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SHORT COMMUNICATION / *COMUNICAÇÃO*

Phytophthora nicotianae causing root and stem rot on *Dieffenbachia picta* in Argentina

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ABSTRACT

Dieffenbachia picta is a worldwide appreciated crop due to its ornamental value. In Argentina it is cultivated in warm provinces and in the outskirts of Buenos Aires city, where in spring 2007 a great amount of plants were lost in commercial greenhouses. Symptoms such as wilting and soaked lesions in the basal leaves began in four- to six-month-old plants causing plants to wilt due to basal stem and root rot processes. A Peronosporomycete was consistently isolated from diseased tissues. The pathogen was identified as *Phytophthora nicotianae* based on cultural characteristics, morphology of vegetative and reproductive structures, and on the analysis of the sequence of the nuclear ribosomal internal transcribed spacer (ITS) region. Pathogenicity tests were carried out and Koch's postulates were fulfilled. In complementary studies, the dieffenbachia varieties Alex, Compact, Camilla and Marianne were susceptible to the infection caused by *P. nicotianae*, whereas Tropic snow (*D. amonea*) was resistant. This is the first report of *P. nicotianae* causing stem and root rot of *D. picta* in Argentina and in the Americas.
Key words: *Peronosporomycetes,* ornamentals, susceptibility.

RESUMO

***Phytophthora nicotianae* causando podridão de raiz e caule em *Dieffenbachia picta em* Buenos Aires, Argentina**

Dieffenbachia picta ("comigo-ninguém-pode") é uma espécie difundida em todo o mundo devido ao seu valor ornamental. Na Argentina, é cultivada nas províncias de clima mais quente e nos arredores da cidade de Buenos Aires. Na primavera de 2007 um grande número de plantas apresentou sintomas de murcha e presença de lesões encharcadas (anasarca) nas folhas inferiores, principalmente em plantas de 4 a 6 meses de idade. Em seguida, constatou-se murcha da planta devido ao apodrecimento do caule e raiz. Um organismo peronosporomycete foi isolado de tecidos afetados. O patógeno foi identificado como *Phytophthora nicotianae* com base em características culturais, morfologia das estruturas vegetativas e reprodutivas, e testes moleculares baseados na sequência da região ITS do DNA ribossômico. Os testes de patogenicidade foram conduzidos e os postulados de Koch atendidos. Em estudos complementares, as variedades de *D. picta* Alex, Compact, Marianne e Camilla foram suscetíveis, enquanto a variedade Tropic snow (*D. amoena*) foi resistente. Este é o primeiro relato de *P. nicotianae* causando podridão radicular de *Dieffenbachia picta* na Argentina e nas Américas.
Palavras-chave: *Peronosporomycetes,* ornamentais, suscetibilidade.

Dieffenbachia picta Schott [Syn. *D. maculata* (Lodd. et al.) G. Don = *D. seguine* (Jacq.) Schott var *seguine*] (dieffenbachia, dumb plant, dumb cane), is a perennial ornamental herbaceous plant belonging to the Araceae family and is native to central and tropical forest regions of South America. The leaves are large and bright, in alternating green and white, favorable characteristics for marketing. It is easily cultivated and used as a potted houseplant with elegant indoor foliage. In Argentina it is grown mainly in Corrientes and Buenos Aires provinces but also in other northern warm provinces (JICA-INTEA, 2003). Most of the cultivated plants are hybrids or varieties of *D. picta* such as Exotic, Camilla, Marianne, Rudolph Roehrs and Alex among others. Plants with larger leaves belong to *D. amoena*, and the var. Tropic Snow is the most popular.

In spring 2007, plants of *D. picta* var. Marianne cultivated in some commercial greenhouses located in the vicinity of Buenos Aires city were affected by a new disease. (Grijalba et al, 2008). Although at low prevalence, the disease caused losses up to 70% in the affected greenhouses. The symptoms began early, at 2 to 3 months from the first transplant, coinciding with excess water in the substrate due to excessive irrigation. In spring 2008 and 2009, plants of *D. picta* var. Alex were also affected. Symptoms were chlorosis and wilting of the basal leaves (Figure 1A). They began as water-soaked lesions on the petioles and stem at the soil level. As the disease progressed

the whole plant wilted suddenly due to the soft stem and the root decay (Figure 1B).

Similar symptoms were described in different countries on plants of dieffenbachia infected by different species of the genus *Phythophtora*. The first report of this disease was in USA by Tompkins and Tucker (1947), who cited *P. palmivora* affecting *D. maculata* var Rudolph Roehrs. It was also cited in Puerto Rico (Wellman, 1977). In Europe, stem rot of plants of *D. maculata* was recorded in Germany for the first time and the causal agent was identified as *P. mexicana* Hots. *et* Hart. (Kröber et al., 1983). Later *Phytophthora* sp. was reported in Greece on *Dieffenbachia* sp. (Holevas et al., 2000), *P. nicotinae* on *D. maculata* in Poland (Orlikovsky et al*.,* 2001), and *P. citrophthora* on *Dieffenbachia* sp. in the United Kingdom (Jones & Baker, 2007). In Asian countries *P. nicotianae* was reported in Taiwan causing leaf blight (Ann, 1992) and in India on *D. picta* and *D. amoena* (Nema & Sharma, 2000).

The objectives of this research were to determine the etiology of stem and root rot of *Dieffenbachia picta*, and to assess the resistance of dieffenbachia varieties to the infection of the pathogen.

Diseased dieffenbachia plants were collected during 2007 to 2009, from four commercial greenhouses in the green belt of Buenos Aires city. Roots, basal stems and petioles were washed with tap water for pathogen isolation. Each sample was split in half and small sections of diseased tissue were placed on potato dextrose agar (PDA) and V8 juice agar media (V8A) + Pimaricin-ampicilin-rifampicin-pentachloronitrobenzene-hymexazol (PARPH), and incubated at 23 to 25°C. After 5 to 7 days of incubation, five colonies with cultural and morphological features of a peronosporomycete were transferred to PDA and V8A. One colony (BDF110) was morphologically and molecularly characterized and used to carry out the pathogenicity test.

Cardinal temperatures were determined on PDA. Petri dishes were inoculated in the centre with a 7 mm diameter agar plug. After 24h two lines were drawn perpendicularly to each other at the bottom of the Petri dish which intersected at the place of the inoculum and colony margins were marked on these lines. Petri dishes were subsequently incubated in the dark at temperatures of 0, 5, 10, 15, 20, 25, 30, 35 °C. After 24 and 48 h the radial growth was determined in all four directions. If growth stopped before the edge of the Petri dish was reached, the culture was returned to 20 °C to check if growth could resume and if the culture was still viable.

The plants were grown on pots with a mix of peat, perlite and 20% of soil. A 9 cm disc of V8A with a 10-day-old mycelium colony fully grown was cut and mixed with the soil around the root of ten plants of each variety. Control plants were inoculated with agar discs that did not contain fungal growth. Plants were covered with plastic bags for 48 h and incubated at 22 $\pm$ 2°C and 100% relative humidity, before being transferred to growth chambers at 25°C with natural light for 15 days.

DNA extraction was made from a pellet containing mycelium and reproductive structures of the oomycete grown in PDA, using the Nucleon™ PhytoPure™ Genomic DNA extraction kit (GE Healthcare) according to manufacturer's instructions. The ITS region of the nuclear rDNA was amplified using primers ITS4 and ITS5 (White et al. 1990). Amplification was carried out in 50 µl of PCR Buffer (Boehringer Mannheim) in the presence of 150 ng of each primer, 200 mM of each dNTP (Boehringer Mannheim), 10 ng of DNA and 2 U of Taq polymerase (Boehringer

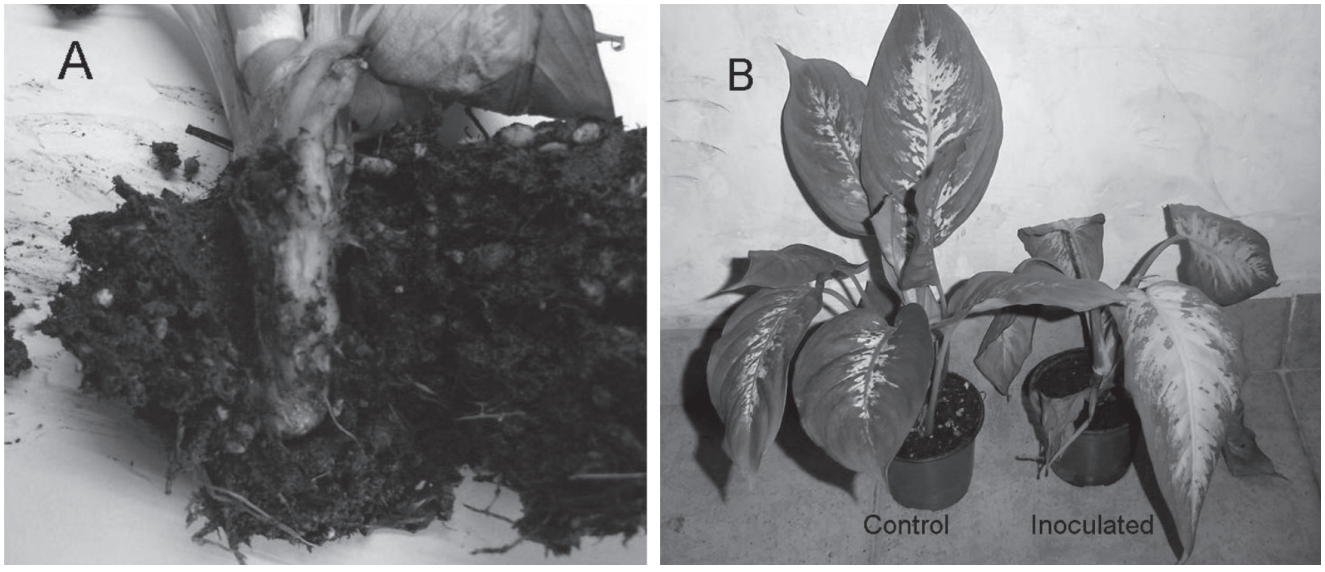

FIGURE 1 - Root and stem rot on *D. picta* caused by *P. nicotianae*. A. Destroyed roots by natural infection on cv Marianne. B. Symptoms of inoculated plant on cv Compact (grade 2 of severity).

Mannheim). Amplified DNA was electrophoresed on 1% (w/v) agarose gels run at 100 V for 1 h in tris-acetate buffer, stained with ethidium bromide, and photographed under UV light. PCR products were purified with the Qiaquick PCR purification Kit 50 (Qiagen) according to the manufacturer's instructions. Purified DNA was quantified by measuring the absorbance at 260 nm. Sequencing reactions with primers ITS4 and ITS5 were carried out using the dye terminator procedure with fluorescent dideoxynucleotides on a 7700 ABI Prism Sequence System (Applied Biosystems). In order to minimize sequencing errors, both strands were sequenced. The consensus sequence was inferred after assembling using the GeneTool Life 1.0 program (Layon M., 2000).

The rDNA sequences obtained were compared with oomycete sequences deposited in GenBank, using the Basic Local Alignment Search Tool (BLAST) program (Altschul et al., 1997). Results were validated against morphometric data, literature information and phylogenetic analysis.

To test varietal behavior of dieffenbachia against *P. nicotianae*, plants of approximately 30-40 cm in height (according to the variety) were placed in pots of 12 cm in diameter. The substrate and inoculation method were the same as in the pathogenicity test. For each variety, ten plants were randomly selected and inoculated with *P. nicotianae* and five plants were left as controls. The varieties studied were: Camilla, Compact, Alex, Marianne and Tropic Snow (*D. amoena*). After inoculation, pots were flooded for 48 h and kept at 25ºC in a growth chamber. Normal irrigation was carried out thereafter. Disease symptoms were recorded at 10, 17 and 28 days after inoculation, using the following scale: Grade 1 = no symptoms, healthy plant; grade 2 = yellowing of leaves and grade 3 = basal and root rot. The data were subjected to ANOVA for a completely randomized design and DGC post-hoc multiple comparison test (Di Rienzo et al*,* 2002). The assumptions of normal distribution and homoscedasticity of errors were tested using Shapiro-Wilks and Levene tests, respectively. Kruskal-Wallis nonparametric test and Dunn's multiple comparison test (Dunn, 1964) were used when the assumptions were not met.

Five isolates of the pathogen associated with the disease had the same growth pattern and morphological features: white colonies with abundant aerial mycelia. Cardinal temperatures were 1 and 35ºC, with the maximum growth rate at 30 °C on PDA. Sporangia were papillate, spherical, ovoid or obpyriform, terminal or intercalary (Figure 2A), formed singly or in a loose sympodium on long sporangiophores mostly persistent, or caducous with short pedicel. Measurements of sporangia were (30) 37.5 (49) µm x (18) 26 (37) µm with a length: breadth ratio from 1.4:1. Spherical chlamidospores were terminal or intercalary and formed singly (Figure 2B). Oospores were not observed**.** These characteristics are consistent with those described for *P. nicotianae* (syn*. P. parasitica* Dast.) (Erwin and Ribeiro, 1996).

Leaf chlorosis symptoms were observed seven days after inoculation. The disease progressed and caused stem breaks at the point of infection and whole wilting of the plants. When plants were pulled, rot and desegregation of the roots were observed. Control plants remained symptomless. Koch's postulates were confirmed by re-isolating the same microorganism from diseased plants.

Three sequences of *P. mexicana* [acc. # FJ801253 (holotype), FJ746654 and HQ261620], two reliable sequences of *P. nicotianae* [AF266776 (neotype) and AY423299] and the sequence obtained in this study were aligned using the Clustal W program. A phylogenetic tree was constructed with the MEGA program version 5.0, using the Neighbor-joining method with 1000 bootstrap replications (Figure 3). Sequence FJ801253 corresponds to the holotype of *Phytophthora mexicana* Hotson & Hartge 1923, type species WPC P0646 (CBS554.88, IMI92550, ATCC46731) isolated from tomato (*Solanum lycopersicum*) in Mexico (information provided by Z.G. Abad, USDA-APHIS-PPQ). Our sequence (GenBank HQ615719) has 100% identity with *P. nicotianae* specimens (Abad, 2010).

Resistance of the varieties differed ($P \leq 0.01$) in the three assessment dates (Table 1) Symptoms appeared 10 days after inoculation. In the most susceptible varieties

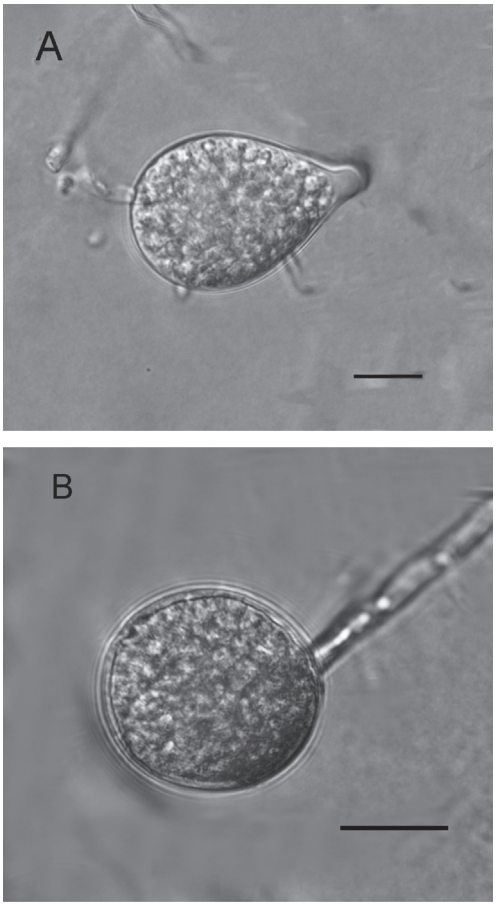

FIGURE 2 - *P. nicotianae.* A: Terminal papilate sporangia. Bar = 10 µm. B.Terminal spherical chlamidospore. Bar = 14 µm.

TABLE 1 - Comparison of severity scores for each variety at different days after inoculation

Variety	Days after inoculation					
	10		17		28	
	Mean	SD	Mean	SD	Mean	SD
Alex	2.10a	0.74	2.20a	0.79	2.30a	0.82
Camilla	2.20a	0.63	2.70a	0.48	3.00a	0.00
Compact	2.20a	0.42	2.70a	0.48	3.00a	0.00
Marianne	2.10a	0.57	2.90a	0.32	3.00a	0.00
Tropic snow	1.30b	0.48	1.30b	0.48	1.30b	0.48
H *	10.91		21.03		24.46	
p-value	0.0077		< 0.0001		< 0.0001	

Values followed for the same letters are not significantly different at P≤ 0.05.
* Kruskal Wallis Statistic
SD=Standard Deviation

(Camilla, Compact, Alex and Marianne) symptoms appeared on stems as irregular narrow, sunken, brown lesions near the soil line and evident chlorosis of lower leaves. Later, stem lesions enlarged rapidly. The root and crown rots were particularly destructive because the main stem weakened at the point of infection, collapsed and the plant wilted and died 7 to 10 days after the third observation. Leaves remained attached after the plants died. All plants of Camilla, Compact and Marianne varieties presented basal stem and root rot (grade 3) in the third assessment date, while only 50% of the 'Alex' plants presented this grade. Only 30% of the 'Tropic snow' plants yellowing of the leaves at the second assessment date. The lesions did not progress. 'Tropic snow' was highly tolerant, showing only rot of the secondary roots and darkening of the taproot. These plants were not killed by the pathogen but were stunted and slightly chlorotic.

According to previous reports, *P. nicotianae* is the prevalent species affecting dieffenbachia worldwide (Ann, 1992, Nema & Sharma, 2000, Orlikovsky et al., 2001). In Argentina this oomycete has been reported as an important pathogen in ornamentals, horticultural and fruit crops (Nome et al., 2010), but there was no report in plants of the Araceae family. The combination of molecular tests, morphological and cultural characteristics, and the phylogenetic tree showed that the BDF110 isolate clearly belongs to *P. nicotianae*. To our knowledge this is the first report of *P. nicotianae* causing dieffenbachia stem and root rot in Argentina and in the Americas. The isolate (BDF110) is kept in the culture collection of the Phytopathology Chair of the Facultad de Agronomia de Buenos Aires (FAUBA).

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