

Corm and root rot of *Colocasia esculenta* caused by *Ovatisporangium vexans* and *Rhizoctonia solani*

Received for publication, June 10, 2014

Accepted, July 20, 2014

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Abstract

Ovatisporangium vexans (de Bary) Uzuhashi, Tojo & Kakis. and *Rhizoctonia solani* Kühn were isolated from the decayed parts of roots and corms of wilted taro (*Colocasia esculenta* (L.) Schott.) plants. Although both pathogens were identified according to morphological and pathogenicity characteristics, *O. vexans* identification was further confirmed based on the internal transcribed spacer (ITS) sequences of 18S ribosomal RNA (rRNA) and 28S rRNA genes using the ITS6 and ITS4 primers. Early symptoms on leaves appeared as pale yellow color, partial rolling of leaf margins and withering of seedlings. Rotting or decay started at the collar region and it spread to roots and corms. In severe cases, the collar region broke off and the seedling collapsed. Inoculating these isolates separately into mature taro plants and corms resulted in symptoms similar to root and corm rots observed on naturally infected plants. Both disease agents were re-isolated from the inoculated tissues. Since symptoms caused by co-inoculation of *O. vexans* with *R. solani* together were more severe, combination of two pathogens induced the greatest plant mortality. This is the first report of corm and root rot disease caused by *O. vexans* and *R. solani* complex on taro plants in Turkey. This is also the first record of *O. vexans* in Turkey.

Keywords: *Colocasia esculenta*, root and corm rot, *Ovatisporangium vexans*, *Rhizoctonia solani*

1. Introduction

Colocasia esculenta (L.) Schott. (taro, elephant ear or cocoyam) is an emergent, perennial, aquatic and semi-aquatic herbaceous species of the Araceae family, native to Asia. As a root vegetable, plant is grown primarily for its edible corms. Nowadays, *C. esculenta* is considered the fifth most consumed root vegetable worldwide. Taro is also used as an ornamental plant [1]. Taro is a new root crop of the southern provinces of Turkey for its edible corms (the root vegetables).

Fungal and oomyceteous plant pathogens of taro have been reported to cause losses in taro fields. Taro leaf blight disease, caused by *Phytophthora colocasiae*, foliar oomyceteous diseases agent, is a major limiting factor in taro production worldwide [2]. One of the major fungi that can be associated with taro root rots is black rot (*Ceratocystis fimbriata*) causing rot of storage corms of taro worldwide [3]. Root rot of taro may also be caused by any one or a combination of several common soilborne microorganisms such as *Phytophthora citricola*, *Phytophthora nicotianae*, *Pythium* spp., *Fusarium oxysporum*, *Fusarium solani*, *Sclerotium rolfsii* and *Rhizoctonia* sp. [4-5]. The relative importance of these pathogens and their effect on yield vary among countries or disease conditions. *Pythium* root and corm rots are probably the most widely distributed disease of taro [4-6]. *Pythium carolinianum* is mostly associated with wetland taro rather than dryland taro. In wetland situations, while *P. carolinianum* is

known to infect taro under favorable conditions, it is a less aggressive pathogen than other species such as *Pythium myriotylum* [7] that are more commonly associated with corm rot. *Pythium* root rots usually develop into corm rots where the interior of the corm is progressively transformed into a foul smelling soft mass [6]. Spread occurs via zoospores that are carried in irrigation water and are attracted to chemical exudates from the root tips [6]. *Pythium* species can also be transferred to new areas on infected vegetative planting material. Several disease complaints came from taro-growing areas in Antalya and Mersin provinces. Although it has been acknowledged that a number of diseases are present in taro crops worldwide, the causes of problems arise in Turkey have not been determined. Thus, the main objective of the study was to determine the disease agents causing root and corm rotting on taro plants cultivated in major taro growing locations in Antalya and Mersin provinces.

2. Materials and Methods

Isolation and identification of disease agents

Taro (*C. esculenta*.) plants and corms showing root and corm rot symptoms were collected from the taro growing locations in Gazipaşa district of Antalya province and Anamur ve Bozyazı districts of Mersin of Turkey during the wet season (May through December) in 2011 and infested samples were examined for associated fungal and oomyceteous pathogens. Diseased roots and corms were collected from taro, cut into 1 cm² pieces, washed in several changes of sterile water, and plated on potato dextrose agar (PDA), PDA with rifampicin (25 mg/ml) and pimarinic (10 mg ml⁻¹), V-8 Agar, Water (WA) and Carrot Agars (CA), incubated in Petri dishes at 25°C. When the mycelia appeared the hyphal tips were subcultured again on selective media. Isolates were then transferred to CA and PDA slants for storage. Basically, morphological characteristics were used for identification. For *Pythium* sp. identification, to produce sporangia and sex organs, mycelial discs taken from the edge of colonies were transferred to a sterile petri dish with enough sterile deionized water to submerge the discs. Within five days, a small piece of mycelium bearing sporangia and sex organs was cut off and mounted in 0.05% cotton blue/lactophenol. The morphological characteristics of sporangia, oogonia, oospores and antheridia were examined under a light microscope. At least 10 measurements were taken from each type of fungal and oomyceteous structure observed. Culture of *R. solani* isolate was identified on the basis of morphological features including right-angled branching of brown hyphae and the presence of sclerotia.

Pathogenicity tests

The pathogenicity of *O. vexans* and *R. solani* isolates obtained from taro plants with typical root and corm rot symptoms in Mersin and Antalya provinces were evaluated on healthy taro seedlings and corms. To test the pathogenicity of the both organisms, the fungus and oomyceteous species were cultured on PDA (*R. solani*) and CA (*O. vexans*) in Petri dishes. Three isolates of *O. vexans*, three isolates of *R. solani* and mix inocula including discs of both *R. solani* and *O. vexans* species were used for pathogenicity tests. Small mycelial agar blocks taken from the edge of a young colony (4-days-old) were used as inoculum sources. Two replicated pathogenicity trials involving two treatments (corm inoculations and root inoculations) were conducted in the laboratory and greenhouse. Six mature and healthy 1-year-old taro plants were selected for greenhouse inoculations. A few wounds were made with a sterilized knife on the underground roots randomly and simultaneously agar disks were placed near to cuts of each of four plants with a scalpel. Four plants were left as controls. For the control, sterile agar blocks were used instead. Three to four weeks after inoculation, roots were cut longitudinally and examined for symptoms. Pathogenicities of the fungus and the oomycete were confirmed by satisfying Koch's postulates.

For laboratory trials, the presence of rots (average diameter, cm) produced in taro corms stored at 27°C for up to 4 weeks were observed. Medium-sized corms free from wounds were selected for uniformity from a large collection. The corms were cleaned and surface sterilized with 5% Chlorox solution for 2 minutes. The inocula consisted of discs of mycelium 4 mm in diameter cut from actively growing cultures. Discs were placed inside the hole in the corm tissues after the core of corms had been removed with a sterile 5-mm cork borer. The core of tissue was cut in two, and the halves were placed in the hole as plugs for both ends. The replaced core of tissue at each end was sealed with parafilm. Discs of sterile PDA were used as control. Each isolate was used to inoculate 10 taro corms. The inoculated corms were kept on a raised platform for 10 days. The laboratory temperature during the period was 30°C. The corms were then cut and the presence of rot was assessed. Both greenhouse and laboratory studies were carried out two times.

Molecular characterization of *Ovatisporangium vexans* isolates

DNAs of two representative *O. vexans* isolates were obtained from scrapings of mycelia growth of petri dish culture. Around 200 mg mycelia were ground with liquid nitrogen and 1 ml of extraction buffer (0.2 M Tris 8.5, 0.25 M NaCl, 25 mM EDTA, 0.5% SDS) was added. Following that, phenol/chloroform clarification and ethanol precipitation were applied. DNA's were diluted in 50 µl TE (10 mM Tris 7.5- 1 mM EDTA) buffer.

ITS region from DNA samples were amplified using primers ITS6 (5' GAA GGT GAA GTC GTA ACA AGG 3') located on 18S rRNA [8] and ITS4 (5' TCC TCC GCT TAT TGA TATGC 3') located on 28S rRNA [9]. PCR amplifications were performed in 50 µl reaction mix containing 1.5 mM MgCl₂, 1 mM of the four dNTPs, 0.1 mM of each primer, 0.5 U of Taq DNA polymerase, 1X polymerase buffer (100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl, 0.8% (v/v) Nonidet P40) (Fermentas, Germany) and 1 µl of template DNA (20–50 ng). Amplification condition was 1 cycle of 94°C for 3 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 60 s; and a final cycle of 72°C for 10 min. PCR products were separated in 1.5 % agarose gels, stained with ethidium bromide, and visualized under UV light. Sequence analysis was carried out by IONTEK (Istanbul, Turkey). Isolates were identified by blasting against sequences already present in NCBI GenBank.

3. Results and discussions

During the field examinations, early symptoms of root rots on leaves appeared as pale yellow color, partial rolling of leaf margins and withering of seedlings. Rotting or decay started at the collar region and it spread to roots and corms. In severe cases, the collar region broke off and the seedling collapsed. Pathogen(s) of corm rots caused dry to soft and powdery whitish to black rot or lesions inside corms, inadequate corms, rot of root tissues, defoliation, and sometimes killed plants.

Ovatisporangium vexans (de Bary) Uzuhashi, Tojo & Kakish. 2010 (previously *Pythium vexans*)'s main hyphae were 3-6.1 µm wide. Sporangia were (sub)globose, ovoid or pyriform, intercalary or terminal, 19-22 µm long and 18-21 µm broad, producing short protuberances which developed into zoospore discharge tube(s). Oogonia were mostly terminal on short side branches, sometimes lateral or intercalary, globose, 18-23 µm diam. Antheridia were monoclinal. Antheridial cells were large, typically bell-shaped. Oospores were aplerotic, 16-20 µm diam. Based on these characteristics, the oomycete pathogen was identified as *O. vexans* using the keys of van der Plaats-Niterink [10] and Uzuhashi et al. [11]. The identification of pathogenic isolate of *O. vexans* was further confirmed by molecular studies. The amplified ITS region of the nuclear ribosomal DNA of the fungus isolates comprises 724 bp. The resulting 724 bp sequences of two representative isolates of *O. vexans* were submitted

to GenBank (Accession numbers of KF669887 and KF669888). A comparison with other sequences available in the GenBank database revealed that the ITS sequence of *O. vexans* shared 99% similarity with *Pythium vexans* sequences deposited in the GenBank. The two isolates were clustered with *P. vexans* isolates in phylogenetic analysis (Fig 1).

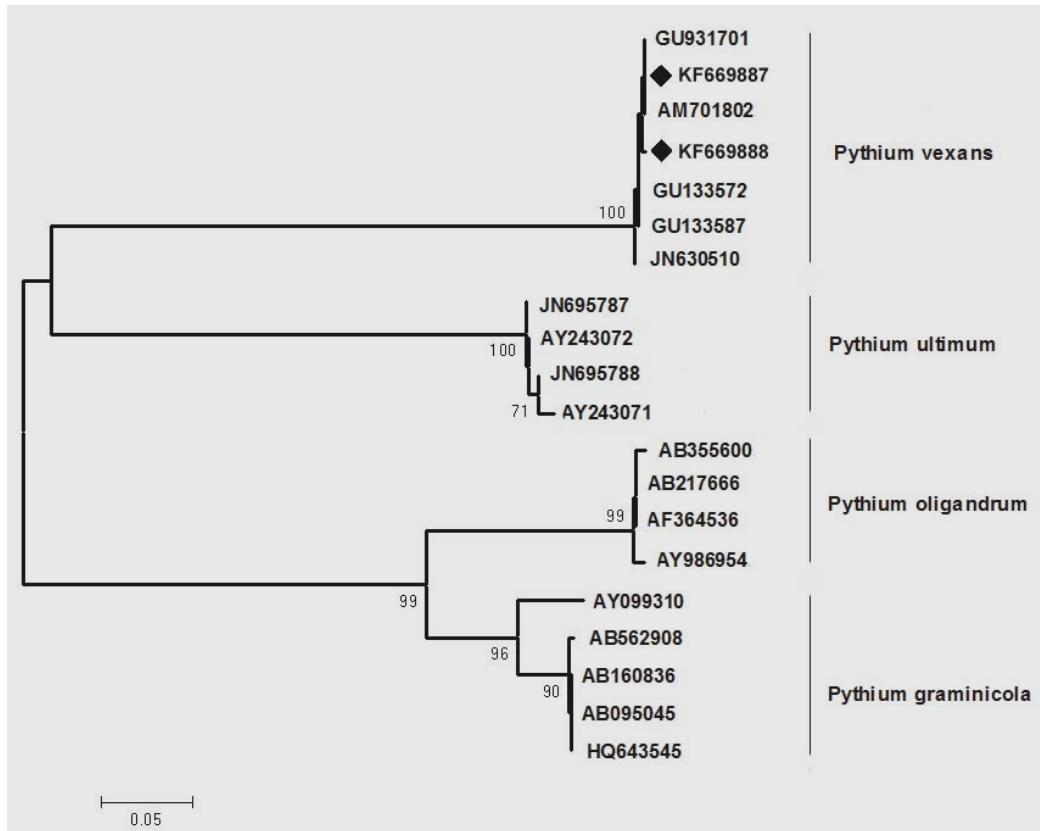


Figure 1. Neighbour-joining tree of genetic relatedness for two isolates of *O. vexans*, (pointed with diamond) together with several *Pythium* isolates provided from Genbank, based on ITS sequences of rDNA.

O. vexans has been previously recorded on taro plants in Fiji, Samoa, Solomon Islands, New South Wales, Queensland, South Australia and Western Australia [12-18]. Other *Pythium* species, which were recorded on taro plants, were *P. myriotylum* from South Africa [19]; *Pythium* sp. from Ghana [20], Brazil [21], California [22], Papua New Guinea [23] and Hawaii [24]; *P. aphanidermatum* from Hawaii [25]; *P. graminicola* from Hawaii [25]; *Globisporangium carolinianum* from Hawaii [25] and Papua New Guinea [23]; *G. debaryanum* from California [22] and Hawaii [25]; *G. ultimum* from Puerto Rico [26], Virgin Islands [26] and West Indies [27]. Although *P. vexans* has been recorded from both soil and plants in many countries, its pathogenicity has been proved on limited hosts including geranium [28], ginger [28], cardamon [28], holly [29], carnation [30], pecan seedlings [31], potato and sweet potato [32], *Metrosideros* [33], some Proteaceae [34], *Aframomum* [34] and peach [35].

Mycelium of *R. solani* was branched at right angles with a septum near the branch and a slight constriction at the branch base and had sclerotia typical of *Rhizoctonia* spp. [36]. Cream-colored colonies produced irregular, light brown sclerotia that were 4.0 to 7.5 mm (average 4.2 mm) in diameter. Hyphae were 6.5 to 7.5 μm (average 7.0 μm) wide and multinucleate (7 to 14 nuclei per cell) when stained with 1% safranin O and 3% KOH solution. No *Phytophthora* or other pathogen-like species was isolated from naturally infected taro plant samples. *Rhizoctonia solani* J.G. Kühn 1858 has been recorded on taro from Ghana [20] and *Rhizoctonia* sp. from Brazil [21] and Malaya Peninsula [37].

A total of 10 isolates of *O. vexans* and 15 isolates of *R. solani* were obtained from the diseased corms and roots of taro plants. All main corms and roots infected with representative *O. vexans* and *R. solani* isolates had moderate to extensive lesions towards the base surrounded by extensive areas of dry and firm to soft rot or lesions. Disease symptoms appeared on all the inoculated plants. Root tissues turned brown and rotted and the lesion reached into the wood. These symptoms were similar to those associated with root rot disease observed in the field. Both *O. vexans* and *R. solani* isolates were recovered in pure culture from advancing margins of infected root tissues. No disease symptoms were observed on control plants. Similarly, inoculating these isolates into healthy, mature taro corms resulted in symptoms similar to corm rots observed in the field and the same species were re-isolated from the diseased corm tissues.

Although three *O. vexans* and three *R. solani* isolates examined in these trials were able to colonize root systems and corms separately, they did cause more severe root rot or enlarged corm rot symptoms in all plants and greater plant mortality when they treated in mix inocula together. Each pathogen alone caused significantly less symptom severity than the mix inocula. Co-inoculation of *O. vexans* with *R. solani* together caused greater disease severity and plant mortality than either pathogen inoculated alone, suggesting synergism.

4. Conclusion

Causal agents of corm and root rot of *C. esculenta* plant growing in Antalya and Mersin provinces of Turkey were isolated and identified. Based on the symptoms, mycological characteristics, ITS sequence analysis and host plant pathogenicity, the causal agents were identified as *O. vexans* and *R. solani*. To our knowledge, this is the first report of corm and root rot of *C. esculenta* caused by *Ovatisporangium vexans* and *Rhizoctonia solani* complex in Turkey.

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