

Bacterial wilt of geranium and portulaca caused by *Ralstonia solanacearum* in Japan

Katsumi Ozaki¹ and Hiroshi Watabe²

¹Laboratory of Plant Pathology, Department of Horticulture, Faculty of Horticulture, Minamikyushu University, Takanabe, Miyazaki 884-0003, Japan; ²Tokyo Branch, Yokohama Plant Protection Station, The Ministry of Agriculture, Forestry and Fisheries of Japan, Aomi, Tokyo 135-0064, Japan

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New bacterial disease was observed on geranium and portulaca in Miyazaki Prefecture, Japan in 2002. The leaves were wilted and yellowed, and the stem and roots were rotted. Faint discoloration of the vascular bundles were observed. When the cut of the stem was dipped into water, white bacterial ooze was visible. The colony appearance of the isolated bacterium was similar to that of *Ralstonia solanacearum*. The bacterium was pathogenic to both geranium and portulaca. Its bacteriological properties agreed with those of the standard isolates of *R. solanacearum*. From these results, the bacterium was identified as *R. solanacearum* biovar 3. Bacterial wilt of geranium and bacterial wilt of portulaca were proposed for the names of the disease.

Key words: *Ralstonia solanacearum*, bacterial wilt, geranium, portulaca, new disease.

INTRODUCTION

Geranium (*Pelargonium x hortorum*) and portulaca (*Portulaca grandiflora* Hook) are commonly cultivated worldwide as popular ornamental flowers. In the summer of 2002, an unknown disease was observed on geranium and portulaca in Takanabe, Miyazaki Prefecture, Japan. The disease was observed on plants in a flower garden. The symptom appeared wilting on leaves and dark brown lesions on stems. When the cut surface of the affected stems were dipped into clear water, bacterial ooze exuded visibly. Bacterial white fluidal and mucoid colonies on potato peptone glucose agar (PPGA) plates were consistently isolated from the infected plants. Our preliminary studies suggested that the disease was caused by *Ralstonia solanacearum* (Ozaki and Watabe 2004). Southern bacterial wilt of geranium was firstly reported in U.S. in 1981 (Strider et al. 1981). And the disease was reported to be caused by *R. solanacearum* race 3 biovar 2 (USDA 2001, 2004). This paper describes the bacterial wilt of geranium and portulaca in Japan.

MATERIALS AND METHODS

Bacterial test. The bacteria were isolated from the stem and root of the infected plants. White fluidal and mucoid colonies resembling those of *R. solanacearum* were isolated on PPGA (Nishiyama and Ezuka 1977) plate by the conventional plating method. In the study, each ten isolates from geranium and portulaca were examined. For long term preservation, bacterial cells were suspended in skim milk broth (10g skim milk, 1.5g sodium glutamate, 100ml distilled water) and lyophilized. For comparison, cultures of *R. solanacearum* MAFF301521 (biovar 4), MAFF301525 (biovar 3), and MAFF730133

(biovar 2 · N2) were used (Horita and Tsuchiya 2002, Horita 2006).

Pathogenicity test. Each five isolates from geranium and portulaca were tested for pathogenicity to geranium (*Pelargonium x hortorum*, cv. unknown) and portulaca (*Portulaca grandiflora*, cv. unknown). The following plants were inoculated to determine the host range: tomato (*Lycopersicon esculentum* Mill. cv. Ponderosa), tobacco (*Nicotiana tabacum* L. cv. Bright Yellow), eggplant (*Solanum melongena* L. cv. Senryo No.2), potato (*Solanum tuberosum* L. cv. Danshaku), sesame (*Sesamum indicum* L. cv. Shirogoma), sweet pepper (*Capsicum annuum* L. cv. New Ace).

For inoculation tests, the plants were grown in 15cm diameter pots for 6 to 8 weeks in greenhouse at 20 to 25°C. The plants at the 5 to 7 true-leaf stages were inoculated with the isolates by the cut-root method (Ozaki and Kimura 1989). For the cut-root inoculation, the potted soil was stabbed deeply with a knife, and then the bacterial suspension (about 10⁸ cfu/ml) was poured into the soil. The inoculated plants were kept in the greenhouse to observe disease development.

Bacteriological properties. Each ten strains of geranium and portulaca isolates were tested for bacteriological properties by the protocol described by Goto and Takikawa (1984). Poly-β-hydroxybutyrate granules were detected by the method of Burdon (Burdon 1946). All tests were done at 28°C except gelatin liquifaction test at 20°C.

RESULTS AND DISCUSSION

Symptoms. Disease symptoms of geranium appeared on leaves as lower leaf wilting and yellowing. The

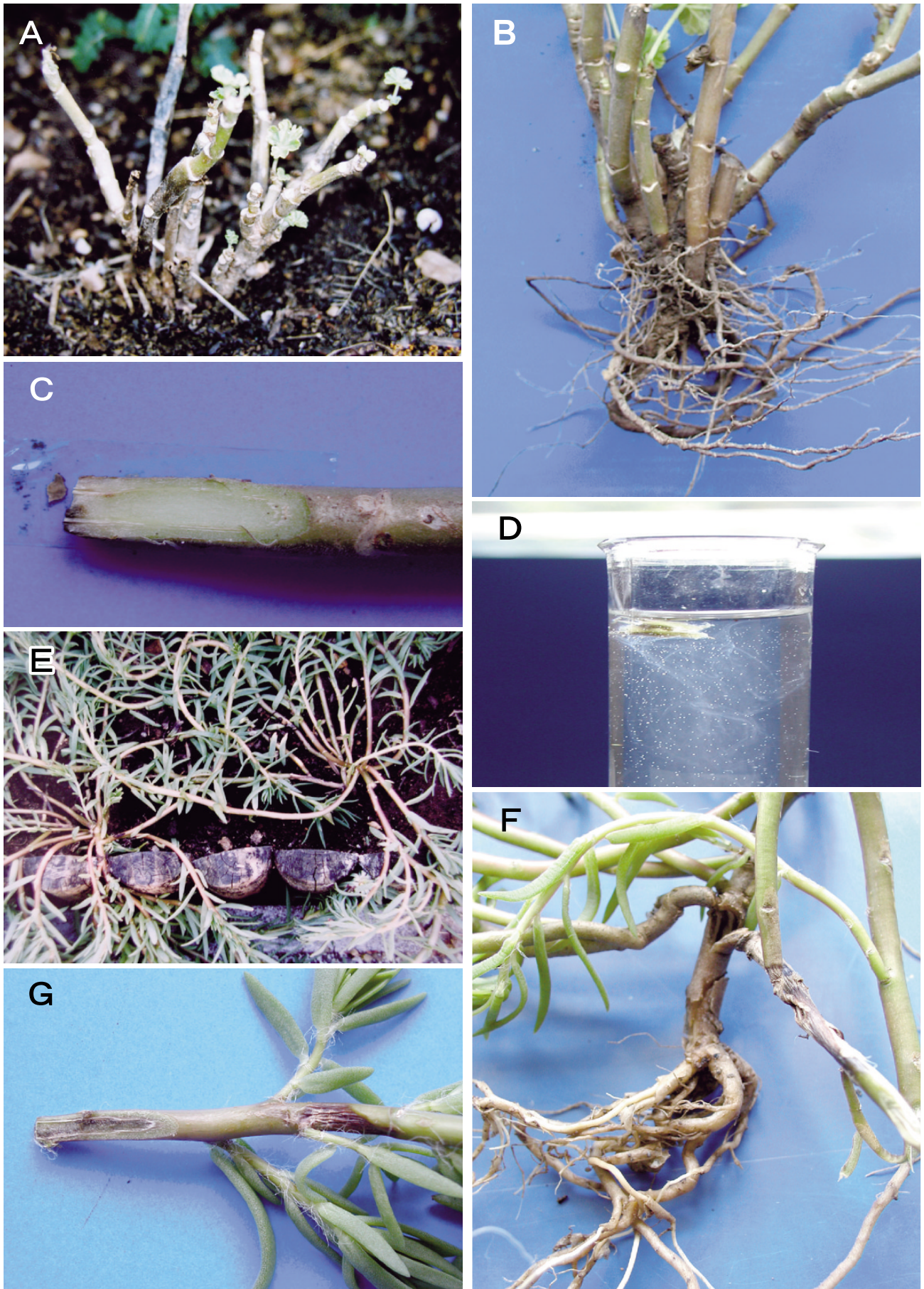


Fig. 1. Naturally occurrence symptoms of bacterial wilt on geranium and portulaca. A) Severe symptoms of geranium; B) Affected geranium roots; C) Browning vascular bundles of geranium; D) Exuded bacterial ooze from affected stems into water; E) Moderate symptoms of portulaca; F) Affected portulaca roots; G) Browning vastems into scular bundles of portulaca.

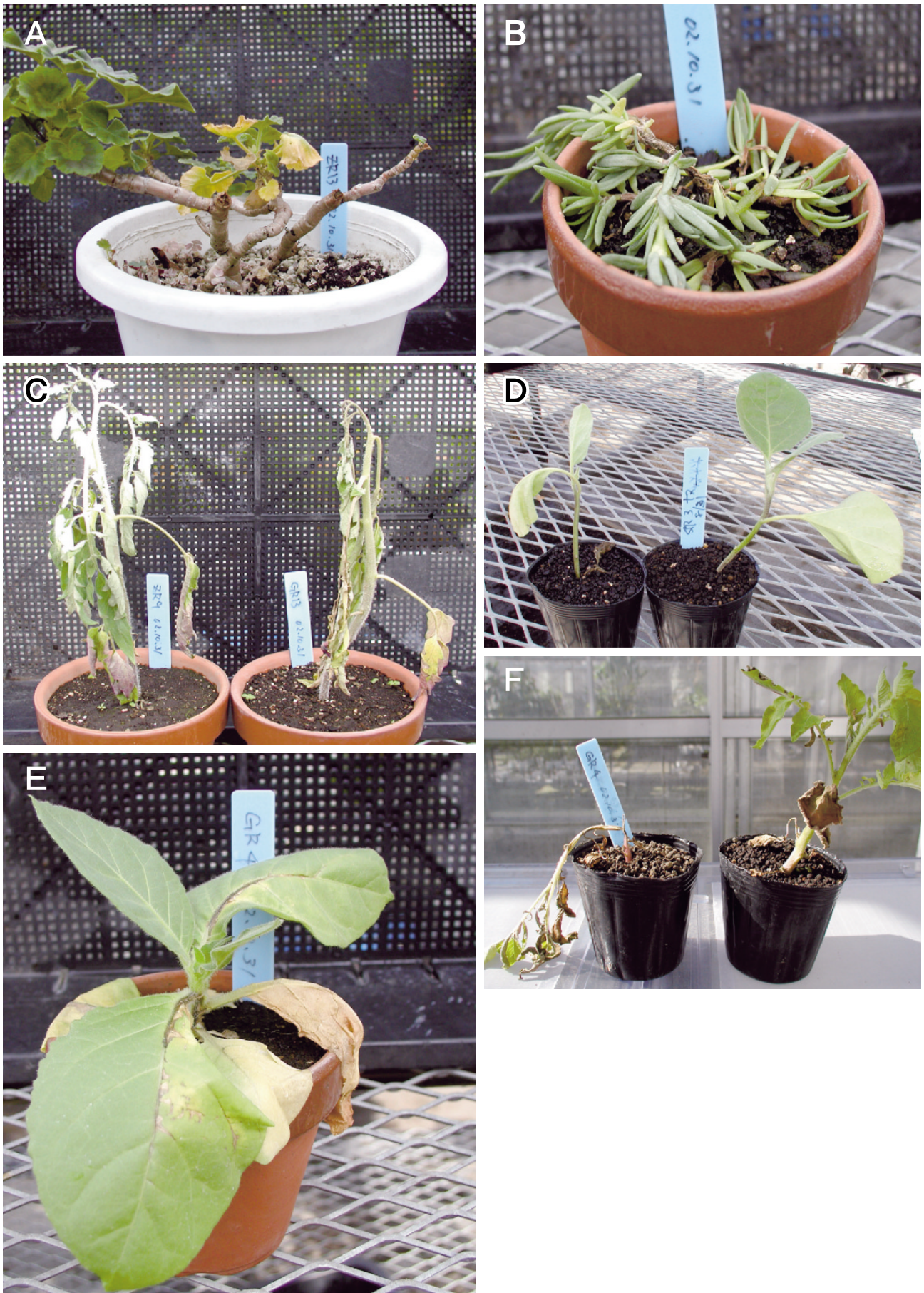


Fig. 2. Symptoms induced by inoculation with the isolated bacterium. A) Geranium; B) Portulaca; C) Tomato; D) Eggplant; E) Tobacco; F) Potato.

Table 1. Pathogenicity of the present isolates to geranium, portulaca and solanaceae plants^{a)}

| Isolates | Geranium | Portulaca | Tomato | Tobacco | Eggplant | Potato | Sesame | Sweet pepper |
|-----------|----------|-----------|--------|---------|----------|--------|--------|--------------|
| Geranium | + | ++ | ++ | ++ | + | ++ | ++ | ++ |
| Portulaca | + | ++ | ++ | ++ | + | ++ | ++ | ++ |

a) 30 days after inoculation

b) ++: Wilted, yellowed and died +: Wilted, yellowed but not died

Table 2. Oxidation of carbohydrate by the present isolates and other *Ralstonia solanacearum* biovars^{a)}

| Oxidation of Carbohydrate | Geranium isolates (n=10) | Portulaca isolates (n=10) | MAFF730133 biovar 2 · N2 | MAFF301525 biovar 3 | MAFF301521 biovar 4 |
|---------------------------|--------------------------|---------------------------|--------------------------|---------------------|---------------------|
| Mannitol | + ^{b)} | + | - | + | + |
| Sorbitol | + | + | - | + | + |
| Dulcitol | + | + | - | + | + |
| Trehalose | + | + | + | + | + |
| Inositol | + | + | + | + | + |
| D-Ribose | + | + | + | + | + |
| Lactose | + | + | + | + | - |
| Maltose | + | + | + | + | - |
| Cellobiose | + | + | + | + | - |

a) Description by Hayward (1964,1994)

b) +: positive -: negative

affected plants of stem and roots were rotted and ultimately died (Fig. 1A, B). When the basal part of the affected plant with shrivelled leaves was cut, faint discoloration of the vascular bundles was observed (Fig. 1C). When the cut surface of stem was dipped into clear water, white bacterial ooze exuded visibly (Fig. 1D). The disease symptoms of portulaca were similar to those of geranium (Fig. 1E, F, G).

Pathogenicity. Each five isolates from geranium and portulaca with white fluidal colony showed pathogenicity on geranium, portulaca, tomato, eggplant, tobacco, potato, sesame and sweet pepper (Table 1). Symptoms on geranium appeared 10 days after inoculation showing a few drooping leaves. Tomato, tobacco, potato, sesame and sweet pepper wilted about 7 days after inoculation showing brownish-colored longitudinal streaks on their stems, and finally died. But eggplant and geranium showed moderate disease symptoms, and did not die within 30 days after inoculation (Fig. 2). The pathogenic bacteria were recovered from the each plants.

Bacteriological properties. The geranium isolates were aerobic and gram-negative. They decomposed glucose oxidatively but did not produce yellowish green fluorescent pigment. Poly- β -hydroxybutyrate accumulation, oxidase and catalase activity were positive. Arginine dehydrolase and growth at 40°C were negative. The bacteria utilized the following organic compounds as the sole carbon source: glucose, fructose, D-mannose, galactose, sucrose, mannitol, sorbitol, dulcitol, inositol, D-ribose, trehalose, maltose, lactose, cellobiose. Negative reactions were obtained in the utilization of raffinose and starch. Different reactions among the isolates were not shown.

The portulaca isolates showed same characteristics as those of geranium isolates. Therefore, the biovar of geranium and portulaca isolates were classified as biovar 3

(Hayward 1964, 1994) (Table 2).

Based on these result, the geranium and portulaca isolates were identified as *R. solanacearum* (Smith 1896) Yabuuchi, Kosako, Yano, Hotta & Nishiuchi 1996, biovar 3. This is the first report of bacterial wilt caused by *R. solanacearum* of geranium and portulaca in Japan.

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