Distribution of Mating Types and the Teleomorph of *Ascochyta rabiei* on Chickpea in Turkey

W. J. Kaiser, USDA-ARS, Western Regional Plant Introduction Station, Washington State University, P.O. Box 646402, Pullman 99164-6402; and I. Küsmenoglu, Central Research Institute for Field Crops, Ministry of Agriculture, Ankara, Turkey

ABSTRACT

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One hundred forty-five isolates of *Ascochyta rabiei*, the cause of Ascochyta blight of chickpea, were collected from chickpea (*Cicer arietinum*) in 23 provinces of Turkey. Each isolate was tested for mating type with compatible MAT1-1 and MAT1-2 tester isolates. Both mating types were found in 18 provinces. Of the isolates tested, 59% were MAT1-1 and 41% were MAT1-2. A great deal of variation in cultural characteristics was observed among the Turkish isolates in mycelial growth, sporulation, and colony appearance. Mature pseudothecia of *Didymella rabiei*, the teleomorph (sexual state) of *A. rabiei*, developed on naturally infested chickpea debris collected in 15 of 20 provinces when incubated under favorable conditions. This is a new geographic record for *D. rabiei*. The teleomorph may play an important role in long-distance dissemination of the pathogen and in increasing genetic diversity in the pathogen population in Turkey.

Chickpea (*Cicer arietinum* L.) is an important food and cash crop in Turkey, where it is cultivated on over 800,000 ha annually (1). In Turkey, various biotic and abiotic stresses adversely affect chickpea seed yields and quality (1-3,10,11,16). The most important disease of chickpea is Ascochyta blight caused by *Ascochyta rabiei* (Pass.) Labrousse. Serious crop losses occur when environmental conditions, especially cool, wet weather, favor disease development and spread (1,3,10,11,16). Dolar and Gürcan (3) identified races 1, 4, and 6 (15) among 20 isolates of *A. rabiei* collected from 11 sites in Turkey. Maden et al. (11) demonstrated that seed infection was important in spread and survival of the pathogen in Turkey. Of 100 seed samples collected from 5 provinces in Central Anatolia, Maden et al. (11) found that 75% were infected with *A. rabiei*. The incidence of seed infection was 16% or higher in 5% of the samples.

Didymella rabiei (Kovachevski) v. Arx (= *Mycosphaerella rabiei* Kovachevski), the teleomorph (sexual state) of *A. rabiei*, was discovered by Kovachevski in 1936 on overwintered chickpea debris in southern Bulgaria. The fungus is heterothallic (17,19). The teleomorph plays an important role in the epidemiology and control of Ascochyta blight in the United States Pacific Northwest (17) and Spain (12,18). The teleomorph of *A. rabiei* has not been reported from Turkey, although it has been reported in Bulgaria (9), Greece (21) and Syria (4), all of which border Turkey.

The objectives of this study were to determine the distribution of mating types of *D. rabiei* in different chickpea-growing regions of Turkey, and to establish whether the teleomorph developed on naturally infested chickpea residue collected after harvest in different provinces of the country.

MATERIALS AND METHODS

Isolates and culture maintenance.

Random samples of chickpeas infected with *A. rabiei* from different regions of Turkey were collected during the growing season in 1985, 1989, 1991, 1993, and 1994. Most samples were collected from farmers' fields. Plant tissues with lesions were surface-disinfected in 0.25% NaOCl for 5 min, dried on paper towels, and placed on 2% water agar (WA) and Difco potato dextrose agar (PDA; Difco Laboratories, Detroit) in 9-cm-diameter plastic petri dishes. The dishes were incubated at 21 to 23°C under fluorescent lights (12-h photoperiod, 77 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to induce abundant sporulation. Single-spored isolates of

A. rabiei were cultured on chickpea seed meal-dextrose agar (CDA; 5% chickpea flour, 2% dextrose, and 2% agar in 1 liter deionized water; 19). Single ascospore and conidial isolates on PDA and CDA were incubated at 21 to 23°C (12-h photoperiod) in plastic petri dishes sealed with Parafilm (American National Can, Neenah, WI).

Two methods were used to store isolates. In the first method, each single-spore isolate was grown on PDA and/or CDA at 21 to 23°C and then stored at 4°C. In the second method, isolates were grown on sterile chickpea stem pieces, 2 to 3 cm long, for 3 weeks on WA at 21 to 23°C, then dried, and stored in sterile plastic Whirl-pak bags (Nasco, Ft. Atkinson, WI) at -18°C.

Matings. The procedure used to determine the mating type of Turkish isolates of *A. rabiei* was similar to that used by Kaiser et al. (8) with *A. fabae* Speg. and *A. lentis* Vassilievsky. Conidial suspensions (1×10^6 conidia/ml) of single-spore cultures of each isolate and compatible mating types, designated MAT1-1 and MAT1-2 according to the genetic nomenclature of Yoder et al. (20), were prepared. Compatible MAT1-1 and MAT1-2 tester isolates used in this study have been deposited with the American Type Culture Collection (ATCC), Rockville, Maryland, as ATCC 76501 and 76502, respectively. Sterile chickpea stem pieces, 6 to 8 cm long, were placed in test tubes (2.2 by 17.5 cm) that contained 20 ml of a conidial suspension of a single isolate, or a mixture of one isolate with each tester isolate (MAT1-1 or MAT1-2). After 1 h, the spore suspension was decanted and the stem pieces were allowed to drain for 10 to 15 min in the tubes. The inoculated stem pieces were then placed on 10 layers of sterile filter paper that were moistened with 15 ml of sterile deionized water in 10-cm-diameter Pyrex petri dishes. After 24 h at 21 to 23°C, the dishes were incubated in the dark at 10°C for 5 weeks. The petri dishes were not sealed with Parafilm or placed in an air-tight container. After 5 weeks, the stem pieces were air-dried at 21 to 23°C in a laminar flow hood.

Ascospores were discharged from inoculated tissues by placing pieces of stem (2.5 cm long) on a 4 cm² WA block that was placed on the inner surface of a petri dish lid which was inverted over a bottom dish that contained WA. Ascospores discharged downwards onto the surface of the

Corresponding author: W. J. Kaiser
E-mail: wjkaiser@wsu.edu

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WA. Plates were incubated at 21 to 23°C for 24 h to observe viability and germination of the ascospores.

Teleomorph development. In nature, the teleomorph of *A. rabiei* develops only on overwintered chickpea debris and not on plants infected during the growing season (17). Naturally infested chickpea stems and pods from 23 chickpea-growing provinces of Turkey were collected and incubated at environmental conditions (described below) that favored development of the teleomorph. Two methods were used. In the first, infested tissue was placed on sterile filter paper (10 layers wetted with 15 ml of sterile water) in 10-cm-diameter Pyrex petri dishes. The dishes were incubated in the dark at 10°C for 5 weeks, at which time the tissue was air-dried at 21 to 23°C. The colonized stem pieces were tested for discharge of ascospores as described above. Single ascospore cultures were made on PDA from tissue samples in which ascospore discharge occurred. This was done to confirm the identity of the ascospore cultures as *D. rabiei*. In the second method, infested tissue was placed in nylon-net bags (11 by 13 cm) with a 1.6-mm mesh permeable to air. The bags were placed outdoors at Pullman, Washington, on the soil surface in October and collected the following April, at which time the tissue was rinsed in running tap water to remove soil particles and air-dried at 21 to 23°C. After drying, the tissue was tested for discharge of ascospores as indicated above. Single ascospore cultures were grown on PDA from the samples that

discharged ascospores to confirm the identity of the fungus as *D. rabiei*.

RESULTS

Mating types. One hundred forty-five isolates of *D. rabiei* were collected from 23 Turkish provinces. When paired with tester isolates of MAT1-1 (ATCC 76501) and MAT1-2 (ATCC 76502), 85 isolates were identified as MAT1-1 and 60 as MAT1-2 (Table 1). Both mating types were found in 18 provinces, including Adana, Amasya, Ankara, Antalya, Balıkesir, Burdur, Çorum, Denizli, Diyarbakır, Eskişehir, İçel, İzmir, Kırşehir, Konya, Sivas, Tokat, Uşak, and Yozgat (Table 1, Fig. 1). Turkish isolates of *A. rabiei* varied in mycelial growth, sporulation, and colony appearance. Similar variations in cultural characteristics also were observed by Dolar and Gürçan (3) in 20 isolates of *A. rabiei* collected from 11 locations in Turkey, and by Reddy and Kabbabeh (15) in 50 isolates of the pathogen collected in Syria and Lebanon.

Distribution of the teleomorph. *D. rabiei*, the teleomorph of *A. rabiei*, is widely distributed in different chickpea-growing regions of Turkey (Fig. 1). Fertile pseudothecia developed on ascochyta-infested chickpea stems and pods when they were incubated on moist filter paper at 10°C or outdoors in nylon-net bags. The teleomorph developed on chickpea residue from 15 of 20 provinces of Turkey, including Adana, Amasya, Ankara, Antalya, Burdur, Çorum, Denizli, Diyarbakır, Eskişehir, Kırşehir, Konya, Sivas, Tokat, Uşak, and

Yozgat. Ascospore discharge did not occur in tissue collected from the provinces of Afyon, Balıkesir, Erzurum, Gaziantep, İçel, and İzmir. Cultures derived from single ascospores discharged from chickpea debris from different provinces were identified as *D. rabiei* based on growth, sporulation, cultural characteristics, colony appearance, and morphology. Single ascospore cultures of *D. rabiei* from different provinces of Turkey differed in mycelial growth, sporulation, and colony appearance. These differences were similar to those observed by the authors in conidial isolates of the pathogen.

DISCUSSION

Both mating types of *A. rabiei* were found in 19 of 23 Turkish provinces from which naturally infested chickpea residue was collected. Fertile pseudothecia of *D. rabiei* developed on overwintered infested debris collected in 15 of 20 provinces. Although only small samples of chickpea debris were available for the overwintering studies, discharge of ascospores of *D. rabiei* from some samples was very high (more than 1,000 ascospores/cm² of tissue). *D. rabiei* will undoubtedly be found in other Turkish provinces as more samples of overwintered chickpea residue are examined for the teleomorph, particularly in those provinces where we found both mating types but where the teleomorph did not develop on the overwintered infested chickpea samples. If the teleomorph is not found in these areas, then its development may be affected by factors other than mating type distribution.

In Spain and the United States, the teleomorph plays an important role in the epidemiology of Ascochyta blight of chickpea (12,17,18). In both countries, it contributes to long-distance spread of ascospores which can serve as primary inoculum for establishing new infection foci in chickpea fields located several kilometers from blighted source fields (17,18). The teleomorph also aids in survival of the pathogen from one growing season to another on infested debris (7,12). In Turkey, the teleomorph is present in many chickpea-growing areas, where it undoubtedly contributes to disease development, spread, and survival. The effect of the teleomorph may be more pronounced in some areas than others due to the cultivation of different chickpea cultivars, differences in temperature and rainfall patterns, and/or use of different cultural practices. More studies need to be conducted in Turkey to establish the importance of the teleomorph in the epidemiology of the disease.

All 145 Turkish isolates of *A. rabiei* mated successfully with one or the other of the two tester isolates used in this study. All isolates were self-sterile. This also occurred with over 550 isolates from 22 other countries that have been paired by the senior author (6). Wilson and Kaiser

Table 1. Distribution of mating types and the teleomorph of *Didymella rabiei* on chickpea in different provinces of Turkey

Province	No. isolates tested	No. isolates		Teleomorph incidence
		MAT1-1	MAT1-2	
Adana	16	9	7	1/27 ^a
Afyon	4	4	0	NS ^b
Amasya	6	5	1	4/4
Ankara	7	3	4	3/7
Antalya	8	6	2	2/7
Balıkesir	4	3	1	0/1
Burdur	5	1	4	3/3
Bursa	2	0	2	NS
Çankırı	1	1	0	NS
Çorum	7	4	3	2/2
Denizli	8	5	3	4/5
Diyarbakır	5	1	4	7/9
Erzurum	5	5	0	0/1
Eskişehir	10	5	5	2/6
Gaziantep	4	4	0	0/1
İçel	5	3	2	0/2
İzmir	6	3	3	0/3
Kırşehir	5	2	3	2/2
Konya	6	4	2	1/5
Sivas	4	1	3	3/3
Tokat	5	3	2	1/3
Uşak	4	2	2	2/3
Yozgat	10	8	2	6/7
Unknown	8	3	5	NS
Total	145	85	60	43/101

^a Numerator = number of samples in which the teleomorph developed; denominator = number of samples tested.

^b NS = not sampled.

(19) demonstrated that *D. rabiei* was heterothallic and had a unifactorial (bipolar) homogenic mating incompatibility system. One gene with two alleles, designated MAT1-1 and MAT1-2, controls its mating type.

In 1985, Reddy and Kabbabeh (15) identified six races of *A. rabiei* from Lebanon and Syria on the basis of their pathogenicity on a set of six differential chickpea lines. The races differed markedly in virulence, with race 1 being the least virulent and race 6 the most virulent. In 1992, Dolar and Gürcan (3) inoculated 20 isolates of *A. rabiei* from 11 locations in Turkey and identified races 1, 4, and 6 using the same set of chickpea differentials as Reddy and Kabbabeh (15). As yet, no race, pathotype, or virulence evaluations have been made with our Turkish isolates. The sexual state may be contributing to the development of new pathotypes of the pathogen in Turkey that differ in virulence. Trapero-Casas (cited in 6) demonstrated that sexual reproduction provides a mechanism for recombining genes for avirulence and virulence in *A. rabiei* in a study involving the cross between a highly virulent isolate and an isolate of low virulence, both from Spain.

In Syria, highly virulent pathotypes of *A. rabiei* have been identified within the last few years (5), particularly in the northeastern province of Hassakeh, which has a border with the Turkish province of Mardin. Fertile pseudothecia developed on 3 of 3 infested chickpea samples from

Hassakeh province that were collected in 1994 (W. J. Kaiser, *unpublished data*). Through sexual recombination, the teleomorph may be involved in generating genetic diversity in the pathogen, which may have contributed to the development of these highly virulent isolates of *A. rabiei* now found in Hassakeh province. Long-distance spread of these highly virulent pathotypes across the border into Turkey could occur via airborne ascospores. Maden et al. (11) demonstrated the importance of infected chickpea seed in spread and survival of *A. rabiei* in Turkey. Movement of infected seed is one method by which these highly virulent Syrian isolates of *A. rabiei* could be moved from Mardin or adjacent provinces into other chickpea-growing regions of Turkey.

In Turkey, the teleomorph of *A. rabiei* is a component of the disease cycle and it may have a profound effect on the spread, survival, and control of Ascochyta blight as follows: (i) the presence of the teleomorph may contribute to increased genetic diversity in the fungus, which could have adverse effects on resistance breeding programs and chemical control strategies; (ii) the sexual state aids in survival of the pathogen from one growing season to the next on infested chickpea debris; (iii) sexual reproduction results in long-distance spread of airborne ascospores in the spring that may provide the primary inoculum source required for development of disease epidemics; and, (iv) the importation of *A. rabiei* on seed for

crop improvement and/or planting purposes may result in the distribution of new and more virulent pathotypes of the fungus, or in the introduction of compatible isolates of the blight pathogen into areas where only one mating type occurred previously, which could lead to the development of the sexual state.

Resistance to Ascochyta blight in chickpea cultivars has historically been overcome by new pathotypes of *A. rabiei* (13,14), as is now occurring in northeastern Syria (5). Implementation of an integrated control program may be needed to reduce the damage caused by the disease in some disease-prone areas, and to prevent or delay the time it takes for resistance to break down. This would include: (i) planting Ascochyta blight-free seed or treating seeds from blighted fields with effective fungicides, such as thiabendazole, that reduce the level of seedborne inoculum; (ii) practicing a three-year crop rotation; (iii) using cultural practices to manage infested crop debris which can serve as an important source of inoculum for the anamorphic (asexual) and teleomorphic states; (iv) identifying new sources of blight-resistant germplasm and using this to develop new cultivars with improved blight resistance; and, (v) planting blight-resistant cultivars when possible.

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Fig. 1. Provinces of Turkey in which both mating types (□) of *Didymella rabiei*, the teleomorph (sexual state) of *Ascochyta rabiei*, were found and where the teleomorph developed on naturally infested chickpea debris (*).

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