Verticillium Wilt of Potato Caused by Verticillium albo-atrum in Daegwallyong Area in Korea

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Verticillium wilt was first observed in 2001 on potatoes (Solanum tuberosum) cv. Superior at Daegwallyong area, one of the major seed potato producing areas in Korea. The wilted potato plants showed typical symptoms including gradual vellowing and interveinal necrosis. There was discoloration in the vascular tissues of the infected stems which turned light brown. Fungal isolates from discolored vascular tissues were whitish to creamy with folding on potato dextrose agar medium, where they used to produce resting dark mycelia but no microsclerotia. Conidiophores were septate with side branches, swelled at the base, and arranged in a whorl. Conidia were 2.5-11.2×2.0-4.5 μm in size and were borne in small clusters at the tips of phialides. Optimal temperature range for mycelial growth was 25-30°C. Based on these cultural and morphological characteristics, the fungus was identified as Verticillium albo-atrum Reink & Berth. Pathogenicity tests by root dipping method revealed that the fungus caused the same symptoms as observed in naturally infected potato plants. This is the first report of Verticillium wilt on potato caused by Verticillium albo-atrum in Korea.

Keywords: potato, soil-borne pathogen, Verticillium alboatrum, Verticillium wilt

Verticillium wilt of potato (Solanum tuberosum) is caused by the soil-borne fungal pathogens Verticillium albo-atrum Reink & Berthier and V. dahliae Kelb. Both pathogens infect many plant species, including trees, vegetables, field crops, ornamentals, and weeds. Characteristic symptoms of Verticillium wilt were recoverable true wilting, unilateral permanent wilting, unilateral chlorosis, and necrosis. In addition, plants infected with these pathogens have reduced growth rates of leaves, stems and tubers, and have premature maturation or senescence, which are commonly referred to as potato early dying. V. albo-atrum originally

recognized as a causal agent of potato wilt, also causes wilt in several plants including hop (John and Heale, 1985; Keyworth, 1953; Sewell and Wilson, 1984), tomato (Kim et al., 2001; Tjamos, 1981), and alfalfa (Jimenezdiaz and Millar, 1988; Keinath and Millar, 1986). V. albo-atrum is generally far less common than V. dahliae and is more virulent at low temperatures (Ludbrook, 1933; Smith, 1965); vascular infection leads to wilt with or without obvious flaccidity; and the infected xylem vessels are commonly browned. In 2001, symptomatic potato plants were collected from major seed producing areas in the highland (Daegwallyong area, Gangwon province, Korea). Wilted potato plants showed gradual yellowing in the field and discoloration of the vascular tissues of the infected stems which turned light brown (Fig. 1A).

Stems and roots from diseased plants were washed with tap water. After removal of the outer stem cortex, small pieces of vascular tissues were surface sterilized in 0.5% NaOCl for 30-60 seconds, and then placed on Petri plates containing 2% water agar or acidified potato dextrose agar (APDA). The vascular tissues were incubated at 22°C for 5-7 days. A number of conidiophores were formed on the discolored vascular tissues (Fig. 1B). Fungal isolates from these vascular tissues were whitish to creamy with folding on potato dextrose agar medium (PDA) (Fig. 1C), where they used to produce dark mycelium as resting structures but no microsclerotia (Fig. 1D). The isolates were identified based on published descriptions by Smith (1965) and Hawksworth and Talboys (1970). Conidiophores were septate with side branches as phialides 15.3-27.9×1.0-2.5 um, swollen at the base, and arranged in a whorl. Conidia were $2.5-11.2\times2.0-4.5 \,\mu\text{m}$ in size, borne in small clusters at the tips of phialides (Table 1 and Fig. 1D).

Four isolates of *Verticillium* spp., isolated from potato (*V*. albo-atrum; PV-01 and PV-03) and tomato (V. albo-atrum, TV-29; V. dahliae, TV-07), were tested for mycelial growth. The isolates were incubated at 15, 20, 25, 30, and 35°C, and colony diameters were measured every 2 days for 2 weeks on PDA in the dark. Optimum temperature for mycelial growth of the V. albo-atrum isolates (PV-01, PV-

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Fig. 1. Morphological characteristics of *Verticillium albo-atrum* and symptoms of Verticillium wilt on potato. Symptoms of Verticillium wilt on naturally infected potato plants (A); conidia and conidiophores on infected potato vascular tissue under stereo microscope (B); colony morphology (C) and dark mycelium as resting structures on PDA medium (D) for 14 days at 25°C incubation. Symptoms of potato leaves (E) and tuber (F) were formed by artificial inoculation with *V. albo-atrum*, and potatoes were taken 3 months after inoculation. Bar sizes: 50 µm (B), 10 µm (D).

03 and TV-29) ranged from 25°C to 30°C, whereas, that of *V. dahliae* isolate (TV-07) was 15°C to 20°C (Fig. 2).

Four isolates of *Verticillium* were tested pathogenic to potato seedlings, cvs. Superior and Atlantic. Inoculum for each isolate was prepared using two 14-day-old cultures on PDA plates blended with 40-50 ml of sterile distilled water to make a thick slurry, and the inoculum concentration was adjusted to 10⁷ conidia/ml. Twenty (20) seedlings of each cultivar were inoculated at growth stage upon appearance of the 3-4th true leaf by a root-dip technique (Bender and Shoemaker, 1984). Uninoculated controls (seedlings dipped in PDA suspensions) were included. The seedlings were

grown for 30 days after inoculation in a greenhouse maintained at 20-25°C, and disease development on seedling were carried out according to disease index (0= healthy, 1=slight vascular discoloration, 2=slight wilting, 3 = severe wilting and death). Two weeks after inoculation, inoculated plants began to exhibit yellowing (Fig. 1E), chlorosis, and defoliation of lower leaves. The pathogenicity of *V. dahliae* and *V. albo-atrum* varied on two potato cultivars; three isolates of *V. albo-atrum*, PV-01, PV-03 and TV-29, were highly virulent to potato cultivars and the disease index ranged from 1.7 to 2.3, whereas one isolate of *V. dahliae*, TV-07, only had vascular discolor-

Table 1. Comparison of morphological and cultural characteristics of *Verticillium albo-atrum* isolated from potato, and *V. dahliae* and *V. albo-atrum* isolated from tomato

Isolate	Source	Color of colony	Resting structure -	Size (µm)		
				Phialides	Conidia	
PV-01	Potato (V. albo-atrum)	Hyaline to white grey	Dark mycelium	15.3~27.9×1.0~2.5	2.5~11.3×2.0~4.8	
PV-03	Potato (V. albo-atrum)	Hyaline to white grey	Dark mycelium	15.7~ 26.9×1.0~2.5	2.6~11.2×2.0~4.5	
Kim et al. (2001)	Tomato (V. albo-atrum)	Hyaline to white grey	Dark mycelium	17.5~27.5×1.0~2.5	2.5~10.0×2.3~3.5	
	Tomato (V. dahliae)	Hyaline to black	Microsclerotium	17.5~35.0×1.0~2.5	2.5~8.8×2.0~3.0	
Hawksworth & Talboys (1970)	V. albo-atrum	Hyaline to white grey	Dark mycelium	14.0~26.0×1.0~2.5	3.5~10.5×2.0~4.0	
	V. dahliae	Hyaline to black	Microsclerotium	16.0~35.0×1.0~2.5	2.5~8.0×1.4~3.2	

Table 2. Pathogenicity of *Verticillium dahliae* (V-d) and *V. albo-atrum* (V-a) against two different potato cultivars

Potato cultivar	Disease index (0-3) ^a						
	Isolates from potato		Isolates from tomato		Con.b		
	PV-01(V-a)	PV-03(V-a)	TV-29(V-a)	TV-07(V-d)	-		
Superior	2.1°	2.3	2.0	1.5	0.5		
Atlantic	2.3	2.0	1.7	1.3	0.4		

^aDisease index: 0=healthy; 1=slight vascular discoloration; 2=slight wilting; 3=severe wilting and death.

Mean number of disease index with symptoms 4 weeks after inoculation, for 20 replicates of each cultivar. Error mean square=0.4.

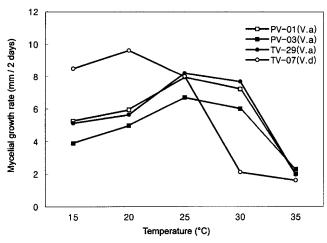


Fig. 2. Mycelial growth rate of *Verticillium dahliae* and *V. alboatrum* on PDA at different temperatures 2 weeks after incubation. Verticillium isolates were isolated from potato (PV-01, 03) and tomato (TV-07, 29).

ation of stems but no wilt symptom of plants (Table 2). The vascular ring of infected tubers from the inoculated potato plant had slightly brown discoloration after 3 months of inoculation (Fig. 1F).

It has been reported that *V. albo-atrum* is a dominant species on cool climates (Folsom et al., 1955), and more pathogenic than *V. dahliae* (Campbell and Griffiths, 1973; Botseas & Rowe, 1994). Under cool temperatures and high moisture conditions, especially late in the growing season,

infected plants may not actually wilt, but they turn yellow, wither, and die from the base upward. It is easy to confuse plant symptoms with those of black leg, ring rot, southern bacterial wilt, and Fusarium wilt.

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^bControl: seedlings immersed in a pathogen-free agar slurry were used as uninoculated controls.

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