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BRIEF COMMUNICATION

Pepper morphological traits related with resistance to *Phytophthora capsici*

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Abstract

Inheritance of 10 morphological and quantitative traits related to plant and fruit development and resistance to the pathogen *Phytophthora capsici* was studied in an intraspecific cross between a non-pungent, susceptible *Capsicum annuum* parent (cv. Americano) and a wild, pungent and resistant line (Serrano Criollo de Morelos-334). Data were obtained from the segregation of 166 F_2 plants and 50 F_3 plants in four years. Three of the traits analyzed (necrosis length, leaf width and leaf length) exhibited a transgressive segregation. A multiple linear regression analysis was applied in order to establish a relationship between necrosis length and some of the morphological traits measured such as length and width of leaf, length, diameter and mass of fruit, capsaicin content in fruits, and presence of hair on leaves and stems. The results identified a linear dependence between necrosis length (as an inverse measurement of resistance) and leaf width, fruit diameter and hair presence in the stem. Pungency was not related with resistance.

Additional key words: *Capsicum annuum*, pungency, necrosis length, breeding program.

Phytophthora capsici, causing root rot and shoot blight (Barksdale *et al.* 1984), is one of the most devastating field or greenhouse diseases of pepper plants (Ristaino *et al.* 1994). There are many studies about the interaction between different plants and *P. capsici* in order to get cultivars with durable resistance to late blight (Baysal *et al.* 2005, Egea *et al.* 1996, Evers *et al.* 2006). Many agronomical important traits in crop plants exhibit a continuum of phenotypic variation in a segregating population, suggesting that they are under the control of several genes, each of which may account for only a small portion of the existing phenotypic variation. One of these genes provides resistance to *P. capsici* in pepper (Smith *et al.* 1967, Pochard *et al.* 1983, Kim *et al.* 1989) probably with epistatic effects (Palloix *et al.* 1990).

In order to study the inheritance of fruit and plant characteristics and to examine the potential use of wild varieties of pepper as donors of valuable genes encoding agriculturally important traits, such as disease resistance to *P. capsici*, we crossed a non-pungent pepper (cv. Americano), with a wild pungent line (Serrano Criollo de Morelos-334). The cv. Americano is characterized by its spherical and non-pungent fruits, which exhibit a high degree of colour and are used in the production of paprika (Candela *et al.* 1984), and a very high susceptibility to *P. capsici* (Egea *et al.* 1996). Serrano Criollo de Morelos-334 is a wild type pepper characterized by small, conical and pungent fruits and a very high resistance to *P. capsici* (Gil Ortega *et al.* 1991). In this paper, we proposed a theorical correlation between some morphological traits and the resistance to the pathogen that may be used to accelerate breeding programs.

Pepper plants were grown from seeds in a *Fisons* (Vancouver, Canada) chamber with a 16-h photoperiod at 25 ºC and a humidity of 75 - 80 % in trays containing a steam-disinfected substrate (clay-loam soil, sand and humus-substrate; 2:2:6). After flower bud formation, the inflorescences were covered with perforated bags to prevent cross-pollination. Crossing was made by bud

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pollination of emasculated flowers, using line Serrano Criollo de Morelos 334 (SCM-334) as masculine parent and cv. Americano as female parent, and *vice versa*. The main raceme of individual F_1 plants was self-pollinated to obtain F_2 seed. F_2 plants were self-pollinated to obtain F_3. Both populations were cultivated as described above.

The oomycete used was *Phytophthora capsici* Leonian, isolate 17, which was maintained in the dark on potato dextrose agar (PDA) at 25 ºC. The tops of the stems of the plants were cut off and inoculated with plugs of actively growing mycelium of *P. capsici*. The stems of control plants were inoculated in the same way, but only with culture medium (without mycelium) (Candela *et al.* 1995).

Plants were measured at maturity when they were bearing fruit. The characteristics measured were: leaf length, leaf width, leaf length to width ratio, hair presence or absence on leaf and on stem, fruit length, fruit diameter, ratio of fruit length to fruit diameter, fresh fruit mass, presence or absence of capsaicin, extracted of *Capsicum* fruit powder using acetonitrile and measured by HPLC as described by Collins *et al.* (1995), and necrosis length 6 d after inoculation with *P. capsici* as described by Candela *et al.* (1995). All fruit and leaf dimensions and necrosis length were determined with a digital calliper.

Twenty plants from each of the two progenitors and their F_1 progeny, 166 F_2 plants (originating from a single F_1), and 50 F_3 plant (originated from resistant F_2 plants to *P. capsici*) were used in order to collect the data. The statistical analyses were performed using the R program (http://cran.r-project.org). An *ANOVA* based on fixed effects was carried out to compare the means of the four populations (Americano, SCM-334, F_1, F_2 and F_3), in order to study the differences between each morphological characteristic measured. The following conditions were assumed for the *ANOVA* test: *1*) The samples are taken randomly, *2*) the populations have normal distribution (K-S´s test, and *3*) variance was homogeneous. When these conditions were verified, a statistical analysis was carried out using the statistical package *SPSS version 10.0.7* (http://www.spss.com). When the homogeneity of variances could not be verified, the *BMDP version 1985* package was applied; however, the 7D program computed one additional one-way *ANOVA* as described in Brown and Forsythe (1974). A multiple linear regression analysis was made using the statistics program *SPSS 10.0.7* in order to correlate necrosis length and some of the variables measured.

The most viable cross was that made using line SCM-334 as male parent and cv. Americano as female. The reciprocal cross did not produce mature fruit. The two progenitors differed significantly in leaf length. The mean length of F_1 leaves pointed to the dominance of alleles coding for shorter leaves, while the mean length of F_3 leaves exhibited heterosis towards shortness, indicating a transgressive segregation for this trait. The leaf width segregated in a manner similar to leaf length, with narrow leaves being dominant. SCM-334 fruit were significantly longer than Americano fruit. The mean value of F_1 fruit length was similar to that of Americano, indicating a dominance of alleles coding for reduced fruit length. The mean value of the F_3 fruit length was similar to that of SCM-334 fruit. SCM-334 had significantly narrower fruit than Americano. There was a dominance of narrow fruits, meaning that most of the phenotypic variation of this trait is additive. The fruits of the two progenitors showed a large difference in their respective masses. The mean fresh mass of the F_1 fruit indicated a dominance of alleles coding for decreased fruit mass.

The measurement of necrosis length after inoculation by decapitation is a valid and easily repeated form of evaluating the resistance of a plant, which also permits the use of those which survive for other purposes. The two progenitors differed significantly from each other in necrosis length. The mean necrosis length of the F_1 generation indicated a dominance of alleles coding for shorter necrosis length. The mean F_3 leaf necrosis length pointed towards shortness, indicating a transgressive segregation since some plants of the F_3 generation showed a lower length of necrosis than SCM-334 and, therefore, greater resistance to the pathogen (Table 1).

In the present study we have analyzed how different morphological characteristics (of the plant and fruit) typical of each of the progenitor used (SCM-334 and Americano) are transmitted to the F_1 generation, and how these characteristics segregate in successive generations (Figs. 1 and 2). The aim was to relate these characteristics with the resistance to the pathogen *P. capsici* in order to predict resistance in pepper against this pathogen.

Table 1. Morphological traits and necrosis in parents and an intra-specific cross of *Capsicum annuum* line SCM-334 with *Capsicum annuum* cv. Americano. Means ± SE, $n = 3$. Means marked by different letters in each row are significantly different at $P = 0.01$.

Parameters	Americano	SCM-334	F_1	F_3
Leaf length [mm]	111.2 ± 1.0 c	88.0 ± 1.0 b	85.1 ± 1.2 b	59.0 ± 1.0 a
Leaf width [mm]	58.0 ± 1.6 c	37.0 ± 0.8b	34.6 ± 0.4 b	24.7 ± 0.5 a
Fruit length [mm]	32.3 ± 0.6 a	42.5 ± 0.4 b	33.2 ± 0.3 a	43.6 ± 3.3b
Fruit diameter [mm]	43.0 ± 0.5 d	15.4 ± 02 a	25.0 ± 0.2c	20.2 ± 0.6b
Fruit fresh mass [g]	15.1 ± 0.3 b	3.8 ± 0.1 a	3.7 ± 0.1 a	3.9 ± 0.3 a
Necrosis length [mm]	12.1 ± 0.3 d	1.7 ± 0.1 b	3.0 ± 0.1c	1.3 ± 0.1 a

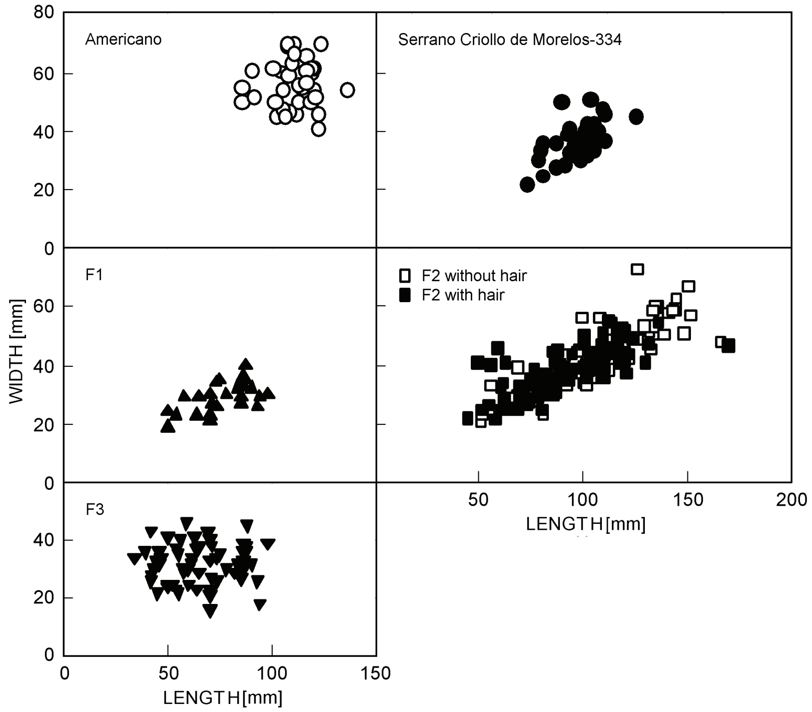

Fig. 1. Relationship between width and length of leaf, together with presence or absence of hair on stems or leaves. Bold shapes represent the presence of hair in stems and leaves.

The resistant plants (SCM-334, F_1 and F_3) will show small leaves, very similar to those of the SCM-334 progenitor and hair on stems and leaves. Thus, the presence of hair would be associated with resistance to *P. capsici* (Fig. 1). Capsaicin, which confers spicy flavour, was detected in SCM-334 fruits, and it was absence in Americano fruits. The F_1 generation conserved the capsaicin, while the F_2 and F_3 generations showed pungent and sweet fruits. Hence the presence of capsaicin in fruit is not linked with resistance to *P. capsici* (Fig. 2).

The relationship between resistance to the pathogen *P. capsici* (measured as necrosis length) and the morphological characteristics in pepper plants was proposed. Of the ten traits studied, the most closely correlated with resistance were the presence of hairs on the stem and leaf and the fruit width. Of these, the presence of hairs on the stem is an exclusion trait, all the resistant plants show that but not the susceptible plants (Fig. 1) and it can also be evaluated very early in 2 - 3 months old plants. Our results are similar to those obtained in other plant-pathogen interactions as *Gerbera jamesonii* and the parasitic *Encarsia formosa* (Sutterlin and Van Lenteren 1997), cotton and cotton leaf curl virus (Ashraf *et al.* 1999), where the higher leaf hair density improved the resistance to the pathogen.

In order to establish the relationship between necrosis length (dependent variable) and leaf length, leaf width, fruit length, fruit diameter, fruit weight, capsaicin presence in fruit and hair presence in stem (independent variables) a multiple lineal regression analysis (Draper and Smith 1998) was applied.

In the correlation between necrosis length (NL) and presence of stem hair (SH) [$NL = 12.105 - (10.130 \times SH)$] we see that the presence of hair in stem explains 98.2 % of the variance in necrosis length. In the correlation of NL with two traits SH and leaf width (LW) [$NL = 8.551 - (8.541 \times SH) + (0.061 \times LW)$], the two variables, SH and LW, explain 98.5 % of the variance of necrosis length. The variables, SH, LW and fruit width (FW) [($NL = 3.232 - (4.673 \times SH) + (0.073 \times LW) + (0.003 \times FW)$], explain 99 % of necrosis length. A linear equation relating the resistance to the fungus *P. capsici* with the morphological characteristics of each plant was obtained.

There is great disparity among different authors concerning the number of genes involved in the interaction pepper-*P. capsici*. The most convincing results suggest that resistance to *P. capsici* in SCM-334 pepper plants is polygenic and that the three genes that confirm resistance provoke a susceptible-resistant gradient in the segregating generations (Palloix 1986, Gil Ortega *et al.* 1992). However, other researchers have suggested that resistance in SCM-334 might be based on a two gene model (Guerrero and Laborde 1980), while, more recently, Walker and Bosland (1999) observed that the segregation of resistance in F_1 was dominant, but the number of genes required in the F_2 plants is dependent on the susceptible parent.

Our results show that resistance to the pathogen is dominant. The progenitor SCM-334 always showed 100 % survival and, in addition, all the members of F_1 were resistant (Table 1). These results agree with those of Walker and Bosland (1999) as regards the dominance of resistance in the F_1 generation.

One of the traits that is not included in the correlations, but which is important from an agronomic point of view, is the pungent taste of the fruit resulting from the presence of capsaicin (Blum *et al.* 2003, Minamiyama *et al.* 2005). This is a characteristic of SCM-334, but is not necessarily related with resistance to *P. capsici*, since F_3 fruits were both non-pungent and pungent (Fig. 2) and all of them were resistant. For this reason it is not included in any of the resistance prediction models. As far as we know there is no any direct relationship between capsaicin presence and resistance to any biotic stress, but capsaicin level are increased by abiotic stresses as water deficit (Sung *et al.* 2005).

Our result confirm SCM-334 might be considered a potential source for donating genes to promote resistance to *Phytophthora capsici* in pepper breeding programs as it was described earlier by Gil Ortega *et al.* (1992). According with the relationship established between different traits, plants phenotypically closest to this resistant progenitor would themselves be resistant.

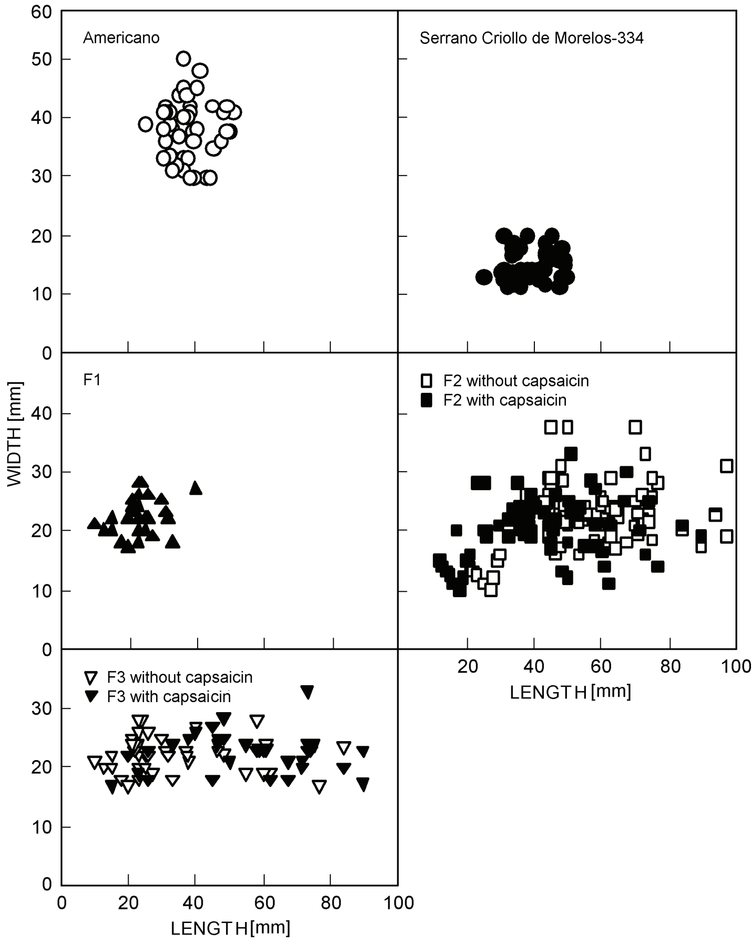

Fig. 2. Relationship between diameter and length of fruit, together with presence or absence of capsaicin. Bold shapes represent presence of capsaicin in fruit.

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