Investigation note

Inheritance of resistance to *Phytophthora parasitica* Dastur in Jamaica

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Abstract

To determine the genetics of resistance to *Phytophthora parasitica* in Jamaica, the generational means of five resistant and five susceptible lines were analyzed to estimate the genetic parameters of resistance in Jamaica crosses. The analysis showed that the additive effects were more important than dominance effects for resistance to *P. parasitica*. Heritability, broadly speaking, was 37%. The results obtained indicate that a pedigree program can be effective and the most appropriate to increase genetic resistance to *P. parasitica*.

Keywords: additivity, crosses, genetic advance, heritability.

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Jamaica (*H. sabdariffa* L.) is a short-growing annual plant, the Malvaceae family, shrub growth, native to East Africa (Omalsaad *et al*., 2012). It is autogamous, chromosomal number 2n= 4x= 72. In Mexico it is cultivated to obtain calyces for the preparation of infusion, juice, jam, etc., with a high content of vitamin C, anthocyanins and other antioxidants (Anel *et al*., 2016). One of the problems of the crop is the diseases caused by pathogens that affect root and stem, especially *Phytophthora parasitica*, *Fusarium oxysporum* and *Fusarium equiseti* (Hassan *et al*., 2014).

The infection by the omicet *Phytotphthora parasitica* D. causes necrotization of the stem known as 'pata prieta', which causes the death of the plant (Hernández and Romero, 1990). The disease occurs in warm conditions of high relative humidity (Erwin and Riveiro, 1996) and in varieties susceptible to the disease.

The few studies of genetic inheritance to diseases in Jamaica have focused on characters especially in the analysis of morphological characters, acidity, anthocyanin content, earliness and yield (Bandi and Appalaswamy, 2014). Regarding genetic resistance to diseases in Jamaica, only Boccas and Pellegrin (1976) report that resistance to *P. parasitica* is polygenic in nature.

Generational means analysis (GMA) is a simple method that allows estimating the gene effects of a polygenic character (Mather and Jinks, 1971), which calculates and interprets the mean of different gene effects resulting from the crossing of two lines. Bernardo (2002) reported that the calculations and interpretation of non-allelic interactions (epistasis) in GMA is carried out more continuously than when variances are used, since the effect of the means are frequently identified and the experiments required for analysis of means are smaller and easier to implement than those necessary to study the variances.

Similarly, the study of gene effects can be based on mean generation contrast, for example, the comparison of mean differences at two different levels of genetic factors, in which the t-test is normally used (Piepho and Mohring, 2010). Some researchers report the role of gene action in yield and other agronomic characters using GMA (Said, 2014).

GMA is also used in disease resistance studies where parents have a high level of resistance and a high degree of contrasting susceptibility (Acquaah, 2007), as occurs in pathosystems involving *Sphacelotheca reiliana* in corn (Bernardo *et al*., 2002) and *Alternaria alternata* in tomato (Cassol and Clair, 1994). Heritability is a parameter used to assess the degree to which a character is transmitted from parents to their descendants (Akhshi *et al*., 2014). A high heritability value and high genetic advance suggest the conditions for choosing the selection method (Hussein *et al*., 2017).

In Mexico, there are no reports in the search for genotypes or native varieties of Jamaica resistant to *P. parasitica*, so it is important to study the type of gene action involved in genetic resistance to implement a program for genetic improvement of the crop. The objective of the present investigation was to analyze the generational means of inter-varietal crosses of native jamaica collections to estimate the genetic parameters of resistance to *P. parasitica*.

Vegetal material

To achieve the crosses between native jamaica materials, five resistant and four susceptible varieties identified by their response to *P. parasitica* were used as parents (Table 1). The sowing of the parents was done using three seeds of each parent in greenhouse conditions in Montecillo, State of Mexico. When the seedlings reached 20 cm in height, they were transplanted into black polyethylene bags 35 cm in diameter by 40 cm in height, with sterile soil. Pest control and plant management was conventional with irrigation using 75% nutrient solution (Steiner, 1961). Seven months after sowing, when all the plants started the flowering stage, the crosses were made in diallelo (Griffing, 1956).

Table 1. Name, response and origin of the lines used as parents for the analysis of resistance to *P. parasitica*.

Name	Response	Origin
UAN 6 (novillero)	Susceptible	Nayarit
UAN 23-1	Susceptible	Nayarit
3Q3	Susceptible	Guerrero
UAN 6-1	Susceptible	Nayarit
UAN 13	Resistant	Nayarit
Jersey acriollada	Resistant	Puebla
UAN 13-1	Resistant	Nayarit
10	Resistant	Guerrero
UAN 8	Resistant	Nayarit

In the following cycle, five plants of each F1 were established in a greenhouse in polyethylene bags with sterile soil for self-pollination (Table 2) and obtain F2 seed.

Table 2. Direct crosses obtained to obtain F1 and F2 seeds for the analysis of resistance to *P. parasitica*.

Crosses
UAN 6 (novillero) x Jersey creole
UAN 6 (novillero) x UAN 13-1
UAN 6 (novillero) x 10
UAN 6 (novillero) x UAN 8
UAN 23-1 x UAN 13
UAN 23-1 x Jersey creole
UAN 23-1 x UAN 13-1
UAN 23-1 x 10
UAN 23-1 x UAN 8
3Q3 x UAN 13
3Q3 x Jersey creole
UAN 6-1 x Jersey creole
UAN 6-1 x UAN 13-1
UAN 6-1 x 10

Endurance assessment

The four generations were established in the greenhouse to assess resistance, so seeds of the susceptible parent (P1), resistant parent (P2), F1 and F2 were sown in 250 ml pots with sterile soil. The four generations were established in a completely randomized design with three replications, 25 plants per repetition of each parent, 20 of the F1 and 30 of the F2, with an experimental unit of 10 to 25 plants. When the plants reached 20 cm in height, they were inoculated with zoospores of *P. parasitica* at the base of the stem with a concentration of 375 000 zoospores per plant, making a second inoculation 10 days later to avoid leaks.

Measurement of severity

The severity of the disease was recorded on two dates, starting six days after inoculation using an arbitrary scale with five severity levels, where 1) healthy plant with turgid leaves and green color; 2) necrosis <2 cm at the base of the stem and some leaves with chlorosis; 3) necrosis of 2 to 3 cm at the base of the stem and chlorotic leaves; 4) necrosis >3 cm in the stem, curved plant with chlorotic, wilted or defoliate lower leaves; and 5) dead plant. 56 genotypes were evaluated: 9 parents (with 28 combinations), 14 direct F1 and 14 F2 crosses, in a completely randomized design with three replications.

Statistical and genetic analysis

Data analysis was done with all four generations (susceptible and resistant parents, F1 and F2). First, the analysis of variance of the average severity of the four generations was performed. The significance of three contrasts of interest was evaluated, two of which are orthogonal. These contrasts allow evaluating the effect of the mean severity in the four generations.

Because the model proposed by Mather and Jinks (1971) did not fit the present study due to the lack of backcrossing and the absence of some crosses, the analysis of generational means with the available crosses was carried out according to Steel and Torrie (1990), resulting in the ANOVA in Table 3. The significance of the gene effects was calculated using the Student's t-test. All statistical analyzes were performed with the SAS 9.3 program (SAS Institute, 2012).

Table 3. Model used for the analysis of variance of severity in the four generations of Jamaica.

Variation sources	Degrees of freedom	Average squares
Dates (R)	(r -1)	$\sigma_\varepsilon^2 + b\sigma_\delta^2 + ab\sigma_\mathfrak{p}^2$
Crosses (A)	(a-1)	$\sigma_\varepsilon^2 + b\sigma_\delta^2 + r\sigma_{\alpha\beta}^2 + rb\sigma_\alpha^2$
Error (a)	(r -1)(a-1)	$\sigma_\varepsilon^2 + b\sigma_\delta^2$
Generations (B)	(b -1)	$\sigma_\varepsilon^2 + r\sigma_{\alpha\beta}^2 + ra\sigma_\beta^2$
Crosses x generations (AXB)	(a -1)(b -1)	$\sigma_\varepsilon^2 + r\sigma_{\alpha\beta}^2$
Error (b)	a(b -1)(r -1)	σ_ε^2

Estimation of genetic parameters

Heritability in the broad sense was calculated by applying the formula proposed by Warner (1952): $H^2= [V_{F2}-(V_{P1}+V_{P2}+V_{F1})/3]/V_{F2}$ where: H^2= heritability in the broad sense, V_{P1} = phenotypic variance of the susceptible parent, V_{P2}= phenotypic variance of the resistant parent, V_{F1}= phenotypic variance of F1, V_{F2}= variance phenotypic of F2.

Genetic advancement was calculated according to Johnson et al. (1955), with a selection intensity of i= 1% and i= 5% as follows: $AG= i \times H_A \times \sqrt{VF_2}$ where: H_A= heritability in the broad sense, i = level of selection intensity, V_{F2}= phenotypic variance of F2 .

The means of the four generations were significantly different (Table 4). The mean of the resistant parent (P2) and the susceptible parent (P1) were 1.43 and 2.43, respectively. A threshold was defined to decide whether the parents' crosses have dominant or recessive characteristics to the susceptibility, so the average of the parents [(P1+P2)/2] was used for this case was 1.94. The presence of dominance was observed when comparing the mean of the F1 and F2 generations with an average of 2.24 and 2.37, respectively. Because the mean of the F1 generation was 2.24, close to the value of the susceptible parent (2.4285), it can be deduced that the resistant x susceptible cross resulted in progenies with dominant characteristics to susceptibility (Table 4).

Table 4. Generational means of severity in jamaica plants inoculated with *P. parasitica*.

Generation	Average severity
P1	2.4285
P2	1.4357
F1	2.2464
F2	2.3678

P1= susceptible parent; P2 = resistant parent.

The results in Table 5 show that there were no significant differences for the main effect of the crosses or for the dates x crosses interaction. However, the analysis of variance of the four generations (P1, P2, F1 and F2) showed that the generations compared in pairs were highly significant (1% significance). The orthogonal comparison between the parents, P1 vs P2, showed that they were significantly different at 1%; while the comparison between F1 and F2 showed no significant difference. The mean of the two parents [(P1 + P2)/2] was significantly different (significance level 1%), compared to the mean of the F1.

The differences between resistant and susceptible parents (P1 *vs* P2) represented 77% of the sum of squares due to the generations (13.8 and 17.94, respectively), being the most important source of variation between the four generations. These results show the importance of additive effects in determining resistance to *P. parasitica*. Similar responses were reported in resistance to *Sphacelotheca reiliana* (Bernardo *et al*., 1992 and *Aspergillus flavus* (Hamblin and White, 2000), in corn.

The sum of squares corresponding to the contrast [(P1+P2)/2] *vs* F1, which could reflect the presence of heterosis is small, but significant and the lack of significance of the difference between F1 and F2 suggests the low importance of the effects. dominance, indicating that the presence of heterosis was mainly due to additive effects, evidence of the lack of inbreeding indicated by the absence of a significant difference between the means of F1 and F2.

Table 5. Components of variance for severity of *P. parasitica* in jamaica.

Variation sources	Degrees of freedom (gl)	Sum of squares (SC)	Squares media (CM)
Dates (a-1)	1	52.94	52.94*
Crosses (c-1)	13	4.17	0.32 ns
Dates x crosses (a-1)(c-1)	13	1.095	0.08
Generations (g-1):	3	17.94	
P1 *vs* P2	1	13.8	13.8*
F1 *vs* F2	1	0.21	0.21 ns
(P1+P2)/2 *vs* F1	1	1.84	1.8*
Crosses x generations (A x B)	39	6.84	0.175
Error C(a-1)(g-1)	42	10.99	
Total error	111	93.58	

*= significant at the 1% probability level; ns= not significant.

The heritability value, in the broad sense, was relatively high (37%) compared to values of 9 and 16% reported in tomato for resistance to *Alternaria alternata* suggest that the resistance character can be improved using a selection program (Wannows *et al*., 2015) and the predicted gains by selection intensity of 1% or 5% are 0.96 and 0.74 units, respectively. These values suggest that selection by the pedigree method may be an effective method (Marquez, 1988) in the development of varieties resistant to *P. parasitica*.

Conclusions

The additive effects were more important than the dominance effects for resistance to *P. parasitica*. Heritability, broadly speaking, was 37%. The results indicate that a pedigree breeding, program can be effective in developing superior varieties of Jamaica with genetic resistance to *P. parasitica* and improve existing characters.

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