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Field resistance of selected potato clones to Early blight

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ABSTRACT
Six potato clones, selected *in vitro* for their resistance to *Alternaria solani* Sor. culture filtrates, were evaluated for their field response to early blight infection. Field screening were performance under artificial inoculation and natural conditions. Early blight response was evaluated based on lesion size, disease severity, and area under disease progress curve (AUDPC). One clone displayed reduced lesion area (0.35 cm^2) and AUDPC values compared to cv. 'Desirée' (susceptible control) (0.58 cm^2) but those values were higher than that of the resistant control *Solanum chacoense* 'PI 275136' (0.14 cm^2). On the other hand, no differences in lesion number were detected between the susceptible control and the selected clones. This variable showed values between 21.82 and 23.87 lesions in two leaves per plant. Although early blight resistance in potato is generally associated to late maturity, the mutant IBP-27 displayed increased resistance to early blight with medium-late maturity. The six clones presented medium-early to medium-late maturity, similar to parental cv. 'Desirée' (vegetative cycle ranging from 90 to 110 days). One clone was found to have higher levels of resistant to early blight than cv. 'Desirée' but lower than the levels of the resistant control *S. chacoense*. The resistance in this clone was characterized by the reduction in lesion area, disease severity, and AUDPC values in both artificial inoculation and natural infection screening.

Keywords: *Alternaria solani,* components of resistance, *Solanum tuberosum*

Resistencia en campo de clones de papa al tizón temprano

RESUMEN
Seis clones de papa, seleccionados por su resistencia *in vitro* al filtrado de cultivo de *Alternaria solani* Sor. se evaluaron para determinar su respuesta de campo a la infección por el tizón temprano. Los ensayos de campo se realizaron mediante inoculación artificial y en condiciones naturales. La respuesta al tizón temprano se evaluó en función del tamaño de la lesión, la severidad de la enfermedad y el área bajo la curva de progreso de la enfermedad (AUDPC). Un clon mostró menor área de la lesión (0.35 cm^2) y valores de AUDPC en comparación con el cv. 'Desirée' (control susceptible) (0.58 cm^2) pero esos valores fueron superiores a los del control resistente *Solanum chacoense* 'PI 275136' (0.14 cm^2). Por otro lado, no se detectaron diferencias en el número de lesiones entre el control susceptible y los clones seleccionados. Esta variable mostró valores entre 21.82 y 23.87 lesiones en dos hojas por planta. Aunque la resistencia al tizón temprano de la papa se asocia generalmente a la madurez tardía, el mutante IBP-27 mostró una mayor resistencia al tizón temprano con madurez media. Los seis clones presentaron una madurez a media temprana a media tardía, similar al cv. progenitor 'Desirée' (ciclo vegetativo que van desde 90 a 110 días). Se encontró un clon con niveles más altos de resistencia al tizón temprano que el cv. 'Desirée', pero inferiores a los niveles del control resistente *S. chacoense*. La resistencia en este clon se caracterizó por la reducción en el área de la lesión, la severidad de la enfermedad, y los valores AUDPC tanto en la inoculación artificial como en la infección natural.

Palabras clave: *Alternaria solani,* componentes de resistencia, *Solanum tuberosum*

Abbreviations: EB- Early blight, AUDPC- area under disease progress curve

INTRODUCTION

Early blight (EB) caused by *Alternaria solani* Sorauer is one of the most important foliar diseases of potato (*Solanum tuberosum* L.) (Pelletier and Fry 1990; van der Waals *et al.,* 2003). Fungicide application is the main control practice used worldwide (Gent and Schwartz, 2003). However, an increase in fungicide insensitivity in *A. solani* populations

has been reported (Pasche *et al.,* 2004). Genetic resistance offers an attractive alternative to chemical control because it reduces production costs and reduces the negative impact of fungicides in the environment (Shtienberg *et al.,* 1995).

Adequate levels of EB resistance are not known within cultivated potato species (Boiteux *et al.*, 1995; Cassells and Kowalski, 1998). However, sources of EB resistance have been identified in accessions of wild *Solanum* species. However, natural crossing barriers are present making difficulty the introgression of this resistance from wild species accessions into commercial varieties (Jansky, 2006). For that reason, selection of EB resistant cultivars with attractive commercial characteristics is not easily achieved (Cassells and Kowalski, 1998).

In vitro mutation treatment to induce variability followed by *in vitro* selection for EB resistance is considered an important tool in potato breeding (Cassells and Kowalski, 1998). *Alternaria solani* culture filtrates could be used to select EB resistant genotypes from susceptible ones (Lynch *et al.,* 1991; Martinez and Sinclair, 1994). Lynch *et al.* (1991) reported that results of culture filtrate assays did not correspond with the results from greenhouse or field resistance screenings. In contrast, Martinez and Sinclair (1994) described a high correlation between *in vitro* selection plants with *A. solani* culture filtrates and *in vivo* response to EB infection in greenhouse. However, part of the studies published were mainly focused on the evaluation of the effect of culture filtrates on either cells or tissues cultured *in vitro* and comparing them with the *in vivo* response of plants in greenhouse (van den Bulk, 1991).

Pelletier and Fry (1989) and Boiteux *et al.* (1995) characterized the response of different potato varieties and clones based on components of resistance. However, to the best of our knowledge, field characterization of early blight response of potato clones generated from irradiated tissue with increased levels of resistance to *A. solani* culture filtrate is not described yet.

We previously reported the selection of six potato clones, derived from the early blight susceptible cv. Desirée, with lower levels of infection under natural infection in both greenhouse and field conditions (Veitía *et al.,* 2007). The six clones were selected from a population of 1 000 putative mutants, obtained from irradiated callus cultures which were inoculated *in vitro* with culture filtrates of *A. solani*. In the present study, we describe the assessment of EB field resistance of the six potato clones under both artificial and natural inoculums conditions.

MATERIALS AND METHODS

Plant material

Six potato clones (IBP-27, IBP-30, IBP-38, IBP-93, IBP-101 and IBP-107) were used in this study. They were selected from irradiated callus cultures of the susceptible cv. 'Desirée' inoculated with *A. solani* culture filtrates (Veitía *et al.,* 2007). Two sets of field experiments were performed to assess the EB resistance of the mutant clones. Cv. 'Desirée' was used as the susceptible control (Lorenzo *et al.,* 1992) and wild potato accession *Solanum chacoense* BIT type 'PI 275136' as the resistant control (Estévez *et al.,* 1993). One experiment was conducted with artificial inoculation of the pathogen using mycelial suspensions and a second experiment was carried out under natural inoculum conditions.

Both experiments were planted in a field with a red ferralitic soil under irrigation at Pedro Lantigua Experimental Station (Remedios, Cuba). Tubers with 35-45mm diameter originated from *in vitro* cultured plants were used as seeds.

Artificial inoculation

Tubers of the six potato clones as well as of the susceptible and resistant controls were planted (for each experiments) in a randomized complete block design with four replicates. Each block was divided into eight plots corresponding to the six clones plus the susceptible and resistant controls (128 experimental plots in total). Each plot consisted of five rows with six plants (30 plants per plot). Seed tuber were planted 0.20 m apart

within and 0.90 m between rows. Fertilizer was applied at the rate of 1.5 t ha^{-1} in NPK formulation of 9:17:13. No fungicide was applied to control EB incidence. The 12 central plants of each plot were considered for evaluation.

An *A. solani* isolate (CCIBP-VAs_4) obtained from infected potato leaves in Las Antillas, Villa Clara, Cuba was used for artificial inoculation. Inoculum preparation was done as described by Leiva-Mora *et al.* (2006).

Plants (35 days after planting) were inoculated with a mycelial suspension. The inoculation was performed early in the morning by spraying until run off with a suspension containing $2x10^5$ mycelia fragments per milliliter supplemented with 1% (w/v) gelatin.

Two leaves of the middle section of the plants were evaluated weekly post inoculation for lesion number and diameter. The lesion diameter was measured with a ruler and the lesion area was estimated using the ellipse area formula (A= (a/2*b/2)* π), where, *a* is the length, *b* the width, and π=3.14.

EB progress was assessed weekly using the scale described by Horsfall and Barrat (1945). Disease severity was estimated using the Towsend and Heuberger formula **$I= (\sum(n* v)/ i*N)*100\%$** (where *n* is the total plants per degree of the scale, *v* is the rating of diagrammatic scale, *i* is the highest rating of diagrammatic scale, and *N* is the total plants evaluated).

Disease severity values were plotted against time and the area under disease progress curve (AUDPC) for each genotype was calculated using the mean disease severity values of each genotype (Jerger and Viljanen-Rollinson, 2001). Also, the vegetative cycle of plants was evaluated through tuber maturity.

Natural infection

This experiment was performed using the same design as previously described, except that four guard rows of cv. 'Desirée' were used around the entire the experimental field aiming to provide a source of natural inoculum of *A. solani* conidia.

Reaction to EB was assessed weekly starting from 45 days after planting. The visual estimation of disease damage (using the Horsfall-Barratt grading scale). Disease severity and AUDPC were estimated as described above. The vegetative cycle of plants, tuber yield and number were also evaluated.

Statistical analysis

The Statistical Package for Social science (version 13.0) for Windows was used to analyze the lesion size, disease severity and AUDPC data. Kruskal–Wallis non-parametric test was applied in the analyses.

RESULTS

Artificial inoculation

At seven days post inoculation (DPI), the lesions number was higher in all mutant clones (lesion number mean of 24.6-29.5) and in the susceptible control cv. 'Desirée' (lesion number mean of 29.3) when compared with the resistant control *S. chacoense* (lesion number mean 5.2) (Table 1).

The lesion area parameter allowed better discrimination of the mutant clone from the susceptible control (cv. 'Desirée'). One clone, IBP-27, displayed an average lesion area (cm^2) lower than the susceptible control but higher than the resistant control (*S. chacoense*) (Table 1).

The lesions in the *S. chacoense* accession were restricted to the infection sites with no expansion throughout the evaluation period. IBP-27 clone reaction was inferior to that observed in the resistant control, but lesion expansion was more restricted to the infection site than in the other clones as well as in cv. 'Desirée' (Figure 1).

Five out of six potato clones evaluated had diseases severity indexes (%) similar to the susceptible control, but IBP-27 clone displayed a disease severity index significantly lower than cv. 'Desirée' and significantly higher than *S. chacoense* (Figure 3).

Table 1. Lesion number and lesion area (cm^2) in six potato clones artificially inoculated with *A. solani* (7 DPI).

Clones	Lesion number in two leaves per plant	Lesion area (cm^2)
IBP-27	21.97 a ±0.296	0.35 b ±0.0329
IBP-30	21.82 a ±0.337	0.56 a ±0.0514
IBP-38	22.27 a ±0.455	0.53 a ±0.0433
IBP-93	23.20 a ±0.351	0.59 a ±0.0428
IBP-101	22.85 a ±0.357	0.61 a ±0.0579
IBP-107	23.57 a ±0.295	0.56 a ±0.0415
cv. 'Desirée'	23.87 a ±.726	0.58 a ±0.0549
Solanum chacoense	5.57 b ±.270	0.14 c ±0.0116

Data are means ± standard Error. Values followed by different letters are significantly different (p< 0.05), according to Kruskal Wallis test

Figure 1. Early blight lesions on leaves of IBP-27 clone, at 30 DPI. (a) Susceptible control (cv. 'Desirée'), (b) IBP-27 and (c) resistant control (*S. chacoense* 'PI 275136').

The six clones presented medium-early to medium-late maturity, similar to the parental cv. 'Desirée', with a vegetative cycle ranging from 90 to 110 days.

Natural infection

Under natural infection conditions, IBP 27 clone also displayed higher levels of EB resistance and with lower disease severity (%) when compared to the susceptible control and the other five mutant clones (Figure 3). Once more, the resistant control showed lower disease severity values (%) than IBP-27clone.

The clones IBP-30, IBP-38, IBP-93, IBP-101 and IBP-107 showed AUDPC values similar to that of the susceptible control cv. 'Desirée'. Only IBP-27, presented lower AUDPC values than cv. Desirée, but higher than resistant control (Figure 3).

IBP-27 showed similar response in both experimental conditions (artificial inoculation and natural infection screenings) with lower disease severity and AUDPC values compared to the susceptible control, although higher than in the resistant control. The six potato clones evaluated presented a similar vegetative cycle to the parental cv. 'Desirée'.

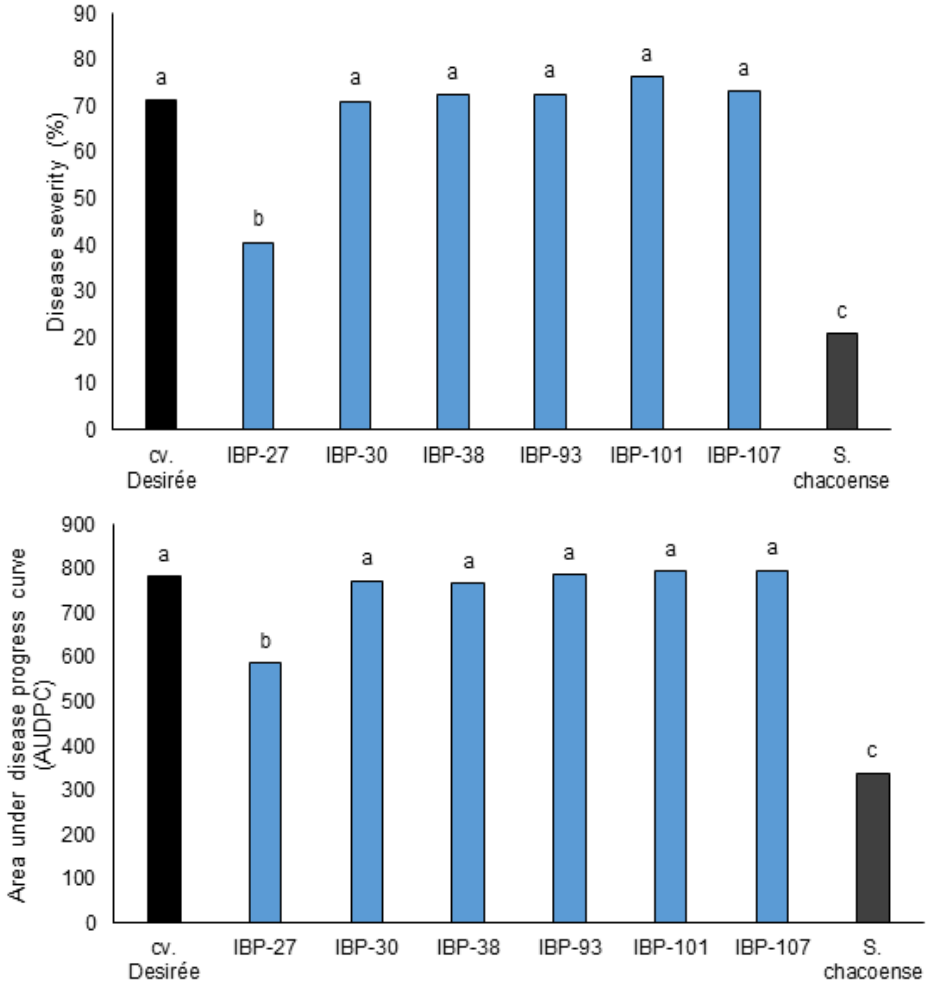

Figure 2. Disease severity (%) and area under the disease progress curve (AUDPC) in six potato clones artificially inoculated with *Alternaria solani* (30 DPI).Bars with different letters indicated significative differences at p< 0.05, according to Kruskal Wallis test.

DISCUSSION

In field trial under artificial inoculation one mutant clone (IBP-27) expressed improved levels of EB resistance. This clone was characterized as having lower lesion area values than the susceptible control and the other five mutant clones (Table 1). Similar results were described by Pelletier and Fry (1989) for a resistant potato cultivar 'Rosa'. These authors informed that the resistant response which delays tissue colonization after fungi penetration plays an important role in potato-*Alternaria solani* interaction. However, the smallest lesion area values were observed in the resistant control *S. chacoense*, confirming that this accession of this wild potato relative as being an excellent source of resistance genes to EB. This resistance can be introgressed into the cultivated *S. tuberosum* using 4x-2x (using synaptic mutants with 2n-pollen formation by first division restitution) breeding strategy (Peloquin *et al.,* 1999; Buso *et al.,* 2000).

It is interesting to note that no differences between lesion number of the susceptible control and the IBP-27 clone were detected. But, lower lesion number values in *S. chacoense* suggest that activation of resistant mechanisms might occur after the penetration stage.

For the parameter disease severity (%) and AUDPC the IBP-27 clone was found to have lower values than the susceptible control under both artificial inoculation (Figures 2) and natural infection (Figures 3). The others mutant clones had presented diseases severity similar to that

of the susceptible control. The results are in agreement with a recently reported study involving this group of potato clones (Veitía *et al.,* 2007). In that study, the IBP-27 clone was the only one identified displaying a significantly decreased early blight rating of affectation under natural infection under field conditions and had tuber yield similar to the susceptible control (cv. 'Desirée').

As described by Johanson and Thurston (1990) early blight epidemics initially progresses slowly, but it accelerates with the plant maturity. However, this increase in IBP-27 was less, as shown, by lower values of disease severity (%) and AUDPC at 30 DPI. Several authors described the use of diseases severity integrated over time as AUDPC to characterize cultivars according to the resistance level (Boiteux *et al.,* 1995; Christ and Haynes, 2001; Liatukas and Ruzgas, 2011; Duarte *et al.,* 2014) since it allows a better description of the resistance response in epidemiological terms (Pandey *et al.,* 2003).

No differences were found in the maturity cycle between the mutant clones and the parental cv. 'Desirée'. The identification of one potato clone with a reduced lesion area, an AUDPC value and identical maturity rating to cv. 'Desirée' suggests the existence of some mechanisms that acts regardless of the physiological maturity of the tissue. The association of late maturity with EB resistance has been documented for potato (Johanson and Thurston, 1990). These authors observed that late maturity clones were the most resistant ones but, not all early maturing clones were susceptible to EB pathogen. Also, they found one medium maturity clone that retained a resistant level to EB. In potato about half of the genotypic variation for EB resistance is also linked to maturity (Chaerani and Voorrips, 2006). However, different responses between potato cultivars with the same type of maturity had been found. For example, Boiteux *et al.* (1995) found one clone with high commercial production, lower values of AUDPC and early maturity.

The phytotoxicity of *A. solani* culture filtrates have been demonstrated in biotest on potato and tomato plants (Lynch *et al.,* 1991; Maiero *et al.,* 1991; Martinez and Sinclair, 1994). Most of secondary metabolites contained in *A. solani* culture filtrates are non host selective phytotoxins that interact with different target of the physiology of plants (Chaerani and Voorips, 2006). They act as virulence factor and intensify disease symptoms severity (Thommas, 2003). In the present work, *A. solani* culture filtrates (CCIBP-VAs$_4$) could be a factor of virulence because, a potato mutant selected *in vitro* showed improved field performance to EB which was characterized by a reduction in the lesion area, disease severity, and AUDPC values when compared with the susceptible control cv. 'Desirée'.

In summary, our studies allowed to characterize the resistance of potato clones selected *in vitro* via *A. solani* culture filtrates. Field evaluation of the selected clones allowed better characterization of EB resistance in the clones. Further studies are needed to extend our knowledge about other mechanisms which may be involved in the resistance to EB. These studies must be focused on the investigations on the role of *A. solani* metabolites in triggering resistance manifested as reduced lesion area.

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