

# First Report of *Verticillium tricorpus* from Potato Tubers and Plants in North Africa

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## ABSTRACT

In field surveys in the potato growing areas in Tunisia, a dry rot of potato tubers cv. 'Spunta' was observed at harvest but at a low frequency (2%) compared to the other typical dry rots caused by *Fusarium* spp. Based on macroscopic and microscopic characters of the collected isolates, the fungus was identified as *V. tricorpus*. This fungus was also isolated from many wilted potato plants, alone or together with *V. dahliae*. Pathogenicity tests were performed on wounded and unwounded potato cv. 'Spunta' tubers and on plants. A dry rot was observed on wounded inoculated tubers and moderate but typical *Verticillium* wilt symptoms were occasioned on inoculated plants within 30 days of incubation. Some of the collected isolates grown on PDA amended with benomyl at different rates (0, 0.1, 1, 10 and 100 mg/l), 10 were resistant to benomyl as their minimum inhibitory concentration was greater than 10 mg/l.

## EXPERIMENTAL

Wilted potato plants were collected following a diagonally sampling method at the second half of the growing season when symptoms are more prominent. Rotted tubers were collected after harvest and before final storage.

For pathogen isolation, plant parts (rotted tuber sections, roots or stems) were rinsed thoroughly in tap water and cut into 0.5 cm<sup>2</sup> pieces. After surface-disinfecting in sodium hypochlorite (10%) for 3 min, the plant pieces were rinsed three times in sterile distilled water and dried on sterile filter paper. Plant pieces were plated on PDA (Potato Dextrose Agar) medium with streptomycin sulphate (300 mg/l). Four pieces per plate were used for each plant tissue. Fungal cultures were incubated for two weeks at 20°C. The monoconidial isolates were identified based on published descriptions (Hawksworth 1970).

Pathogenicity tests of *V. tricorpus* were performed by surface inoculation of wounded and unwounded healthy disinfected tubers cv. 'Spunta' (ten per each isolate) with a 6 mm colonised agar piece, using 10 isolates from potato tubers and plants. Ten non-inoculated wounded and unwounded tubers served as controls where a non-colonised agar piece was used. The tubers were kept at room temperature (20-30°C) for 30 days.

*V. tricorpus* isolates were also used to inoculate potato plants cv. 'Spunta', grown singly in 25 cm-pots containing a sterile 2: 1 mixture of peat/perlite (v/v), one week after emergence by adding 100 ml of a 10<sup>7</sup> spores/ml of a conidial suspension to the each of the pots. Control plants were watered with 100 ml of sterile distilled water only. Plants were maintained in a glasshouse at temperature 10-30°C for 30 days.

To assess their resistance to Benzimidazole fungicides, *V. tricorpus* isolates were grown, for 9 days at 20°C, on PDA amended with benomyl (commercial product, Benlate) at 0, 0.1, 1, 10 and 100 mg/l. Each isolate was considered resistant if the minimum inhibitory concentration (the lowest concentration that prevented radial fungal growth) was greater than 10 mg/l.

## RESULTS AND DISCUSSION

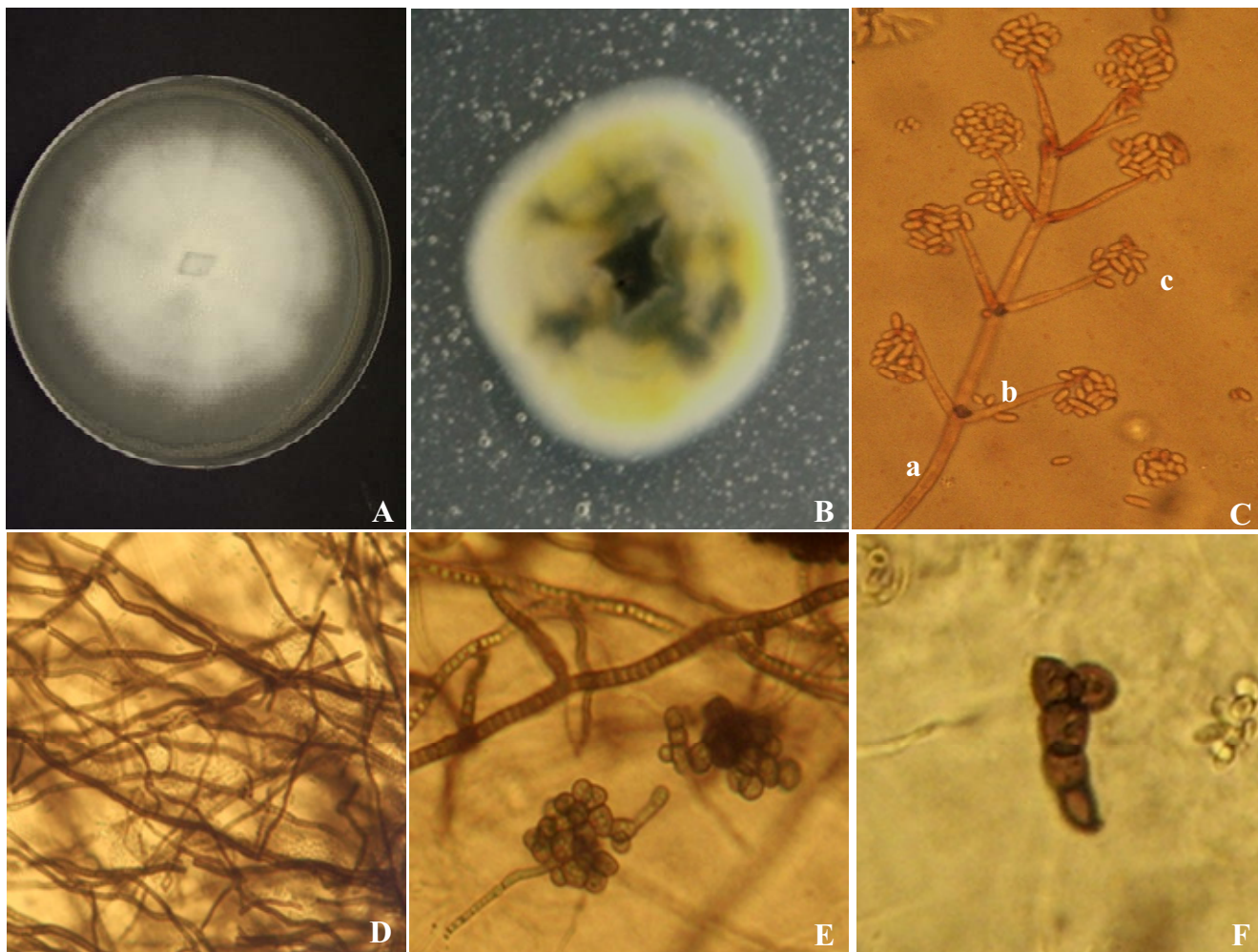
In field surveys a dry rot of potato (*Solanum tuberosum* L.) cv. 'Spunta' tubers was observed at harvest in the potato growing areas of Téboulba, in the eastern part of central

Tunisia. This rot penetrated tubers to 7 to 10 mm and had an internal grey to black area. On potato dextrose agar (PDA), the fungus isolated from rotted areas produced white to black floccose colonies (Fig. 1A) and a yellow-orange mycelium (Fig. 1B). Under optical microscope, conidiophores are abundant, verticillate, hyaline and erect with two to four phialides arising vertically at each node (Fig. 1C), characteristic of the genus *Verticillium*. Conidia were hyaline, ellipsoidal to sub-cylindrical (3.75-6.25 µm × 2.5 µm), produced singly at the apices of the phialides (Fig. 1C). The isolates produced three types of resting structures: microsclerotia (Fig. 1E), dark resting mycelia (Fig. 1D) and chlamydospores (Fig. 1F). Morphological characters of these isolates were indistinguishable from the descriptions of *V. tricorpus* Isaac (Hawksworth 1970). This rot was observed at a low frequency (2%) compared to the other dry rots which were due mainly to *Fusarium* spp. in the same potato cultivar, cv 'Spunta' (Daami-Remadi *et al.* 2006). In tubers, *V. tricorpus* was not found in association with other pathogenic or saprophytic organisms. *V. tricorpus* was also isolated from many wilted potato plants, alone or together with *V. dahliae*, in the regions of Chott-Mariem and Sidi Bou Ali. In fact, following a diagonally sampling method, *V. tricorpus* was isolated from 12% of the symptomatic potato plants collected (Fig. 2A).

Pathogenicity tests performed on tubers showed that dry rot development was evident 9 to 13 mm into the flesh of wounded tubers only (Fig. 2C) and that wounded and unwounded control tubers remained healthy. Inoculated to potato plants, *V. tricorpus* isolates produced, after 30 days of incubation at 10-30°C, moderate but typical *Verticillium* wilt symptoms on the inoculated potato plants but non-inoculated control plants remained symptomless (Fig. 2B).

Of 15 *V. tricorpus* isolates tested and collected from three regions situated in the Tunisian eastern coast (Téboulba, Sidi Bou Ali and Chott Mariem), 10 were resistant to benomyl as their minimum inhibitory concentration was greater than 10 mg/l.

Although generally less common on potato than other *Verticillium* species, *V. tricorpus* has been reported from several countries, causing a variable tuber rot, as from Greece (Thanassoulopoulos and Giapanoglou 1994) and



**Fig. 1** Morphological features of *V. tricorpus* on PDA. Colony formed after 21 days at 20°C (A); yellow-orange mycelium observed after 7 days of incubation at 20°C (B); conidiophore (a), phialide (b) and conidia (c) (X 40) (C); dark resting mycelia (X 40) (D); microsclerotia (X 40) (E) and chlamydoconidia of *V. tricorpus* (X 40) (F).



**Fig. 2** Potato plants naturally infested with *V. tricorpus* and *V. dahliae* (A); Comparison between a noninoculated potato plant (left) and a plant inoculated by an isolate of *V. tricorpus* (right) (B) (cv. 'Spunta', 60 days after inoculation; 5°C<T<28°C); Dry rot developed on a potato tuber cv. 'Spunta' 30 days after its inoculation with *V. tricorpus* (C).

Japan (Ebihara *et al.* 2003). In Tunisia, *V. tricorpus* has been isolated from wilting tomato and melon plants (Jabnoun-Khiareddine *et al.* 2005, 2007) but this is the first report from potato plants and tubers in North Africa.

## REFERENCES

Daami-Remadi M, Ayed F, Jabnoun-Khiareddine H, Hibar K, El Mahjoub M (2006) Comparative susceptibility of some local potato cultivars to four *Fusarium* species causing tuber dry rot in Tunisia. *Journal of Plant Sciences* 1, 306-314

Ebihara Y, Nagao H, Uematsu S, Moriwaki J, Kimishima E (2003) First re-

port of *Verticillium tricorpus* isolated from potato tubers in Japan. *Mycoscience* 44, 481-488

Hawksworth DL (1970) *Verticillium tricorpus*. CMI Descriptions of Pathogenic Fungi and Bacteria, 260. Commonwealth Mycological Institute Kew, UK

Jabnoun-Khiareddine H, Daami-Remadi M, El Mahjoub M (2005) Emergence in Tunisia of new pathotypes of *Verticillium tricorpus* able to infect tomato, aubergine and potato. *Bulletin OEPP/EPPO Bulletin* 35, 497-503

Jabnoun-Khiareddine H, Daami-Remadi M, Ayed F, Jebari H, El Mahjoub M (2007) Incidence of *Verticillium* wilt of melon in Tunisia. *The African Journal of Plant Science and Biotechnology* 1, 10-15

Thanassouloupoulos CC, Giapanoglou (1994) Two new and unusual dry rots of stored potatoes in Greece. *Plant Disease* 78, 924