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Occurrence of Root Rot Caused by *Phytophthora cryptogea* on Common Sage (*Salvia officinalis*) in Turkey

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Abstract: Common sage or Dalmatian sage (*Salvia officinalis* L.) is a perennial plant (subshrub), native to the Mediterranean region. This research was conducted to identify the fungi species which cause a sudden damping-off disease in some common sage plantation, in the coastal experimental areas of Antalya and Izmir provinces of Turkey. The infected plant materials were collected from Mediterranean and Eagean regions which showed root and crown rots typical of *Phytophthora* sp. symptoms. Ten plants having those symptoms were used for identification of the causal agent by *Phytophthora* selective medium. A new *Phytophthora* species was isolated and identified as *P. cryptogea* as a result of morphological and molecular characteristics of DNA base sequences of internal transcribed spacer (ITS) regions. Pathogenicity of *P. cryptogea* was proved on rooted cuttings of common sage. This is the first report of *P. cryptogea* on common sage in Turkey.

Key words: Sage, *Salvia*, *Phytophthora* root rot, cutting.

1. Introduction

More than 90 sage (*Salvia* spp.) species are found in Anatolian flora of Turkey. Although *Salvia officinalis* is not native to Turkey, it has been well adapted to Central Anatolian climatic conditions. It has been used in indigestion, treatment of inflammation of the mouth and throat, and excessive sweating, including that associated with peri-menopause and as a food flavoring. In order to meet increased industrial demands, new common sage lines and cultivars having desease tolerance should be bred to help in provision of high quality raw material for industrial use without depending on natural flora. Extensive collection from natural flora has resulted in the extinction of some *Salvia* species and has let to use of the undesired materials, like *Phlomis* species instead of common sage. To protect the natural flora, attempts to cultivate these species have been made since 1980s, starting with the Agricultural Research Institutes. First cultivation effort was started by Menemen Agricultural Research Institute in 1981 and later on some efforts were made at two other places, in Turkey. Severe damping-off was observed at the above mentioned experimental plots and the pathogens causing the disease were determined by Çarkacı and Maden [1], where many fungi were isolated from the root rots and pathogenicity of 191 isolates was performed by inoculating sterile soil by fungal spore suspensions and planting with sage seedlings grown in sterile soil. Some isolates of *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *Rhizoctonia solani* produced 90%-100% damping-off. Although the diseases of common sage have not been investigated in natural flora of Turkey, a few reports are available from cultivated areas. Root rot on cultivated common sage was also found common in Poland, and *Fusarium*

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spp., *R. solani*, *Phoma exigua* var. *exigua* were isolated from roots and the lower parts of stems with the symptoms of necrosis and tissue disintegration. In the same research, *Phomopsis sclarea* was obtained from the stems with the symptoms of necrosis, peeling off and bark breaking for the first time for Poland [2].

In India, Mallesh et al. [3] reported that root rot of sage was mainly caused by *F. solani* and *R. solani* and sage was so vulnerable against root rot.

Phytophthora root and crown rot of common sage (*S. officinalis*) was first reported by Koike et al. [4] in commercial plantings in the Salinas Valley in Monterey County, CA, USA. The pathogen was identified as *Phytophthora cryptogea* based upon its morphological characteristics. The authors also detected this disease in experimental plantings of sage in Stanislas County in 1990. *P. cryptogea* was isolated from potted sage plants grown for ornamental aim and they identified the *Phytophthora* species by specific PCR primers and sequence analysis of their ITS1 and ITS4 regions [5].

In 2015, severe damping-off symptoms having dark necrosis at collar regions of the plants similar to *Phytophthora* root rot were observed at two fields planted with common sage in İzmir, Turkey. When uprooted, they also showed dark necrosis at the crown and root regions, which is a typical symptom of *Phytophthora* root rot. Rooted cuttings produced from the material collected from the fields in İzmir also showed similar symptoms. The aim of this study was to find out the causes of the damping-off occurring recently on common sage in Turkey.

2. Materials and Methods

2.1 Sampling and Isolation

Infected plant samples were collected from 10 different locations of the experimental plots of common sage plantations in Izmir (Menemen county) and Antalya (Aksu county). All the samples were collected from various locations showing similar symptoms of root and crown rots and dark necrotic areas on the stems. Ten plants having those symptoms were used for identification of the causal agent. Since these symptoms resembled *Phytophthora* root and crown rot, a selective medium was used for isolations. The plant parts having both necrotic and intact tissues were washed under running tap water, dried with paper towels and disinfected in 0.5% NaOCl for 5 min. After rinsing with sterile distilled water, small sections about 0.5 cm of stem pieces having both the intact and necrotic areas were dried and plated on the modified selective P_5ARPNH-agar, i.e., 5 mg/L pimaricin, 250 mg/L ampicilline, 10 mg/L rifampicin, 50 mg/L PCNB, 50 mg/L nystatin, 50 mg/L hymexazole [6], with carrot agar (CA) (40 g/L thinly grated carrot and 18 g/L agar) as the basal medium [7]. Pure colonies were obtained by transferring mycelia tips from the cultures growing on the selective medium. Pure cultures were transferred to CA amended with 30 µg/mL β-sitosterol, 1 µg/mL thiamine hydrochloride and 20 µg/mL tryptophan to stimulate oospore and sporangia production [8]. Cultures were incubated in the dark at 20 ± 1 °C and examined within 2-8 d. Petri plates were incubated at 20 ± 1 °C for 3-5 d in the dark.

2.2 Identification

Morphological characteristics of the *Phytophthora* isolates were studied on the cultures grown on CA as previously described. Formation of oospores was checked in cultures on amended CA in darkness for four weeks [9]. Identification of *Phytophthora* sp. was performed by using morphological and physiological criteria, such as sporangial shape, their measurements, growth at some temperatures, by going into the published keys [10, 11]. Identification of the pathogen was also verified by comparing DNA base sequences of ITS1 and ITS4 regions of one isolate with the ones deposited in Gene Bank as described by Jeffers and Aldwinckle [12].

2.3 Testing Pathogenicity

Pathogenicity of the present isolate was performed by inoculating 10-12 cm rooted sage cuttings, which were kept in 100 µg/mL indolebutyric acid and planted in sterile perlite filled tray pots. The cuttings were grown for a 3-4 weeks period in sterile perlite. Each tray pots had 30 cuttings (six rows in each five cuttings) and left to the greenhouse set to 22 ± 5 °C and 60% relative humidity. The inoculum was prepared by blending 10 cultures of the fungal isolate in Petri dishes with 500 mL sterile water for 2 min. Inoculation was done by adding 20 mL macerated fungal culture to the root region of each cutting. Control cuttings were treated with the same amount of sterile water. Cuttings were kept in the greenhouse for two weeks and uprooted at the end of this period.

Evaluation of the pathogenicity was done by symptom onset on the inoculated cuttings, such as wilting, dwarfing and necrosis on the roots compared to the controls.

3. Results

3.1 Occurrence of Disease in the Fields

The disease appeared as sudden wilting in the field. All of the infected plant samples showed root and crown symptoms (Figs. 1a and 1b). Some samples having intensive root rots also showed dark discolorations on the crown and stem sections (Figs. 1c and 1d).

3.2 Isolation and Identification of the Causal Agent

All the samples yielded mycelial growth on the *Phytophthora* selective medium. The colonies had a uniform, fluffy growth on amended CA medium (Fig. 2a). Sporangia were observed on culture discs grown on amended CA and submerged in sterile and non-sterile soil extracts and incubated in daylight at 22 ± 1 °C [9]. Sporangia formation started after 24 h and the formation increased after 48 h. They were non-papillate and non-caducous, mostly ovoid, but

Fig. 1 Plants having dark discolorations on stems bases, sage (a), mountain tea (b) due to *P. cryptogea* infection; crown sections having dark discolorations, sage (c) and mountain tea (d).

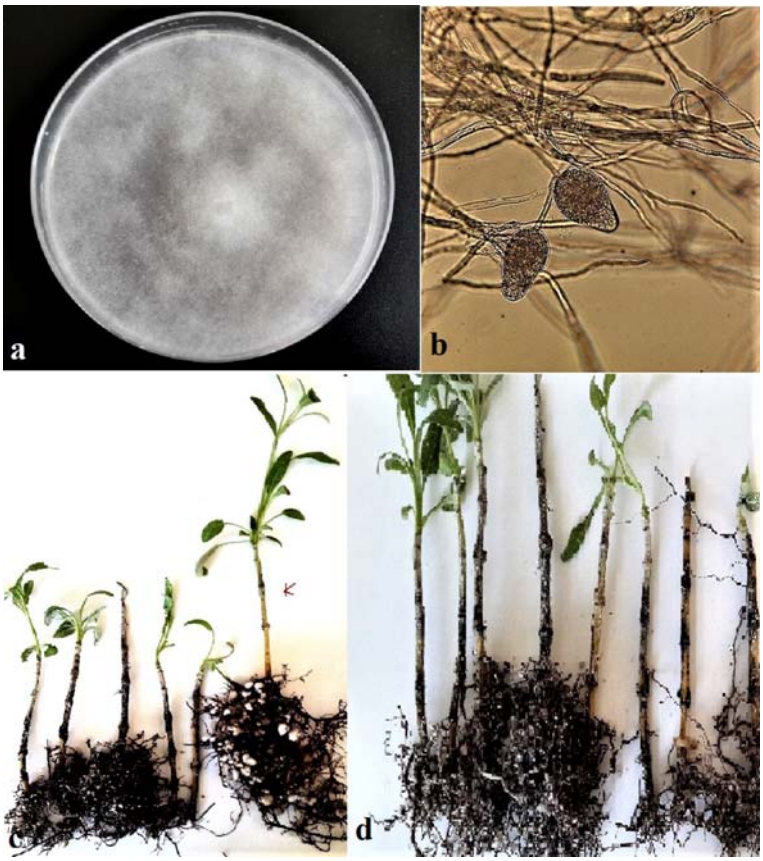

Fig. 2 Some aspects related with *P. cryptogea*: colony growth on CA medium (a); non-papillate, ovoid and obpyriform sporangia (b); symptoms on the inoculated cuttings, with the marked K one as control (c); some other cuttings showing various type of necrosis (d).

some were obpyriform (Fig. 2b). The mean size of the sporangia was 22.5-35.0 × 25.0-55.0 (average 29.2 × 42.3) µm. Oospores were not formed on amended CA medium.

The Oomycete was identified as *P. cryptogea* Pethybr. & Laff., according to its morphological characteristics [10, 11]. The identification of the isolate was confirmed by sequence analysis of the ribosomal DNA internal transcribed spacer region using primers ITS1 and ITS4. The ITS sequence matched 99% to many of the isolates of *P. cryptogea* strains deposited in Gene Bank (Accessions No. KC695697 and KR011187.1).

3.3 Pathogenicity of the Isolate

All the inoculated 30 rooted cuttings showed dwarfing and wilting at various degrees, and when they were uprooted, they showed dark necrosis at the root and crown regions in various severity (Figs. 2c and 2d). Re-isolations on P_5ARPNH-agar yielded *Phytophthora* sp..

4. Discussion

With this study, *P. cryptogea* was isolated for the first time from common sage grown at the experimental field near İzmir. *P. cryptogea* was also reported from potato previously in Turkey [9]. Not only *P. cryptogea* but also other *Phytophthora* species might infect sage as well.

Phytophthora species cause rapid decline on many plant species and several species have been reported on various crops in Turkey. *P. cryptogea* on the other hand, was also reported from chestnuts (unpublished results of us). *Phytophthora* root rot on common sage could be present beforehand but the difficulty on the isolation and identification might delay its earlier

report. Although the symptoms caused by *Phytophthora* spp., such as dark rapid wilting and dark necrosis on the root and collar regions is very typical, the isolation and identification of them need elaborated work and experience. Other fungi, such as *Fusarium* spp. may be obtained from those symptoms when standard media are used since *Fusarium* spp. were also found causing root rots on the sage plantations [1, 13]. Root rots may cause severe damage and they were reported in a few countries, such as India [3], Italy [5], Poland [2] and Turkey [1].

The symptoms observed on the samples studied in this paper were typical as given by Garibaldi et al. [5] and Gallegly and Hong [11]. Identification of these isolates based upon the formation of hyphal swellings, morphology of sporangia and growth at cardinal temperatures was easily made. DNA base sequence of ITS region of the isolate also showed 99% homology with the sequences of *P. cryptogea* with the accessions of KC695697 and KR011187.1 preserved in Gene Bank (NCBI).

Koike et al. [4] proved pathogenicity of their isolates by applying 2 mL of a zoospore suspension (2.0×10^5 zoospores/mL) to roots and crowns of 3-month-old potted sage plants. In this paper, the pathogenicity of isolate was tested by applying macerated cultures around crown regions of rooted cuttings, and identical symptoms were obtained in about 20 d. The method worked well, since the mycelia of the pathogen produce zoosporangia and zoospores in potting mixture in time. This method is also applicable for pathogenicity as far as the reactions of some sage cultivars are not tested, since this procedure needs quantitative evaluation.

Phytophthora species, including *P. cryptogea*, are usually non-host specific and might affect various annual and perennial crops when they are heavily watered. When, in greenhouse sage cutting cultivation is intended, sanitation practices of using disease free material should be considered in detail.

5. Conclusions

Various fungal diseases have been reported on common sage so far in Turkey, but *Phytophthora* root rot, caused by *P. cryptogea* is reported the first time by this study. To determine *Phytophthora* root rots selective media and special methods should be used.

Although zoospore suspensions are used for inoculation of *Phytophthora* species, the method of using macerated mycelial suspension gave quick results for pathogenicity testing.

Phytophthora species require abundant water in order to infect plants and are especially common in agricultural soils having water saturation. For this reason, cultivation of sage or other spice species should be avoided from the soils planted agricultural plants, especially vegetables and ornamental plants. Irrigation practices are also essential when considered the spread of the disease. Plants should not be watered heavily, especially by flooded irrigation. Preferably treated seeds with fungicides should be used for sage reproduction. If rooted cuttings are going to be used, sanitation in nurseries be practiced properly and the cuttings should be treated by phosphorous acid for probable *Phytophthora* infections.

Phosphorous acid is environmentally and toxicologically safe and very effective against *Phytophthora* root rots, but its type of application and rates should be investigated. The other diseases causing root rots, such as *Fusarium* spp. should also be considered.

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