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Pith necrosis associated with *Pseudomonas viridiflava* in tomato plants in Brazil

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Abstract

This is the first report of the bacterium *Pseudomonas viridiflava* as the aetiological agent of tomato pith necrosis (TPN) in Brazil. The strain was identified by a scheme of tests for bacteria that emit fluorescence, known as LOPAT, and by sequencing the 16S rDNA region.

Key words – fluorescent bacteria – *Solanum lycopersicum* – vascular disease

Introduction

Pith necrosis is a general name for a plant disease which can be caused by different bacteria species (Passos et al. 2005). Up to now, only *Pseudomonas corrugata* and *P. mediterranea* were reported as the aetiological agent in Brazil. The former was reported in the States of São Paulo (Rodrigues et al. 1990), Rio Grande do Sul (Martins et al. 1990), Goiás (Quezado-Duval et al. 2007) and the latter was reported in the State of São Paulo (Rodrigues et al. 2010).

The disease affects tomato plants causing necrosis and destruction of the pith. Attacked plants present yellowing of the leaves and less development that may be related to lower productivity. However, damage associated with the disease are not clearly known since some authors report the disease with low aggressiveness and few expressive damage (Lopes & Quezado-Duval 2005) while others suggest that pith necrosis may cause huge losses (Fiori 2002).

Pseudomonas viridiflava, also reported as *Phytomonas viridiflava*, is a Gram-negative resident in the soil. The bacterium is classified as *Gammaproteobacteria*, order *Pseudomonadales*, family *Pseudomonadaceae*, and it has a wide range of hosts (Wilkie et al. 1973), but it was not previously reported as the aetiological agent of tomato pith necrosis in Brazil. Therefore, the aim of this study is to report the first occurrence of *P. viridiflava* associated with pith necrosis in Brazil.

Materials & Methods

Bacteria were isolated from tomato plants with pith necrosis symptoms, using nutrient agar (APHA 1917). The isolated strain was identified by a scheme of tests for bacteria that emit fluorescence, known as LOPAT, which is a series of determinative tests – L, levan production; O, oxidase production; P, pectinolytic activity; A, arginine dihydrolase production; and T, tobacco hypersensitivity (Lelliott et al. 1966), and by sequencing the 16S rDNA region. The molecular identification was performed by amplifying the V3-V4 region of the 16S rDNA, using the primers

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341F – CCTACGGGRSGCAGCAG (Wang & Qian 2009) and 806R – GGACTACHVGGGTWTCTAAT (Caporaso et al. 2012).

Koch's postulates were performed with Paronset tomato plants grown in a greenhouse and in the field. In the greenhouse, tomato seeds were sown directly in the soil. At age 30 days, using a needle, the plants were inoculated with 1 ml per plant of a suspension of 10^6 CFU/ml. Plants were grown for 7 days after inoculation. In the field trial, the bacteria were inoculated with a wood prick at 15 cm height from the soil, and kept growing for 60 days. The stems were then cut and sliced to see any possible colour modification of the pith compared to the control healthy plants, and the bacteria were re-isolated to complete Kock's postulates.

Results

In March 2017, Paronset tomato plants with pith necrosis symptoms were observed in commercial and experimental fields in Caçador, Santa Catarina State (Southern Brazil). The incidence of the disease was above 90% in affected fields. The symptoms observed, as a reaction of the affected plants, were brown spots and cracks in the stem, and adventitious roots in unusual places, even inside the stem. Cut stems revealed brown coloured pith. The disease caused necrosis and destruction of the pith of tomato plants (Fig. 1).

Fig. 1 – Symptoms of pith necrosis: A Advanced pith necrosis at the end of the plant cycle. B Pith necrosis progress at wounds done while cutting shoots, a putative entrance for *P. viridiflava*. C An atypical place for root formation due to pith necrosis.

Isolated bacterial colonies were white to cream in colour and reasonably circular in shape. Since the fluorescence emission in King's B medium (King et al. 1954) was positive, the LOPAT method for fluorescent *Pseudomonas* (Lelliott et al. 1966) was performed. The oxidase, the arginine dihydrolase and the levan tests were negative, while the tobacco hypersensitivity and the potato rot tests were positive, which suggests the presence of *P. viridiflava*. Sequencing of the 16S rDNA region and the use of the basic local alignment search tool (BLAST) confirmed the bacteria identity as *P. viridiflava*. The sequence was deposited in Genbank with the accession number MG396956. Using two storage methods, the isolate was kept in the culture media collections of the Phytopathology Laboratory at Caçador Experimental Station, Santa Catarina State, Brazil, identified by the accession number EPAGRI BacPvT1. The isolate was also stored in the Phytobacteria Culture Collection of the Instituto Biológico, São Paulo State, Brazil (WDCM 110) as IBSBF 3287.

After bacteria inoculation, the disease progressively spread from the inoculation point. In both the greenhouse (Fig. 2) and in the field (Fig. 3) inoculated plants had stem darkening and necrosis in the pith, while healthy stems had whitish green or white colour (Figs 2, 3).

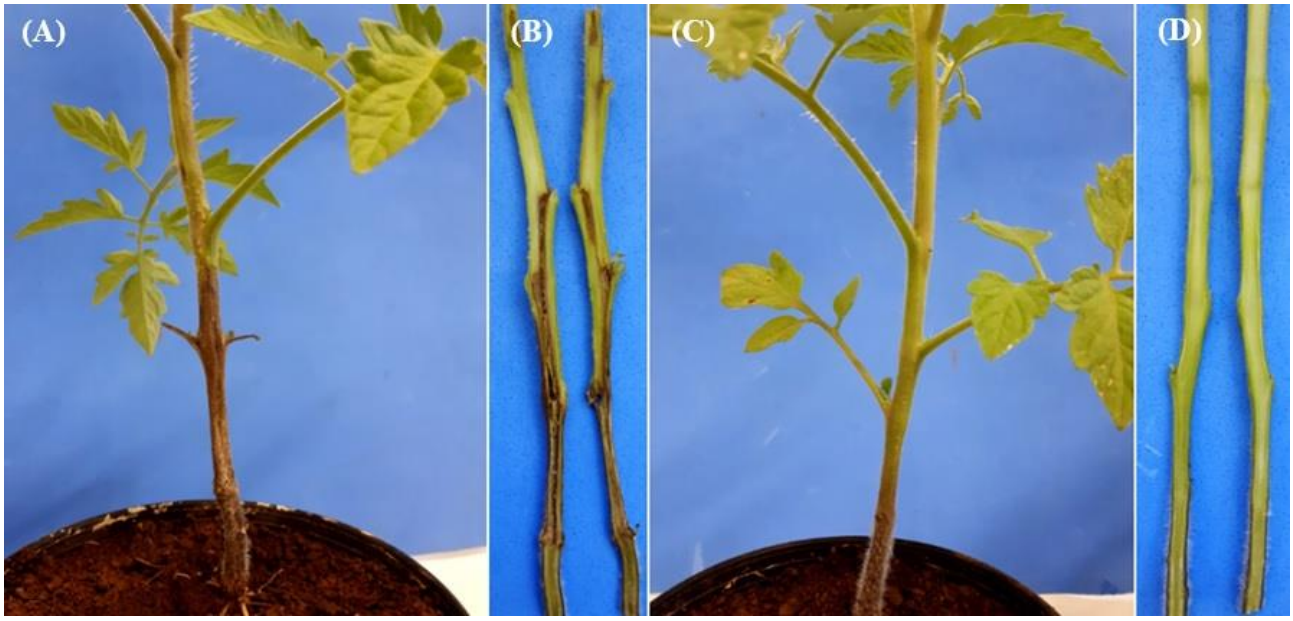

Fig. 2 – Symptoms of pith necrosis seven days after bacterial inoculation in tomato plants grown in greenhouse: A Diseased plant inoculated with *P. viridiflava* EPAGRI BacPvT1 showing the darkening of the stem. B Diseased stem showing the darkening in the inner tissue. C Healthy tomato plant with normal development. D Healthy stem showing normal whitish green coloured inner tissue.

Discussion

Affected plants may show yellowing in the leaves and poor growth, which may reduce yield and fruit quality. The disease has been spreading in the region of Caçador – Santa Catarina, Brazil. The main gateway for bacterial infection is the wounds caused after secondary sprout removal, which is a common practice in staked tomato fields. Research is in progress to test control measures, including protection of the wounds caused by sprout removal. The relationship established herein between *P. viridiflava* and tomato plants was also observed in Serbia (Popović et al. 2015), Argentina (Alippi et al. 2003), Portugal (Passo et al. 2008), Republic of Macedonia (Mitrev et al. 2014) and Turkey (Yildiz et al. 2004).

Maringoni et al. (2009) previously reported necrotic leaf spots caused by *Pseudomonas viridiflava* in tomato in the Brazilian States of Bahia and São Paulo. However, their report did not state the pith necrosis disease. Moreover, in further tests, we sprayed the bacterial suspension with isolate *P. viridiflava* Epagri BacPvT1 onto 60 days-old tomato plants, until runoff point, similar to

the procedure performed by Maringoni et al. (2009), but no symptoms were observed after 2 weeks. This result allows us to conclude that *P. viridiflava* Epagri BacPvT1 is not a foliage pathogen, which seems to be the conclusion reported by Maringoni et al. (2009). Therefore, these results allow us to say this is the first report of the pith necrosis in tomato plants caused by *P. viridiflava* in Brazil.

Fig. 3 – Symptoms of pith necrosis in tomato plants cultivated in the field, 60 days after bacterial inoculation: A Healthy tomato plant without *P. viridiflava* inoculation. B Inoculated tomato plant with pith necrosis caused by *P. viridiflava*.

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