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Epistasis and heritability of resistance to *Phytophthora nicotianae* in pepper (*Capsicum annuum* L.)

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Abstract Gene effects of resistance to two isolates of *Phytophthora nicotianae* in two crosses of pepper were investigated using separate generation means analysis. Additive-dominance models were inadequate in all cases. Digenic parameter models were adequate in three cases and the probability of goodness of fit of models was negatively correlated with the aggressiveness of the pathogen. None of these models explained variation among generation means in the combined cross Beldi × CM334 with *P. nicotianae* isolate Pn_2. Additive × additive, dominance × dominance and dominance × additive effects were significant in most cases. Additive and dominance effects (of negative sign) contribute more to resistance than to susceptibility. Additive variance was greater than environmental and dominance variance and ranged from 0.038 to 0.224. Narrow-sense heritabilities were dependent upon the cross and inoculate and ranged from 86 to 92%. The results of this study indicate that selection with more aggressive isolates of the pathogen will be useful for enhancing resistance in pepper.

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Introduction

In Tunisia *Phytophthora nicotianae* is one of the most economically destructive diseases of pepper (*Capsicum annuum* L.). This pathogen causes many symptoms such as wilting, necrosis and root rot. The environmental problem caused by the continuous use of chemicals for control, make breeding for resistance increasingly important. The resistance to *Phytophthora* spp. has been the subject of intensive studies in recent years (Boukema 1983; Miller et al. 1996; Förster et al. 1998; Man in 't Veld et al. 1998; Matheron and Porchas 2000; Allagui and Lepoivre 2000; Grote et al. 2002). *Phytophthora* spp. are among the most serious threats to agriculture and food production, causing devastating diseases in hundreds of plant hosts (Howard and Flavio 2005).

Recent studies suggest that epistatic effects are present for resistance to pests or diseases in pepper and other species. Examples are wheat and leaf rust (Ezzahiri and Roelfs 1989), wheat and yellowberry (Bnejdi and El Gazzah 2008), common bean and anthracnose (Marcial and Pastor 1994), barley and Fusarium head blight (Flavio et al. 2003), chickpea and *Botrytis cinerea* (Rewal and Grewal 1989), and pepper and *Phytophthora capsici* (Bartual et al. 1994).

Knowledge of the presence of non-allelic interactions is important to a plant breeder in deciding appropriate methodologies for plant improvement (Malhotra and Singh 1989). Information on genetic determination may be obtained by estimation of genetic parameters determining additive (d), dominance (h) and epistatic [additive × additive (i), additive × dominance (j) and dominance × dominance (l)] gene effects. The aim of the present study was to study the nature of gene effect and heritabilities of *P. nicotianae* in pepper.

Materials and methods

Plant material

This study was carried out at the National Institute for Agricultural Research located in Tunis, Tunisia. Parental lines were selected, based on their resistance to *P. nicotianae*. The resistant parent (Pr) used was the cultivar CM334 and the susceptible parents (Ps) were Beldi and Nabeul II. Crosses were made as follows: CM334 (Pr) × Beldi (Ps), CM334 (Pr) × Nabeul II (Ps). Generation means analysis was performed using each resistant parent (Pr) and susceptible parent (Ps), F_1 and F_2 generation and backcrosses of the F_1 to each parent (BC_1 Pr and BC_2 Ps). All crosses were controlled pollinations in a greenhouse.

Inoculum preparation

Two isolates of the pathogen were collected from infected pepper plants in two different regions in Tunisia. The first isolate collected from Korba (Pn_1) and the second isolate from Bizerte (Pn_2). These isolates were identified as *P. nicotianae* according to morphological, biological and molecular characteristics reported by Allagui and Lepoivre (2000). The two isolates tested in the susceptible parent Beldi were revealed aggressiveness.

Pepper seeding and inoculation

Two weeks after sowing seedlings (2-cotyledon stage) were transplanted in the alveolated plates containing the substrate disinfected by heat [mixture of clay soil, sand and peat (2:1:1; v/v/v)]. Plants were grown in a randomised complete block design with two replications. Two weeks after transplantation, seedlings (2-leaf stage) from each replication were inoculated separately by the tow isolates, by dripping a suspension of 280,000 zoospores onto the collar of each plant. Control and inoculated plants were maintained in a greenhouse. Plants were irrigated with tap water every 3–4 days according to substrate humidity.

Assessment of root necrosis

After 3 weeks of incubation, the root system of each seedling was delicately detached from substratum by washing in a water bowl. The root necrosis intensity was evaluated according to the following scale: 0 (healthy plant), 0.5 (necrosis limited at the extremity of radicles), 1 (necrosis only on the lower half of primary roots), 2 (necrosis on all the primary roots), 3 (necrosis reaching the crown and the lateral roots), 4 (hypocotyl rotten), and 5 (whole plant dead).

The number of plants evaluated varied depending on the generation and was greater in generations with greater segregation, such as the F_2 and the BC_1Pr and BC_1Ps. Prior to analysis the intercept and slope coefficients derived from the regressions of SD on mean values were used to calculate Kleckowski transforms (Lynch and Walsh 1998) according to the formula: $k\ (xi) = \log_e\ (xi + \text{intercept/slope})$. This procedure was successful in normalizing distribution and stabilizing the variances.

Statistical analyses

Analyze of variance by population and inoculate using GLM procedures (SAS institute 1990) indicated that replication and generation × replication were not significant. Therefore, generation means analysis was conducted without adjusting the data for replication.

Gene effects

The means of different generations were analyzed by a joint scaling test using the weighted least squares method (Mather and Jinks 1982; Kearsey and Pooni 1996; Lynch and Walsh 1998).

The observed generation means were used to estimate the parameters of a model consisting only of

mean (m), additive (d) and dominance (h) genetic effects. The estimated parameters were used in turn to calculate the expected generation means. The goodness of fit between observed and expected was tested, a significant chi-squared indicating a significant difference between the observed and expected generation means, which implied that a simple additive model was insufficient to explain the data. When the additive-dominance model was found to be insufficient, then the six-parameter model was applied. If a parameter was not significant in the six-parameter model then it was omitted and the best fit model was applied. The weighted least-squares model that incorporates additive, dominance and digenic epistatic effects is (Hayman 1958; Mather and Jinks 1982; Kearsey and Pooni 1996):

$$X = (C'WC)^{-1}(C'WY)$$

where X is the vector of mean, additive, dominance, additive × additive, additive × dominance and dominance × dominance parameters, (m), (d), (h), (i), (j) and (l) respectively. C is the matrix of coefficients from the equation for predicted family means. W is the diagonal matrix of weights (i.e., the reciprocals of the variance of generation means), and Y is the vector of generation means. The variances of the parameter estimates can be obtained from the diagonal elements of $(C'WC)^{-1}$. The expected means of the six generations were calculated using the parameter estimates, the goodness of fit of the observed generation means was tested with the chi-squared statistic. The significance of each parameter was determined by *t*-test.

Heritability

Homogeneity of variances of non-segregation generation was tested for using Bartlett's test (Bartlett 1937), and when the variances were heterogeneous the environmental variance (V_E) was replaced by an adequate number of separate parameters and pooled to produce a single environmental variance. Additive, dominance and environmental variance components were estimated using the maximum likelihood method with the observed variance of the six basic generations being used as the initial weights ($df/2*S^2$) until the chi-squared test value reached a minimum (Lynch and Walsh 1998).

Narrow-sense heritability was calculated as follows: $h_n^2 = V^*_A/V^*_A+V^*_D + V_E$ where V^*_A is the additive genetic component of variance, V^*_D the dominance genetic component of variance and V_E the environmental variance (Kearsey and Pooni 1996). The dominance variance was negative and was set to zero.

Results

Means and their standard errors for parental F_1, F_2 and backcross generations for resistance to pathogen are listed in Table 1. The resistant parent was CM334. Both Nabeul II and Beldi were very susceptible. In all 4 cases, the means of the parents (P_r and P_s) showed a tendency to be more extreme. The backcrosses BC_1P_r and BC_1P_s showed means

Table 1 Root means necrosis ± SE for *P. nicotiana* in parents and offspring populations from two crosses of resistant by susceptible parents (non-transformed data)

Population	Beldi (S) × CM 334 (R)		Nabeul II (S) × CM 334 (R)	
	Pn_1	Pn_2	Pn_2	Pn_1
P_s	3.17 ± 0.47 a (9)[a]	3.07 ± 0.44 a (15)	4.10 ± 0.55 a (10)	4.10 ± 0.46 a (10)
BC_1P_s	1.64 ± 0.10 b (150)	2.70 ± 0.13 ab (173)	1.51 ± 0.18 bc (150)	2.51 ± 0.13 b (181)
F_1	0.45 ± 0.03 c (50)	0.97 ± 0.23 c (43)	1.66 ± 0.35 b (40)	0.54 ± 0.07 c (37)
F_2	0.90 ± 0.05 c (300)	2.22 ± 0.08 b (489)	1.34 ± 0.12 bc (300)	1.01 ± 0.12 c (201)
BC_1P_r	0.58 ± 0.01 c (152)	0.54 ± 0.02 c (121)	0.47 ± 0.02 c (168)	0.54 ± 0.06 c (179)
P_r	0.45 ± 0.05 d (10)	0.33 ± 0.06 c (15)	0.44 ± 0.10 c (9)	0.33 ± 0.06 c (15)

Pn_1, Pn_2 isolates 1 and 2 of *P. nicotianae* respectively

Means, within a column, bearing different letters differ ($P < 0.05$)

[a] Number of plant evaluated in each generation

that tended to be located close to those of their respective recurrent parents. These results confirmed the choice of parents for the present study. The F_1 generation mean was intermediate between the parental means and was not significantly different from the resistant parent in two cases. The F_2 generation mean was not significantly different from the F_1 generation mean. The mean necrosis of F_2 plants in the two crosses caused by isolate Pn_2 was higher than by isolate Pn_1, indicating that isolate Pn_2 was more aggressive than isolate Pn_1 (Table 1).

Gene effects were estimated using the last square methods (Mather and Jinks 1982) and are listed in Table 2. The three-parameter model revealed that additive (d) and dominance (h) effects were negative and significant. For three cases, additive effects were of greater importance compared with dominance. The joint scaling test revealed that an additive-dominance model was inadequate in all four cases ($P < 0.05$). The failure of the model may be due to interaction or linkage among genes governing the resistance to *P. nicotianae*. Therefore, the six-parameter model was invoked and if a parameter was not significant, it was omitted and the best fit model applied. The digenic epistatic model was adequate in three cases (non significant chi-squared).

In the presence of isolate Pn_1 digenic epistatic models adequately explained differences among generation means for both crosses. In the presence of isolate Pn_2 the digenic epistatic model was less appropriate (Table 2). The probability of goodness of fit of these models was negatively correlated with the aggressiveness of the pathogen. Dominance (h), additive × additive (i) and dominance × dominance (l) effects when significant contributed more in the control of resistance than additive effects. In the majority of cases significant additive and dominance effects were negative. The digenic epistatic model failed to explain variation among generation means for resistance to *P. nicotianae* (chi-squared was significant) in the cross Beldi × CM334 with isolate Pn_2. The additive × dominance (j) effect was significant only in the cross Nabeul II × CM334 with isolate Pn_2, where the additive × additive (i) effect was not significant (Table 2).

Table 2 Estimates of gene effects ± (SE × 10) for resistance to *P. nicotiana* in two crosses of resistant by susceptible parents

Model	Beldi (s) × CM 334 (r)		Nabeul II (s) × CM 334 (r)	
	Pn_1	Pn_2	Pn_2	Pn_1
Three-parameter model				
m	0.32 ± 0.15**	0.42 ± 0.23**	0.24 ± 0.3**	0.33 ± 0.22**
d	−0.19 ± 0.14**	−0.38 ± 0.16**	−0.16 ± 0.2**	−0.27 ± 0.20**
h	−0.22 ± 0.23**	−0.18 ± 0.42**	−0.14 ± 0.6*	−0.23 ± 0.36**
X^2 (3*df*)	17.57	67.75	15.17	46.13
$^{(A)}P$	<0.05	<0.001	<0.05	<0.001
Best fit model				
m	0.09 ± 0.11**	0.8 ± 0.6**	0.36 ± 0.5**	−0.19 ± 1**
d	−0.21 ± 0.16**	−0.33 ± 0.2**	−0.28 ± 0.5**	−0.32 ± 0.2**
h	–	−0.95 ± 1.6**	−0.7 ± 1.6**	1.01 ± 2.2**
i	0.21 ± 0.25**	−0.45 ± 0.6**	–	0.55 ± 1**
l	0.37 ± 0.46**	0.32 ± 1.2*	0.57 ± 1.6**	−0.71 ± 1.4**
j	–	–	0.32 ± 1.1*	–
X^2 (*df*)	0.98 (2)	9.14 (1)	0.77 (1)	0.07 (1)
$^{(A)}P$	0.61	0.002	0.38	0.79

Pn_1, Pn_2 isolates 1 and 2 of *P. nicotianae* respectively

"*, **"Indicates means and gene effects are statistically different from zero at $P < 0.05$, 0.01, respectively

Mean (m), additive (d), dominance (h), additive × additive (i), additive × dominance (j) dominance × dominance (l) genetic effect

(A) Probability of adequateness of model, *df* degrees of freedom, calculated as the number of generations minus the number of estimated genetic parameters

Table 3 Estimates of variance components with their SE (× 100) and narrow-sense heritability (h^2_n) for resistance to *P. nicotianae* in two crosses of resistant by susceptible parents

Variance components	Beldi (S) × CM 334 (R)		Nabeul II (S) × CM 334 (R)	
	Pn_1	Pn_2	Pn_2	Pn_1
Phenotypic variance	4.46	14.43	24.31	13.84
Environmental variance	0.60 ± 0.1**	1.61 ± 0.2**	1.85 ± 0.3**	1.56 ± 0.2**
Additive variance	3.85 ± 1.1**	12.82 ± 2.5**	22.46 ± 4.3**	12.28 ± 3.1**
Dominance variance	−1.49 ± 0.5*	−7.14 ± 1.3**	−11.75 ± 2.2**	−4.93 ± 1.7*
X^2 (*df*)	(3) NS	(3) NS	(3) NS	(3) NS
Heritability (h^2_n) (%)	86	88	92	88

Pn_1, Pn_2 isolates 1 and 2 of *P. nicotianae* respectively

"*, **"Indicates Variance components are statistically different from zero at $P < 0.05$, 0.01, respectively

df degrees of freedom, calculated as the number of generations minus the number of estimated variance parameters, *ns* non-significant

Variance components were estimates and used to calculate narrow-sense heritabilities for both cross and inoculate combinations. For all cases the additive variance was positive and ranged from 0.038 to 0.224. Dominance variance was negative and was set to zero. Environmental variance was significant in all cases and ranged from 0.006 to 0.018. Narrow-sense heritability (h^2) averaged 88.5%, with a range from 86 to 92%. (Table 3).

Discussion

Digenic epistatic effects were significant in all cases; therefore, the genetic model which assumes no epistasis does not accurately describe resistance to *P. nicotianae* in pepper. The presence of epistatic effects has been found for different diseases in pepper; for Phytophthora stem blight (Bartual et al. 1994); *P. capsici* Leonian (Lefebvre and Palloix 1996); *P. capsici* (Thabuis et al. 2004), *Leveillula taurica* (Lefebvre et al. 2003) and for cucumber mosaic virus (Caranta et al. 1997).

Depending on the cross and isolate, in most cases the variation in generation means fitted a digenic epistatic model, indicating that improvements in resistance to *P. nicotianae* would be moderately difficult, compared to fitting an additive-dominance model (best from breeders point of view). The dominance effects where significant were more important than additive effects, making the situation more complicated. None of these models explained variation among generation means in the cross Beldi × CM334 with isolate Pn_2 indicating other mechanisms of genetic control such as higher order interaction or linkage effects. A strategy is needed for the early generation selection. To conclude whether the cause of the model failure is the presence of higher order interactions or linkage effects, further analyses in subsequent generations is necessary. The adequacy of models was less in the case of Pn_2, indicating that the mechanism of genetic control was dependent upon the aggressiveness of the pathogen. These results agree with Bartual et al. (1991) who found that epistasis was a principal source of variation in the resistance of pepper to Phytophthora stem blight *(P. capsici*) which was correlated with the level of aggressiveness of the pathogen. The adequacy of models according to isolate were more stable in the cross Nabeul II × CM334. A selection based on this cross was more efficient compared to cross Beldi × CM334. Significant estimates of additive gene effects (d and i) and dominance effects (h) were usually negative, indicating that these effects contribute more to resistance than to susceptibility, in contrast with dominance × dominance effects (l) which were usually positive. When significant the parameters (h) and (l) had opposite signs (Table 2) indicating a situation of complementary epistasis (Mather and Jinks 1982). This situation is more favorable than duplicate epistasis.

In the present study, high values for narrow-sense suggested a considerable participation of genetics on the phenotypic expression of traits and that selection for the traits should be efficient. These results show that both the adequacy of the model of inheritance, as

well as the importance and significance of gene effects were dependent upon the cross and isolates, thus stressing the importance of appropriate selection. Breeding methods, such as recurrent selection, which make the best use of additive variance should be used.

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