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SHORT COMMUNICATION / *COMUNICAÇÃO*

Pathogenicity of Brazilian strains of *Ralstonia solanacearum* in *Strelitzia reginae* seedlings

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ABSTRACT

Twenty four strains of *Ralstonia solanacearum* belonging to races 1, 2 and 3 of biovars I, II and III, isolated from various hosts were investigated for their ability to cause disease on *Strelitzia* seedlings through artificial inoculation. Results revealed that, with one exception, only strains isolated from plants of *Musa* or *Heliconia* (classified as race 2) caused wilt symptoms on *Strelitzia*, indicating their pathogenic potential to that plant species. Seedlings of *Strelitzia* could be used as test plants for presumptive diagnosis for banana Moko disease.
Key words: diagnosis, inoculation, moko, wilt symptoms.

RESUMO

Patogenicidade de estirpes brasileiras de *Ralstonia solanacearum* em mudas de *Strelitzia reginae*

Vinte e quatro estirpes de *Ralstonia solanacerum* provenientes de vários hospedeiros foram avaliadas por meio de inoculação artificial para sua capacidade em causar doença em mudas de *Strelitzia*. Os resultados revelaram, com uma exceção, que apenas as estirpes isoladas de *Musa* ou *Heliconia* (classificadas na raça 2) causaram sintomas de murcha em *Strelitzia*, indicando seu potencial patogênico para essa espécie vegetal, ou pelo menos, que mudas de *Strelitzia* podem ser utilizadas como planta teste para o diagnóstico presuntivo da doença Moko da bananeira.
Palavras-chave: diagnóstico, inoculação, Moko, murcha bacteriana.

Ralstonia solanacearum is considered one of the most important bacterial plant pathogen, causing the bacterial wilt disease in more than 200 plant species belonging to 54 different botanical families (Elphinstone, 2005). This bacterial species has a high pathogenic and genetic diversity and these characteristics are used for the classification at infra-sub specific level into five biovars (Bv) based on carbohydrate utilization, and five races (R) based on the pathogenicity to different hosts (Buddenhagen et al., 1962; Hayward, 1991; Boucher et al., 2006). It can also be grouped into phylotypes or monophyletic clusters, distinguished based on the analysis of nucleotide sequences of multiple genomic regions: the ITS region and the *hrpB* (a regulator of type 3 secretion system) (Poussier et al., 1999), *Egl* (endoglucanase, a virulence factor) and *MutS* (a DNA mismatch repair enzyme) genes (Fegan & Prior, 2005; Prior & Fegan, 2005).

R. solanacearum strains differ considerably in host range as well as on aggressiveness to different hosts. For instance, race 1 strains (R1) have a very wide host range including numerous ornamentals, and are present in most regions of the globe, while race 2 (R2) strains are pathogenic to *Musa* spp and ornamental *Heliconia* spp, occurring in tropical areas of the Central and South America, Hawaii and Philippines (Kelman, 1953; Bradbury, 1986; Elphinstone, 2005). The race 3 (R3) or biovar II (BvII) strains are pathogenic mainly to potato and eventually infect tomato or other solanaceous hosts, including weeds, as well as geraniums; race 4 (R4) affects ginger (Pegg & Moffett, 1971) and race 5 (R5) is pathogenic to mulberry (He et al., 1983). The relationship among them is evident only in the R3 strains which are correlated with BvII (Hayward, 1991). In Brazil, *R. solanacearum* is widely distributed and marked by a great variety of hosts, including several species of ornamental and weed plants (Malavolta Jr. et al., 2008).

Regarding pathogenicity to ornamentals, the literature describes *Strelitzia reginae* (family *Strelitziaceae*, formerly *Musaceae*) as a host of *R. solanacearum* in Hawaii (Quinon & Agaraki, 1963) Japan (Liu et al., 2009) and Australia (Moffett, 1983), and these reports suggest that R1/BvIII was involved with the disease.

To investigate the behavior of *Strelitzia reginae* seedlings in relation to infectivity potential of Brazilian strains of *R. solanacearum*, pathogenicity tests were carried out through artificial inoculations with a set of 24 strains isolated from different hosts and regions of the country. Bacterial strains used in this study were obtained at the IBSBF Culture Collection and are classified as race 1, 2 or 3 and are listed in Table 1. For the inoculation experiments,

TABLE 1 - Origin of *R. solanacearum* strains examined in this study

# IBSBF *	Host	Locality	Race/Biovar	Phylotype
33	*S. lycopersicon*	Indaiatuba - SP	1 / I	IIA
134	*S. tuberosum*	Itutinga - MG	3 / II	II
172	*S. tuberosum*	Santa Juliana - MG	3 / II	-
187	*Musa* sp.	Humaitá - AM	2 / I	IIA
188	*Musa* sp.	Humaitá - AM	2 / I	IIA
615	*Musa* sp.	Pará	2 / I	-
623	*Eucalyptus* hib.	Jarí - PA	1/ I	IIA
891	*Musa* sp.	Manaus - AM	2 / I	-
1543	*Musa* sp.	Itacoara - AM	2 / I	IIA
1559	*Musa* sp.	Coari - AM	2 / I	-
1828	*S. tuberosum*	Bragança Paulista - SP	1 / I	-
1839	*Begonia* hib.	Holambra - SP	1 / III	I
1882	*Begonia* hib.	Atibaia - SP	1 / III	I
2000	*S. lycopersicon*	Manaus- AM	1 / III	I
2001	*S. lycopersicon*	Benjamin Constant - AM	1 / I	-
2131	*Eucalyptus urophylla*	Carbonita - MG	1 / I	IIA
2569	*Musa* sp.	Japoatã - SE	2 / 1	IIA
2644	*Heliconia* sp.	Abreu e Lima - PE	2 / I	IIA
2660	*Heliconia* sp.	Abreu e Lima - PE	2 / I	IIA
2661	*Heliconia* sp.	Abreu e Lima - PE	2 / I	IIA
2714	*S. lycopersicon*	Coimbra - MG	1 / I	-
2715	*S. melongena*	Cruz das Almas - BA	3 / II	-
2725	*Musa* sp.	Japoatã - SE	2 / I	IIA
2834	*S. tuberosum*	Mucugê - BA	3 / II	-

*IBSBF = Phytobacteria Culture Collection of the Instituto Biologico, Campinas, SP, Brazil.
- not determined

freeze-dried cultures were re-hydrated, and cultured on tetrazolium chloride (TZC) medium (Kelman, 1954). Virulent colonies were streaked onto nutrient agar (NA) medium and incubated at 28 ºC for 48 h. Colonies grown on NA were harvested into sterile distilled water and the cell suspensions adjusted to approximately $1x10^8$ colony forming units/mL concentration (OD 600 nm of 0.1) and used as inoculum.

Potted *Strelitzia* seedlings maintained in a greenhouse at the two to three expanded leaves stage were inoculated by wounding the youngest leaf axil with a sterile needle and pouring 30 µL of bacterial suspension over the wounded tissue (Winstead & Kelman, 1952). Control plants were treated similarly with sterile distilled water. Five plants were inoculated with each strain and evaluated weekly for symptom expression until eight weeks after inoculation. Plants showing symptoms were excised for microscope examination of the vascular system to confirm the systemic infection. Results are summarized in Table 2.

All *R. solanacearum* strains originated from *Musa* or *Heliconia* induced leaf chlorosis and/or wilting 4-8 weeks after inoculation (Figure 1A-B). These strains are all classified as R2/BvI. None of the plants inoculated with strains from other hosts (classified as R1 or R3) expressed any external symptom until the end of experiments, except the strain IBSBF 1828 (R1/BvI) isolated from potato, which induced wilt symptoms on leaves and two wilted plants after four weeks (Figure 2). One strain (IBSBF 134, R3/BvII) caused a limited infection in one plant near the inoculation site, eliciting stripes in the leaf (Figure 3), however no wilt symptom developed nor systemic infection was detected. All wilted plants examined under microscope showed bacterial colonization of the vascular system, including the roots adjacent to the cortex.

Although the literature report the occurrence of wilt disease on *Strelitzia* caused by *R. solanacearum* R1/BvIII strains in some countries, the results obtained indicate that with a single exception (strain IBSBF 1828 which showed highly virulence causing wilt four weeks after inoculation), only strains classified as R2 were able to infect the *Strelitzia* plants causing wilt symptoms.

R. solanacearum is a complex bacterial species and its virulence mechanisms are related to their genetic variability, and could be associated to several other factors, such as host range, geographic distribution, physiological properties, adaptation to different temperatures, and even spread by insects (Cellier & Prior, 2010; Milling et al., 2009). In this case, the susceptibility of *Strelitzia* (formerly classified in *Musaceae* family) to *R. solanacearum* R2 strains is probably no due the acquisition of genes or adaptation of

TABLE 2 - Number of the strain according time after inoculation and kind of symptom

Symptom	Nº of the strain	
	four weeks a.i.**	after eight weeks a.i.
Symptomless	33 (R1/BvI) [5]* 134 (R3/BvII) [4] 172 (R3/BvII) [5] 623 (R1/BvI) [5] 1839 (R1/BvIII) [5] 1882 (R1/BvIII) [5] 2000 (R1/BvIII)[5] 2001 (R1/BvI) [5] 2131 (R1/BvI) [5] 2714 (R1/BvI) [5] 2715 (R3/BvII) [5] 2834 (R3/BvII) [4]	33 (R1/BvI) [5] 134 (R3/BvII) [4] 172 (R3/BvII) [5] 623 (R1/BvI) [5] 1839 (R1/BvIII) [5] 1882 (R1/BvIII) [5] 2000 (R1/BvIII)[5] 2001 (R1/BvI) [5] 2131 (R1/BvI) [5] 2714 (R1/BvI) [5] 2715 (R3/BvII) [5] 2834 (R3/BvII) [4]
Leaf chlorosis or distortion	134 (R3/BvII) [1] 1828 (R1/BvI) [1] 2725 (R2/BvI) [4] 2834 (R3/BvII) [1] 2660 (R2/BvI) [1]	134 (R3/BvII) [1] 2834 (R3/BvII) [1]
One or two leaves wilted	187 (R2/BvI) [5] 188 (R2/BvI) [5] 615 (R2/BvI) [5] 891 (R2/BvI) [5] 1543 (R2/BvI) [5] 1559 (R2/BvI) [5] 2569 (R2/BvI) [5] 2644 (R2/BvI) [4] 2660 (R2/BvI) [3] 2661 (R2/BvI) [3]	
Plants wilted	1828 (R1/BvI) [4] 2644 (R2/BvI) [1] 2660 (R2/BvI) [1] 2661 (R2/BvI) [2] 2725 (R2/BvI) [1]	187 (R2/BvI) [5] 188 (R2/BvI) [5] 615 (R2/BvI) [5] 891 (R2/BvI) [5] 1543 (R2/BvI) [5] 1559 (R2/BvI) [5] 1828 (R1/BvI) [5] 2569 (R2/BvI) [5] 2644 (R2/BvI) [5] 2660 (R2/BvI) [5] 2661 (R2/BvI) [5] 2725 (R2/BvI) [5]

* Number in brackets indicates the number of plants for each strain.
** After inoculation

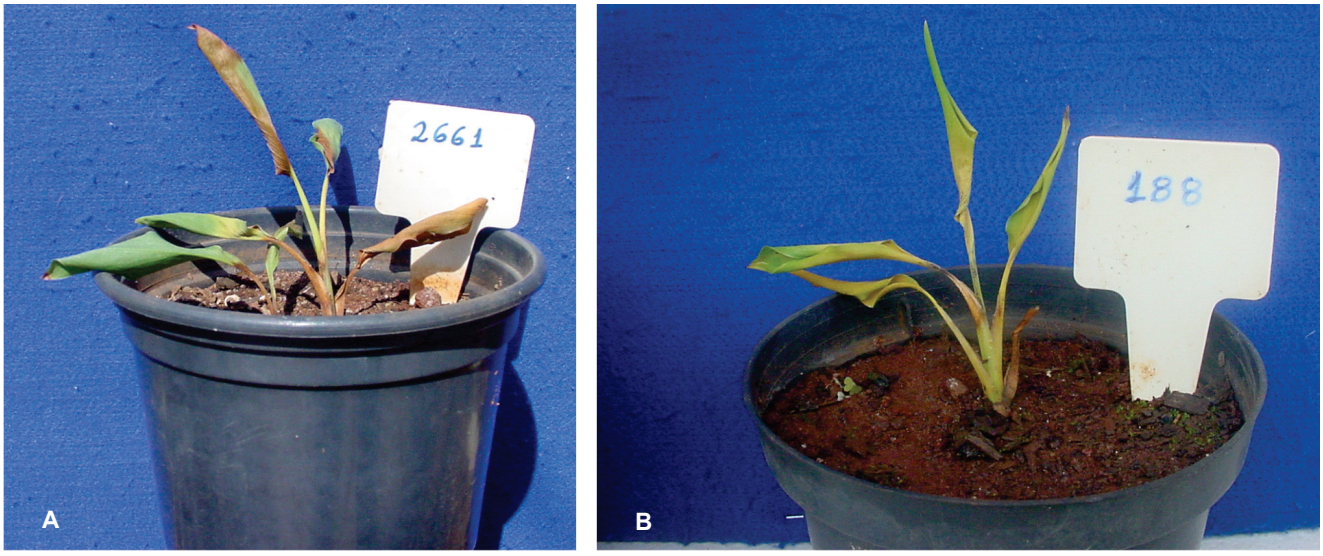

FIGURE 1 - Wilt symptoms induced by *Ralstonia solanacearum* strains IBSBF 2661 (R2/BvI) and IBSBF 188 (R2/BvI) four weeks after inoculation.

FIGURE 2 - Wilt symptoms induced by *Ralstonia solanacearum* strain IBSBF 1828 (R1/BvI) four weeks after inoculation; right, control plant inoculated with distilled water.

FIGURE 3 - Stripe symptoms elicited by *Ralstonia solanacearum* strain IBSBF 134 (R3/BvII).

the pathogen, but to natural host susceptibility to R2 strains. Thus, the results obtained indicated that *Strelitzia* could be used at least, as a test plant for the presumptive diagnosis of banana Moko disease.

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