

A new pathotype of *Synchytrium endobioticum* in Turkey: Pathotype 2

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ÖZ

Türkiye’de *Synchytrium endobioticum*’un yeni bir patotipi: Patotip 2

Patates siğil hastalığı etmeni *Synchytrium endobioticum* (Schilb.) Perc. patatese (*Solanum tuberosum* L.) zarar yapan en önemli organizmalardan biridir. Etmen EPPO’da A2 listesi zararlısı olarak yer almaktadır. Hastalık, sınırlı alanlarda birçok Avrupa ülkesinde görülmektedir. Patates siğil hastalığının şimdiye kadar 40’dan fazla patotipi tanımlanmış olup, Avrupa’daki en önemli patotipler 1(D1), 2(G1), 6(O1), 8(F1) ve 18(T1) olarak belirlenmiştir. Türkiye’de 6(O1), 18(T1) ve 38(Nev) numaralı patotipler tespit edilmiştir. Türkiye’de patates üretimi ve pazarlaması için hastalık artan bir sorun haline gelmiştir. Orta Anadolu’da 2014 yılında hastalıkla bulaşık alan 3.432,5 ha olarak rapor edilmiştir. Bu çalışmada yerel patotipleri belirlemek amacıyla 2014 yılında Nevşehir ili Derinkuyu ilçesinde iki farklı bulaşık alandan toprak örnekleri alınmıştır. Patotiplerin belirlenmesi için Glynn-Lemmerzahl metodu ve saksı testi ile iki farklı araştırma yürütülmüştür. Bu çalışmaların yürütüldüğü iki farklı tarlada bulunan *S. endobioticum* patotipleri 2(G1) ve 18(T1) olarak belirlenmiştir. Bu çalışma Türkiye’de patotip 2’nin belirlenmesinin ilk raporudur.

Anahtar kelimeler: Patates siğil hastalığı, *Synchytrium endobioticum*, patotip, Türkiye

ABSTRACT

Synchytrium endobioticum (Schilb.) Perc., the causal agent of potato wart disease, is one of the most important harmful organisms on potato (*Solanum tuberosum* L.). It is listed in the EPPO A2 list as a pest recommended for regulation. The disease occurs in many European countries in local limited areas. So far, more than 40 pathotypes of *Synchytrium endobioticum* are currently described in Europe, from which, the most important pathotypes are 1(D1), 2(G1), 6(O1), 8(F1) and 18(T1). The pathotypes 6(O1), 18(T1) and 38(Nev) of *S.*

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endobioticum were found previously in Turkey. In the following years, the disease became an increasing problem for Turkey in potato production and marketing. A total 3.432,5 ha area was reported as infested by the fungus in the Central Anatolia Region in 2014. In order to determine the local pathotypes, soil samples were collected from two different fields infested with the fungus in Derinkuyu county of Nevşehir city in June 2014. Two different experiments were carried out, a bioassay according to the Glynne-Lemmerzahl method and a pot test. *S. endobioticum* pathotypes present in the two fields in which the experiments conducted were determined as pathotypes 2(G1) and 18(T1). This paper is the first report of pathotype 2 in Turkey.

Keywords: Potato wart disease, *Synchytrium endobioticum*, pathotype, Turkey

INTRODUCTION

Potato is one of the most important crops in Turkey, with a growing area of approximately 150.000 hectares and an estimated annual average production of 4.6 million tonnes during the 2011-2013 growing periods (Anonymous 2014). Potato wart disease is a major quarantine disease caused by the obligat, biotrophic, soil-borne fungus *Synchytrium endobioticum* (Schilberszky) Percival (Flath et al. 2014). It is recorded as a quarantine pathogen (EPPO 2016) in 47 countries (Anonymous 2017) and losses in susceptible potato cultivars may reach 50–100% (Hampson 1993, Melnik 1998). Potato wart disease symptoms usually appear on tubers, stems and stolons but not on roots which are never affected. On infected tubers, the eyes develop into characteristic warty, cauliflower-like swellings. Tubers may bear more than one warty outgrowth and, in some cases, the whole tuber can be affected. When infected at early stage, tubers can become so distorted and spongy that they are almost unrecognizable (Anonymous 2014). Potato wart disease occurs in many European countries with a restricted distribution. Despite the disease had been observed in cooler areas previously, its occurrence in Turkey shows that the pathogen also adapted to continental climates (Çakır et al. 2009). Many pathotypes of the fungus exist; these are defined by their virulence on differential potato cultivars. The pathotypes 1(D1), 2(G1), 6(O1), 8(F1) and 18(T1) are the most prevalent in the EPPO region (EPPO 2004).

The resting spores of *S. endobioticum* are able to persist in soil for more than 20 years. Due to a lack of chemical control, quarantine measures are used to prevent the spread of the fungus. Cultivation of varieties resistant to *S. endobioticum* pathotypes in safety zone is a statutory obligation in Europe and Turkey according to the EU Council Directive 69/464 on control of potato wart disease. The use of resistant potato cultivars is mandatory around the infested areas. This study aimed to determine *S. endobioticum* pathotypes present in two infested fields under quarantine in Derinkuyu, Nevşehir.

MATERIALS AND METHODS

In 2014 soil samples were collected from two different infested fields (field 1 and field 2) in Derinkuyu district of Nevşehir in order to determine the local pathotypes of *S. endobioticum*. The number of resting sporangia determined according to Hampson and Thomson (1977) was 65 sporangia per gram of soil in field 1 and 87 sporangia per gram of soil in field 2. Two different bioassay methods were carried out in order to determine the presence of the pathogen and isolate them from the soil samples: a bioassay according to the Glynne-Lemmerzahl method (EPPO 2004) and a pot test (EPPO 2003). The inoculum for the Glynne-Lemmerzahl method was produced on the highly susceptible cultivar Marabel (Çakır et al. 2006).

Producing fresh wart material of *Synchytrium endobioticum*

In order to isolate *S. endobioticum* from two different soil samples, 48 tubers of potato cv. Marabel were placed in cell trays in a plastic box and covered with 4 kg of each soil sample. Potato tubers were watered as needed and incubated in climate chambers at 16–18 °C and 60–80% relative humidity for 5–6 weeks. Fresh developing warts were harvested and used for wart multiplication of each isolate separately according to the Glynne-Lemmerzahl method on the highly susceptible potato cv Tomensa and cv. Spunta which is resistant to pathotype 1. The multiplication processes were repeated ten times to obtain sufficient wart material. After the first three multiplications, only the cv. Tomensa was used for the subsequent seven multiplication process, because it produced more fresh warts than cv. Spunta.

Glynne-Lemmerzahl method

The tubers were treated with fungicide copper oxychloride (Miedzian 50 WP (Synthos Agro) in order to inhibit infections of other fungal pathogen *Rhizoctonia solani*. Sprouts and eye areas were cut out from these tubers except one eye. The eye was ringed with heated vaseline, using a plastic syringe. A pieces of fresh wart material (5-10 g) and some water were put on the eye surrounded with vaseline circe. The wart piece was removed after 48 h incubation at 8–12 °C in the dark and tubers were incubated in moist peat at 16–18 °C. The cover mixture (peat) was frequently moistened with distilled water during the incubation period (25 days) to induce wart formation. The reactions of sprouts were evaluated after 25 days incubation in the dark, wart developments were examined and data were recorded. At least three independent tests were conducted for evaluations on 15 eyes of potato tubers per cultivar in each trial (EPPO 2004).

Assessment of reaction types

Differential cultivar set

The most important European pathotypes were designated as 1, 2, 6, 8 and 18. These pathotypes are differentiated using differential potato cultivar set; Deodara,

Tomensa, Eersteling, Delcora, Producent, Combi, Saphir, Miriam, Karolin, Ulme and Belita (EPPO 2004). Differential potato cultivars enclosed cvs. Tomensa, Eersteling, Combi, Saphir, Miriam, Desiree were used in determination of the pathotypes. The reactions of the cultivars used in this experiment against pathotypes 1, 2, 6, 8 and 18 were given in Table 1.

Table 1. Differential potato cultivars for the identification of pathotypes of *Synchytrium endobioticum*.

Cultivar	P 1	P 2	P 6	P 8	P 18
Tomensa, Eersteling	S	S	S	S	S
Combi	R	S	S	S	S
Saphir	R	S	R	R	R
Miriam	R	R	R	R	S
Karolin, Ulme,	R	R	R	R	R

Classification of reaction types

In order to determine reactions, potato sprouts were carefully cleaned and disease symptoms were scored based on the classification table given in the EPPO protocol (EPPO 2004). The sprouts were examined 3-4 weeks after inoculation using a binocular stereo microscope at 40X and 80X magnification. Wart symptoms given in Table 2 were scored according to Langerfeld and Stachewicz (1994). For this purpose, each tuber was assessed according to scale levels given in Table 2. The tubers that had 1(R1), 2(R1), 3(R2) reactions were designated as resistant, tubers showed 4(S1) and 5(S2) reactions as susceptible. The results were obtained by comparison with the reactions in Table 1.

Table 2. Wart development symptoms for scoring according to the classification table laid down in the EPPO protocol (EPPO 2004).

1 (R1) Extremely resistant	2 (R1) Resistant	3 (R2) Weakly resistant	4 (S1) Slightly susceptible	5 (S2) Extremely susceptible
Early defence necrosis; no sorus formation	Late defence necrosis; single necrotic sori	Very late defence necrosis; up to five sori	Scattered infections; sprout can be malformed	Dense infection fields; tumour formation

Pot test

Additional pot trials were conducted with soil sample of field 1 (65 sporangia/g soil) to verify the results of Glynn-Lemmerzähl method with 50 tubers of cv. Saphir which is susceptible only pathotype 2 and 30 tubers of cv. Tomensa, which is

susceptible to all pathotypes of *S. endobioticum*, as controls. The soil samples were taken from infested fields in which potato production have been forbidden according to the EPPO Standard PM 3/59(2) (EPPO 2003). Thirty tubers of cvs Tomensa and Saphir were planted; one tuber was sown in 3-5 cm the depth of soil in each of the pots containing infested soil samples. The pots were located on separate trays in a glasshouse at 16–18 °C and 80% relative humidity (RH) and under 16 h light/8 h dark photoperiod. The soil was watered daily to keep moisture. Sprouts were cut when they reached 40 cm in height. After 8 weeks, tubers were uprooted from the pots and were evaluated in terms of the formation of wart.

RESULTS AND DISCUSSION

To identify the pathotype of *S. endobioticum* in two fields infested with wart disease in Derinkuyu district of Nevşehir, the differential potato cultivars Eersteling, Tomensa, Combi, Saphir, Miriam, and Karolin were inoculated with approximately 5-10 g of fresh warts. The reaction types were evaluated 4–6 weeks after inoculation according to the classification scheme in the EPPO Standard PM 7/28 (EPPO 2004).

Based on the results of isolate field 1 of *S. endobioticum*, cultivars Eersteling and Combi gave an extremely susceptible reaction (predominantly tumor formation); cvs. Miriam and Karolin showed resistant reaction (early and late defence necrosis), and cv. Saphir showed a slightly susceptible reaction (sori-fields and one small wart with numerous resting sporangia). Tomensa showed susceptible reactions in all experiments (Figure 1). To verify these results, additional pot trials were conducted with 50 tubers of cv. Saphir which is susceptible only pathotype 2 and 30 tubers of cv. Tomensa as a control which is susceptible to all pathotypes. Extensive wart formations could be observed on stems, tubers and stolons on both cultivars in eight weeks after planting in field 1 soil. This confirms the susceptible reaction of cv. Saphir to isolate field 1 (Figure 2) in the pot tests compatible with the results of Glynne-Lemmerzähl method. This result suggested that field 1 in Derinkuyu are contaminated with pathotype 2(G1) of *S. endobioticum*. This pathotype was previously recorded in Germany, The Netherlands and Canada (New Foundland) (Baayen et al. 2006). Pathotype 2(G1), one of the most prevalent pathotypes in Europe, was determined in Turkey with this experiment. This is the first record of pathotype 2(G1) in Turkey. Pathotypes 38 (Nev), 6(O1) and 18(T1) previously determined in Central Anatolia and pathotype 1(D1) in Blacksea Region in Turkey (Çakır et al. 2009).



Figure 1. Wart development on the potato sprouts (cv. Tomensa) in Glynne-Lemmerzahl method



Figure 2. *Synchytrium endobioticum* wart formations on stems and tubers of the potato cultivar Saphir

The isolate field 2 caused the reactions expected for pathotype 18. In the earlier, this pathotype has been described in this region. The isolate produced warts on Miriam cv. (positive reaction), but it did not produce warts on cv. Saphir (negative reaction). As reported in previous studies (EPPO 2004) in which pathotype 18(T1) caused sensitive reactions (S) on Eesterling, Combi and Miriam, and resistant reactions (R) on Saphir and Karolin, the results obtained from the present study were compatible with reaction types on the differential set in EPPO (2004). The data indicated that the isolate field 2 belongs to pathotype 18(T1) of *S. endobioticum*.

According to EU 69/464/EEC the member states of EU shall provide that in the safety zone potatoes may be grown only if they are of a variety which is resistant to the races of *S. endobioticum* found on the contaminated plot. Therefore, resistant potato varieties to pathotype 2(G1) as well as present other pathotypes should be cultivated in safety zone surrounding infested areas in Turkey.

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