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DISEASE NOTE

Bacterial wilt of sweet potato caused by *Ralstonia solanacearum* in Taiwan

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Abstract During the last decade, a new bacterial disease has impaired the yield of vegetable sweet potato (30–80%) in Taiwan. Infected plants developed stunting, root and stem rot, vascular discoloration and wilting. Ten bacterial isolates that caused the same symptoms in sweet potatoes after inoculation were reisolated and classified as *Ralstonia solanacearum* phylotype I biovar 4 based on physical and molecular analyses. Moreover, these isolates also caused wilting in convolvulaceous, solanaceaous and cruciferous plants. This report is the first of bacterial wilt of sweet potato caused by *R. solanacearum* in Taiwan.

Keywords Sweet potato · Bacterial wilt · *Ralstonia solanacearum*

Introduction

Sweet potato [*Ipomoea batatas* L. (Lam)] is a high-energy food due to its easily digestible carbohydrate (Clark and Moyer 1988). More than 100 countries produce sweet potato, and countries in Asia produce over 80% of the world's total volume (Anonymous 1998). In Taiwan, sweet potato is a major crop for food and forage, and certain cultivars, normally called vegetable sweet potato, can produce tender shoots with small/non tubers, which are consumed as a leafy vegetable (Hsing et al. 2000; Lai et al. 2000; Villareal et al. 1982). Currently, the annual production area of sweet potato in Taiwan ranges from 10,000 to 12,000 ha, and the yields have a gross value of ca. 60 million US dollars (Lai et al. 2008).

During the first decade of the 2000s, an unknown wilting disease on vegetable sweet potato was observed first in central Taiwan. The disease was later found in many production areas of vegetable sweet potato in Taiwan, such as Taichung, Changhua, Yunlin, and Chiayi, and impaired the yield of cultivars of vegetable sweet potato by ca. 30–80%. Diseased plants developed stunting, root and stem rot, defoliation, vascular discoloration and wilting, similar to those of Fusarium wilt caused by *F. oxysporum* f. sp. *batatas* (Clark and Moyer 1988). However, a bacterial ooze was found in wounds of diseased plants, and the stream of this ooze, that was easily observed in sterile distilled water, was distinct from Fusarium wilt. In the present study, we identified the causal agent of this wilting disease in vegetable sweet potato.

Symptoms

In the field, this disease was usually observed on vegetable sweet potato, rather than the tuber-producing type, causing yield losses over 50% (Fig. 1a). The symptom development in sweet potato started with stunting, then stem rot (Fig. 1b), root rot, plant wilt (Fig. 1c), vascular discoloration (Fig. 1d), followed by death of plants. Furthermore, bacteria oozed from a wound site (Fig. 1e), and a stream of ooze in sterile distilled water could easily be observed (Fig. 1f).

Isolation and identification of the pathogen

Sweet potato plants with wilting and yellowing symptoms were collected from production areas in Taichung,

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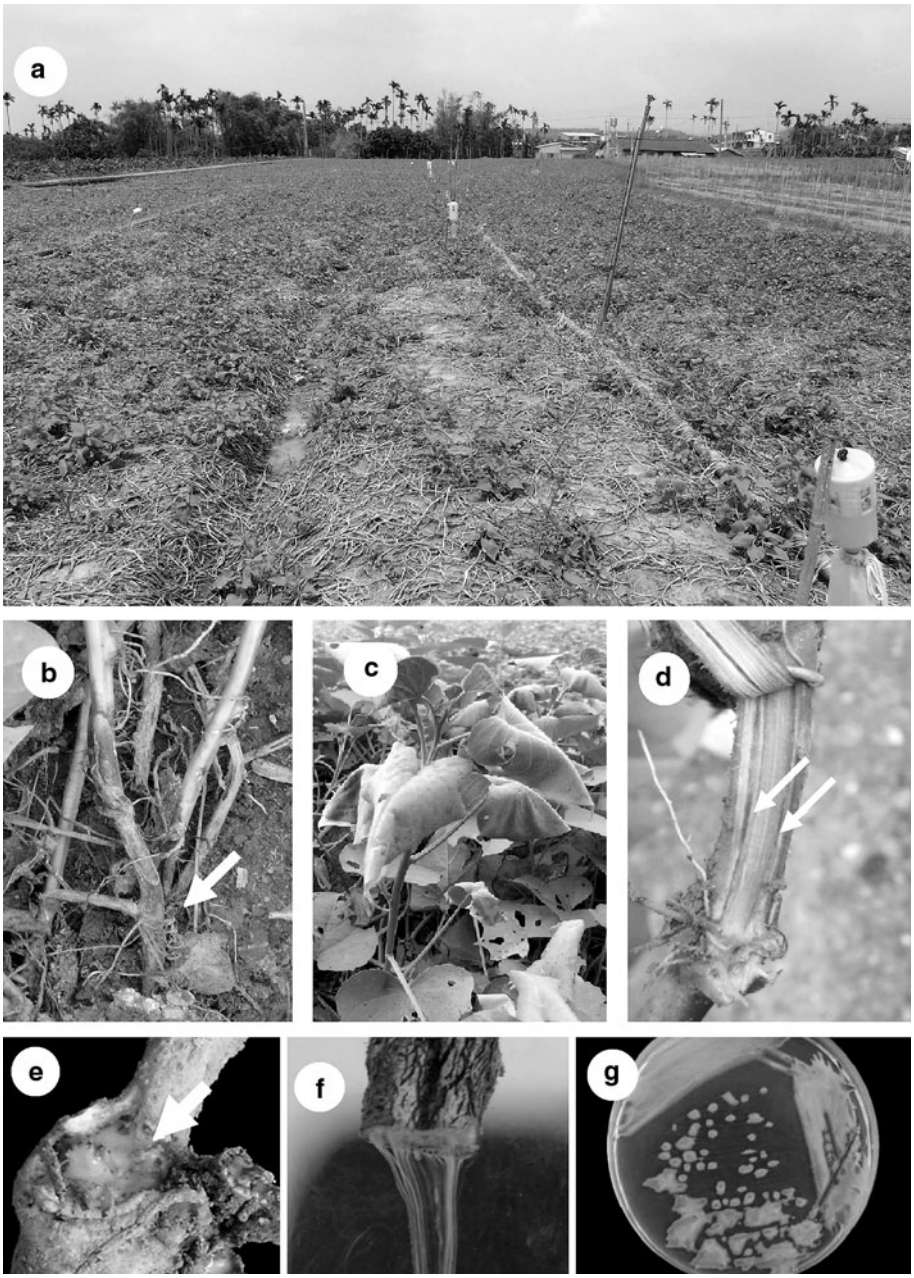

Fig. 1 **a** Bacterial wilt of sweet potato caused by *Ralstonia solanacearum* in fields in Taiwan. Diseased plants had stem rot (*arrow*) (**b**), wilt (**c**), and vascular discoloration (*arrows*) (**d**). Bacteria oozed from a wound (*arrow*) (**e**) and streamed in sterile distilled water (**f**). Colonies of bacterial isolates produced irregularly round, fluidal, white colonies with pink or red centers on TZC medium (**g**)

Changhua, Yunlin, and Chiayi. Symptomatic stems and roots were washed with tap water, then surface sterilized with a mixture of 5.25% sodium hypochlorite and 95% ethanol (1/1, v/v) for 15 s. Segments (0.2–0.5 cm) of surface-sterilized stems and roots were put into tubes containing 9 mL of sterile distilled (SD) water, which was then stirred for 3 min. The suspensions were streaked onto 2% Kelman's tetrazolium (TZC) medium (Kelman 1954) and incubated at 28°C for 48 h. The resultant bacteria-like single colonies were streaked onto TZC medium individually at least two times for purification. Ten bacterial isolates were collected from diseased sweet potato at different locations (Table 1), and all produced irregularly round, fluidal, white colonies with pink or red centers on TZC medium (Fig. 1g). Colony characteristics were observed on TZC and D1M (Perry and Kado 1982) medium after incubation at 28°C for 48 h. Gram staining, tobacco hypersensitivity reaction (HR) and other physiological tests were determined by standard methods (Schaad et al. 2001). Production of acids from the oxidation of carbohydrates used to identify biovars was determined according to the method of Hayward (1964). Strain R10 of *R. solanacearum* from tomato was tested as a control. The results showed that no isolate could grow at 40°C. All were negative for Gram staining, gelatin hydrolysis, starch hydrolysis, arginine dihydrase, growth on D1M medium, and fluorescence on King's B medium, but were positive for

Table 1 Collection information and classifications of the 10 isolates of *Ralstonia solanacearum* and pathogenicity results on sweet potatoes

Isolate	Location	Isolation date	HR induction[a]	Race	Biovar	Phylotype	Mean disease index on sweet potato[b]
RSBA01	Chunghua, Taiwan	Dec. 2005	HR	1	4	I	3.4
RSBA02	Taichung, Taiwan	Jan. 2006	HR	1	4	I	2.7
RSBA03	Yunlin, Taiwan	June 2006	HR	1	4	I	1.1
RSBA04	Chunghua, Taiwan	June 2006	HR	1	4	I	2.5
RSBA05	Yunlin, Taiwan	July 2006	HR	1	4	I	2.3
RSBA06	Taichung, Taiwan	July 2006	HR	1	4	I	4.0
RSBA07	Yunlin, Taiwan	Sept. 2006	HR	1	4	I	3.0
RSBA08	Yunlin, Taiwan	Sept. 2006	HR	1	4	I	2.0
RSBA09	Yunlin, Taiwan	Sept. 2006	HR	1	4	I	3.6
RSBA10	Chiayi, Taiwan	March 2008	HR	1	4	I	4.0

[a] Leaves of 3-week-old tobacco (*Nicotiana tabacum*) plants were infiltrated with 50 μL of 10^8 cfu/mL and monitored daily for disease development. Hypersensitive reaction developed within 24–48 h (HR) or the infiltrated leaves had necrotic lesions by 48–72 h

[b] Disease index at 4 week after inoculation was rated according to Winstead and Kelman (1952): 0, no symptoms; 1, plant stunting or one leaf wilted; 2, two or three leaves wilted; 3, all except the top two or three leaves wilted, stem and root rotted; 4, whole plant wilted

oxidase, catalase, citrate utilization, urease production, and tobacco HR. The bacteria could produce acid in media supplemented with mannitol, sorbitol or dulcitol (Table 2). On the basis of these results, the bacterial isolates were identified as *Ralstonia solanacearum* (Yabuuchi et al. 1995) belonging to biovar 4 (Hayward 1964).

Phylotype identification

For phylotype classification of the *R. solanacearum* isolates from sweet potato in Taiwan, phylotype-specific multiplex-PCR was used to analyze these isolates (Fegan and Prior 2005). The total reaction mixture of 25 μL consisted of 5 μL PCR Master Mix kit RP02 (Gene Mark Co., Taiwan), 2 μL of boiled bacterial suspension, 40 ng of the phylotype-specific primers Nmult:21:1F, Nmult:21:2F, and Nmult:22:InF; 80 ng of the primer Nmult:23:AF; and 40 ng of the *R. solanacearum*-specific primers 759 and 760 (Opina et al. 1997). PCR amplification was performed in a PCR machine (Perkin-Elmer GeneAmp PCR system 2400, Waltham, MA, USA). The PCR reaction program was run for one cycle at 94°C for 10 min; followed by 30 cycles of 94°C for 30 s, 55°C for 1 min and 72°C for 1 min; and a final extension of 72°C for 1 min. Two DNA fragments, one of 282 bp and the other of 144 bp, were amplified from all isolates. The *R. solanacearum* isolates were thus identified as phylotype I (Fegan and Prior 2005).

Pathogenicity tests in sweet potato and other hosts

The ca. 10 cm cuttings of sweet potato seedlings were planted in peat moss (T059, Kekkilä Garden, Finland) in polystyrene plugs with 6 × 6-cm cells. All cuttings were grown in a greenhouse at 25–35°C for 3 weeks. The seedlings were first transplanted into pots (20 cm in diameter, 15 cm in height) of peat moss. The bacterial inocula, enriched on plates of 523 medium (Kado and Heskett 1970), were picked up with a sterile toothpick, which was then inserted into sweet potato stems at the first node above the soil line. Each isolate was tested in seven seedlings with three repeats. Stems inoculated with sterile distilled (SD) water were used as controls. All inoculated plants were grown in a greenhouse. The disease index was evaluated 4 weeks after inoculation according to Winstead and Kelman (1952), and all experiments were done twice. All bacterial isolates from sweet potato were pathogenic to sweet potato and could be reisolated from the diseased plants 4 weeks after inoculation.

One isolate, designated as RSBA01 and originally isolated from sweet potato, was tested with a stem-pricking method for pathogenicity on various hosts. The tested plants included sweet potato (cv. Taoyuan 2), water convolvulus (*Ipomoea aquatica* Forsk cv. Taoyuan 1), loofah [*Luffa cylindrica* (L.) M. Roem. cv. Tung Kuang], tomato (*Solanum lycopersicum* L. cv. Farmer's 301), potato (*Solanum tuberosum* L. cv. Kenebec), eggplant (*S. melongena* L. cv. Nung Yu Chang Chieh), sweet pepper (*Capsicum annuum* L. var. *grossum* Seudt cv. Blue Star), chili (*C. annuum* L. var. *annum* cv. Group Star) and radish (*Raphanus sativus* L. cv. Tameihua). The cuttings of sweet potato and tuber seed pieces of potato were directly planted in plastic pots (20 cm in diameter, 15 cm in height) of peat moss, each pot containing seven cuttings or seed pieces. Seedlings (3 week old) of other plants were also grown in plastic pots (20 cm in diameter, 15 cm in height) with peat moss and inoculated. All inoculated plants were kept in a

Table 2 Physiological and biochemical properties of isolates of *Ralstonia solanacearum* from sweet potato

Character	Isolate	
	RSBA01-10	*R. solanacearum* R10[a]
Gram stain	−	−
Tobacco HR	+	+
Oxidative on glucose	+	+
Kovacs' oxidase	+	+
Arginine dihydrolysis	−	−
Growth at 41°C	−	−
Growth on D1M	−	−
Gelatin hydrolysis	−	−
Starch hydrolysis	−	−
Nitrite from nitrate	+	+
Fluorescent pigment	−	−
H_2S from cysteine	−	−
Catalase	+	+
Citrate utilization	+	+
Urease production	+	+
Indole production	−	−
Salt tolerance		
1% NaCl	+	+
2% NaCl	−	−
Acid production from carbohydrates		
Lactose	−	−
Maltose	−	−
Cellobiose	−	−
Mannitol	+	+
Dulcitol	+	+
Sorbitol	+	+

[a] *R. solanacearum* R10 was isolated from tomato as check

+ positive reaction, − negative reaction

greenhouse at 25–35°C. The disease ratings were recorded 4 weeks after inoculation. The experiment was performed with two replications. Results showed that RSBA01 was pathogenic to convolvulaceous (sweet potato and water convolvulus), solanaceous (tomato, potato, sweet pepper, hot pepper, and eggplant), and cruciferous (radish) hosts, but was unable to cause disease on loofah.

Conclusion

The results revealed that the new wilting disease of sweet potato found in Taiwan was caused by *R. solanacearum* race 1 biovar 4 (Buddenhagen et al. 1962; Hayward 1964), which strongly suggested that the new wilting disease is a bacterial wilt of sweet potato. This report is the first demonstrating bacterial wilt of sweet potato caused by *R. solanacearum* in Taiwan even though it was previously reported in China (He et al. 1983; Zhen and Fan 1962). O'Sullivan et al. (2005) warned that any suspected occurrence should be reported because the potential for the spread of the bacteria to other areas cannot be ignored.

Ralstonia solanacearum is a soil-borne pathogen causing diseases of more than 200 species from 50 families of plants (Hayward 1991). In addition, this bacterium has a high level of genotypic and phenotypic diversity and is considered as a "species complex'' (Gillings and Fahy 1994). Recently, most of the strains of *R. solanacearum* in Asia have belonged to phylotype I (Fegan and Prior 2005; Villa et al. 2005), but a small number of strains have belonged to phylotype II (Xu et al. 2009). Phylotype I strains in Japan can be further classified into many different types on the basis of HR in tobacco leaves and phylogenetic analyses of endoglucanase gene *egl* and DNA gyrase subunit B gene *gyrB* (Liu et al. 2009). In this study, all isolates from sweet potato caused HR in tobacco leaves, and all belonged to phylotype I. In a future study, the genetic diversity among the different isolates from sweet potato or several other hosts for *egl*, *hrpB* and *gyrB* will be analyzed to elucidate differences among isolates from sweet potato and other hosts.

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