprocal differences, (V) no epistasis, (VI) no uncorrelated gene distribution and (VII) no multiple alleles (Hill 1964).

Broad sense heritabilities (H) were calculated by dividing the genetic component (additive + domi-

nance) by the total variance (σ^2) (additive + dominance + environmental components) as follows:

$$H = (\sigma^2_{F2} - \sigma^2_M) / \sigma^2_{F2}$$

The environmental components (σ^2_M) was estimated using the formula:

$$\sigma^2_M = 1/3\ (\sigma^2_{F1} + \sigma^2_{P1} + \sigma^2_{P2})$$

Results and discussion

Accessions P665 and P42 were partially resistant to *M. pinodes*, confirming previous reports (Fondevilla et al. 2005). Thus, 2 weeks after inoculation P665 and P42 showed DRs of 2.5 and 3.2, respectively, while the DR for the highly susceptible 'Ballet' was 4.77 (Tables 1 and 2). The F_1 derived from both crosses were as susceptible as 'Ballet'. That was also the case of the BC_1 obtained from the cross 'Ballet' × P665. In contrast, the BC_2 generation of this cross displayed a DR higher than P665 but lower than 'Ballet'. In the F_2 of both crosses the DR showed a continuous distribution skewed towards susceptibility (Fig. 1). The cross P665 × 'Ballet' fitted the additive-dominance model (Table 1; χ^2 $P > 0.05$; linear regression $P < 0.05$). In this cross, parameters 'd' and 'h' were significantly different from zero showing that both additive and dominance effects were involved in the control of the resistance (Table 3). In addition, 'h' and 'd' gave similar positive values suggesting a complete dominance of susceptibility over resistance. Broad sense heritability displayed a value of 0.43 (Table 5).

Table 1 Summary of conformity of the 'Ballet' × P665 cross to the additive-dominance model

Generation	Number of plants	Observed values[a]	Expected values[b]
'Ballet'	17	4.77	4.79
P665	6	2.50	2.63
F_1	5	4.45	4.83
F_2	153	4.34	4.27
BC_1	7	4.81	4.81
BC_2	3	3.40	3.73
$\chi^2_{(gl = 3)}$		3.71 not significant	
R^{2c}		0.959	

[a] Disease rating visually assessed using a 0–5 scale defined by Roger and Tivoli (1996)

[b] Expected values derived from joint scaling test proposed by Cavalli (Mather and Jinks 1971)

[c] R^2 and significance of linear regression

*** Significance levels $P < 0.001$

Table 2 Summary of conformity of the P42 × 'Ballet' cross to the additive-dominance model

Generation	Number of plants	Observed values[a]	Expected values[b]
P42	20	3.2	3.46
'Ballet'	17	4.77	4.80
F_1	4	4.83	5.11
F_2	167	4.67	4.62
$\chi^2_{(gl = 1)}$ [b]		5.16*	
R^{2c}		0.958*	

[a] Disease rating visually assessed using a 0–5 scale defined by Roger and Tivoli (1996)

[b] Expected values derived from joint scaling test proposed by Cavalli (Mather and Jinks 1971)

[c] R^2 of linear regression

* Significance level $P < 0.05$

Concerning the cross P42 × 'Ballet', although according to the χ^2 value the additive-dominance model should be rejected for this cross, the linear regression showed that the observed values for each generation were highly correlated with the expected values (Table 2). As the F_2 generation values depends on the parental and F_1 values, the χ^2 value could be inflated and, therefore, we cannot rule out the possibility that the cross P42 × 'Ballet' fits the additive-dominance model. If that were the case, the estimation of the additive and dominant effects by Cavalli's test would be accurate and we could conclude that in cross P42 × 'Ballet', as in 'Ballet' × P665, both dominant and additive effects contribute in the control of the resistance (Table 4). In this cross, broad sense heritability was higher than in the cross 'Ballet' × P665 and showed a value of 0.60 (Table 5).

In the two crosses analysed, the distribution of DR was normal suggesting that resistance is a polygenic trait. Other possibilities might be that resistance is controlled by a single or a few major genes whose expression is highly influenced by the environment. Whatever the case, as F_2 individuals could not be

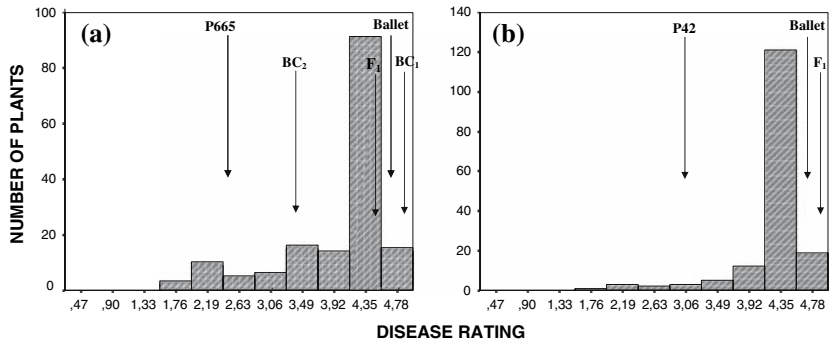

Fig. 1 Histograms of disease rating (Roger and Tivoli 1996) measured in the F_2 derived from the crosses 'Ballet' × P665 (**a**) and P42 × 'Ballet' (**b**). Arrows indicate parental, F_1, BC_1 and BC_2 values

Table 3 Summary of the Cavalli's test for the 'Ballet' × P665 cross

Parameter	Value	S[a]	*t*-student
m[b]	3.712	0.106	34.866***
d[c]	1.077	0.108	9.998***
h[d]	1.122	0.218	5.152***

[a] Standard deviation of the parameter

[b] Mid-parent value

[c] Half of the difference between parental values

[d] Deviation of F_1 from their respective parent values

*** Significance level $P < 0.001$

Table 4 Summary of the Cavalli's test for the P42 × 'Ballet' cross

Parameter	Value	S[a]	*t*-student
m[b]	4.128	0.103	40.162***
d[c]	0.672	0.110	6.125***
h[d]	0.987	0.199	4.962***

[a] Standard deviation of the parameter

[b] Mid-parent value

[c] Half of the difference between parental values

[d] Deviation of F_1 from their respective parent values

*** Significance level $P < 0.001$

Table 5 Estimates of broad sense heritability in two crosses between the pea variety 'Ballet', susceptible to *M. pinodes*, and the partially resistant wild pea accessions P665 and P42

	'Ballet' × P665	P42 × 'Ballet'
Genetic variance (σ_{F2}^2)	0.83	0.41
Environment variance (σ_M^2)	0.47	0.16
Broad sense heritability (H)	0.43	0.60

ambiguously classified in resistance classes, the trait has to be treated as a quantitative character. Our results agree with the majority of previous studies reporting that the inheritance of resistance to *M. pinodes* in pea is controlled by a complex system. Thus, Wroth (1999), using biometric approaches, concluded that resistance to *M. pinodes* in pea was controlled by a polygenic system. Similarly, mapping the resistance to ascochyta blight in several pea crosses has resulted in the identification of numerous genomic regions controlling the trait (Timmerman-Vaughan et al. 2002, 2004; Prioul et al. 2004). In contrast, Clulow et al. (1991b) was able to separate individuals of segregrant populations into discrete resistance classes and concluded that in some crosses resistance was dominant and controlled by single genes.

In this study we report genetic analysis in wide crosses between different subspecies of *Pisum*, where distorted segregations could be expected. However, both crosses gave a good fit to the additive-dominance model showing that, at least for the character studied in this paper, genes are segregating in Mendelian ratios. In addition, as the absence of epistatic effects is an assumption of Cavalli analysis, the conformity of the crosses with the model implies that gene interactions do not play an important role in the control of the resistance. The absence of gene interactions and the presence of additive effects leads to the possibility of enhancing the level of resistance to *M. pinodes* by gene pyramiding.

The analysis performed revealed that the dominance component was also important in the control of resistance. F_1 individuals derived from both crosses were as susceptible as the susceptible parent 'Ballet' and the distribution of the DR in the F_2 were skewed towards susceptibility. These facts show that suscep-

tibility is dominant over resistance in the two crosses. A similar outcome of recessive genes controlling resistance to *M. pinodes* was reported by Ali (1983) while in other pea germplasm the resistance to this pathogen is of dominant nature (Wroth 1999; Clulow et al. 1991b). Although dominance effects will disappear in advanced breeding material, they have to be taken in account in the early stages of breeding programmes including accessions P665 and P42. Thus, the recessive nature of the resistance implies that selection must be performed in selfed generations.

Wroth (1999) found that the inheritance of resistance to *M. pinodes* in leaves fitted the additive-dominance model in some crosses between *P. sativum* accessions. In contrast, the model was rejected for disease response in stems. In two of these crosses, the genetic variance was mainly attributed to additive effects, whereas dominance effects were more important in a third cross.

The moderate value of broad sense heritability showed that resistance expression was influenced by the environment. Several investigations have pointed out the strong importance of environmental factors such as temperature and humidity in the development of ascochyta blight (Wroth 1999; Roger et al. 1999a, b). This result was confirmed in our study where genetic studies were performed under controlled environmental conditions, and differences in the level of resistance were identified within individuals of non-segregating generations. For instance, F_1 showed great variance, contributing to the high value of the environment component estimated. The high variance observed may be caused by the low number of F_1 individuals that were screened in both crosses. Consequently, it is possible that heritability was underestimated in our study. In fact, F_2 individuals at least as resistant as their respective resistant parents were observed in both crosses, suggesting that the heritability values allow for an appropriate strategy of selection for greater resistance.

As resistance is present in the non-adapted pea accessions and is quantitative and recessive, a recurrent selection scheme will be a suitable breeding strategy. In the proposed breeding programme, both wild lines will be crossed to commercial cultivars, both F_1s selfed and the F_2 screened under field conditions to select the best plants showing the highest possible degree of resistance as well as good agronomic features. The best F_2 plants will be backcrossed with the commercial cultivar, starting a new crossing cycle. They will also be advanced to F_3 progenies, where disease and general assessment is more accurate. The lines selected will be intercrossed in as many combinations as possible. The descendants of these crosses will be further selfed, screened for resistance and agronomic characteristics, crossed again and with commercial cultivars. This method has proved to be efficient in several crops (for example, in soybean; Wilcox 1998) in accumulating polygenic alleles for resistance in a common genotype. The method for autogamous species is much more time-consuming than for outcrossers and would be specially tedious when wild accessions are involved. However, in the absence of good levels of resistance to *M. pinodes* in cultivated pea, the effort is worthy to enhance the level of resistance to this worldwide important pea disease.

The studies described here represent the first step towards the development of pea lines with increased resistance. Our conclusions are based on experiments performed under controlled conditions at the seedling stage and using only one isolate. Therefore, our results may differ from those obtained with naturally infected mature field plants. However, previous studies performed with several wild pea accessions showing different levels of resistance to *M. pinodes* have proved that disease assessments under controlled conditions provide a good estimation of field resistance (Fondevilla et al. 2005). Furthermore, accession P665 was found to be resistant against different *M. pinodes* isolates originating from different countries, showing that the resistance present in this accession is not isolate-specific.

The biometric approach performed in early generations was selected from other possible methods because it allows the estimation of the dominance component, providing early and useful information for planning breeding strategies. Future research will include the mapping of genomic regions involved in the control of resistance to *M. pinodes*. This approach would enhance our current knowledge about the genetics of the trait and may be useful to validate the conclusions derived from the present study. With this aim, a population of recombinant inbred lines derived from a cross between accession P665 and the susceptible variety 'Messire' is being developed.

Acknowledgements We thank project AGF2005-01781 of the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) for financial support.

References

Ali, S. M. (1983). Pathotypes of 'black spot' complex pathogens of field peas, selection and inheritance of resistance in pea lines. Adelaide, Australia: Standing Committee on Agriculture, pp. 246–248.

Bretag, T. W., Keane, P. J., & Price, T. V. (1995). Effect of ascochyta blight on the grain yield of field pea (*Pisum sativum L.*). *Australian Journal of Experimental Agriculture, 35*, 531–536.

Clulow, S. A., Lewis, B. G., & Matthews, P. (1991a). A pathotype classification for *Mycosphaerella pinodes*. *Phytopathology, 131*, 322–332.

Clulow, S. A., Matthews, P., Lewis, B. G. (1991b). Genetical analysis of resistance to *Mycosphaerella pinodes* in pea seedlings. *Euphytica, 58*, 183–189.

Fondevilla, S., Ávila, C. M., Cubero, J. I., & Rubiales, D. (2005). Response to *Mycosphaerella pinodes* in a germplasm collection of *Pisum* spp. *Plant Breeding, 124*, 313–315.

Hill, J. (1964). Effects of correlated gene distributions in the analysis of diallel crosses. *Heredity, 19*, 27–46.

Lawyer, A. S. (1984). Diseases caused by *Ascochyta* spp. In D. J. Hagedorn (Ed.), *The compendium of pea diseases* (pp. 11–15). Minnesota, USA: The American Phytopath. Soc.

Mather, K, & Jinks, J. L. (1971). Biometrical genetics. The study of continuous variation. Chapman and Hall Ltd.

Moussart, A., Tivoli, B., Lemarchand, E., Deneufbourg, F., Roi, S., & Sicard, G. (1998). Role of seed infection by the Ascochyta blight pathogen of dried pea (*Mycosphaerella pinodes*) in seedling emergence, early disease development and transmission of the disease to aerial plant parts. *European Journal of Plant Pathology, 104*, 93–102.

Prioul, S., Frankewitz, A., Deniot, G., Morin, G., & Baranger, A. (2004). Mapping of quantitative trait loci for partial resistance to *Mycosphaerella pinodes* in pea (*Pisum sativum* L.) at the seedling and adult plant stages. *Theoretical and Applied Genetic, 108*, 1322–1334.

Roger, C., & Tivoli, R. (1996). Spatio temporal development of pynidia and perithecia and dissemination of spores of *Mycosphaerella pinodes* on pea (*Pisum sativum*). *Plant Pathology, 45*, 518–528.

Roger, C., Tivoli, B., & Huber, L. (1999a). Effects of temperature and moisture on disease and fruit body development of *Mycosphaerella pinodes* on pea (*Pisum sativum*). *Plant Pathology, 48*, 1–9.

Roger, C., Tivoli B., & Huber L. (1999b). Effects of interrupted wet periods and different temperatures on the development of ascochyta blight caused by *Mycosphaerella pinodes* on pea (*Pisum sativum*) seedlings. *Plant Pathology, 48*, 10–18.

Timmerman-Vaughan, G. M., Frew, T. J., Butler, R., Murray, S., Gilpin, M., Falloon, K., Johnston, P., Lakeman, M. B., Russell, A. C., & Khan, T. (2004). Validation of quantitative trait loci for Ascochyta blight resistance in pea (*Pisum sativum* L.), using populations from two crosses. *Theoretical and Applied Genetics, 109*, 1620–1631.

Timmerman-Vaughan, G. M., Frew, T. J., Russell, A. C., Khan, T., Butler, R., Gilpin, M., Murray, S., & Falloon, K. (2002). QTL mapping of partial resistance to field epidemics of ascochyta blight of pea. *Crop Science, 42*, 2100–2111.

Tivoli, B., Beásse, C., Lemarchand, E., & Masson, E. (1996). Effect of ascochyta blight (*Mycosphaerella pinodes*) on yield components of single pea (*Pisum sativum*) plants under field conditions. *Annals of Applied Biology, 129*, 207–216.

Wallen, V. R. (1965). Field evaluation of the importance of the Ascochyta complex of peas. *Canadian Journal of Plant Science, 45*, 27–33.

Wilcox, J. R. (1998). Increasing seed protein in soybean with eight cycles of recurrent selection. *Crop Science, 38*, 1536–1540.

Wroth, J. M. (1998). Possible role for wild genotypes of *Pisum* spp. to enhance ascochyta blight resistance in pea. *Australian Journal of Experimental Agriculture, 38*, 469–479.

Wroth, J. M. (1999). Evidence suggests that *Mycosphaerella pinodes* infection of *Pisum sativum* is inherited as a quantitative trait. *Euphytica, 107*, 193–204.

Xue, A. G., Warkentin, T. D., & Kenaschuk, E. O. (1997). Effect of timings of inoculation with *Mycosphaerella pinodes* on yield and seed infection on field pea. *Canadian Journal of Plant Science, 77*, 685–689.

Zimmer, M. C., & Sabourin, D. (1986). Determining resistance reaction of field pea cultivars at the seedling stage to *Mycosphaerella pinodes*. *Phytopathology, 76*, 878–881.