Virulence Forms of *Ascochyta rabiei* Affecting Chickpea in the Palouse

HAMIDULLAH JAN, Assistant Plant Pathologist, Agricultural Research Institute, Tarnab, Peshawar, Pakistan, and M. V. WIESE, Professor, Plant Pathology Division/PSES, University of Idaho, Moscow 83843

ABSTRACT

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Ascochyta blight, caused by the fungus *Ascochyta rabiei*, is a serious yield-limiting disease of chickpea (*Cicer arietinum*) in the Palouse region of northern Idaho and eastern Washington. To supplement the development of blight-resistant chickpea lines for release in the region, the virulence of local *A. rabiei* isolates was investigated. Thirty-nine isolates of *A. rabiei* were collected from infected chickpea seeds, plants, and residues from different Palouse locations. Each isolate was inoculated onto seedlings of 15 differential chickpea lines in the greenhouse. The resultant spectrum of disease reactions rated on a 1–9 scale on each set of differential hosts distinguished 11 different virulence forms among the 39 isolates. Three virulence forms accounted for 21 of the 39 isolates. Two isolates produced unique virulence spectra. Seven isolates closely resembled *A. rabiei* International Center for Agricultural Research in the Dry Areas (ICARDA) race 3. None of the isolates resembled ICARDA race 6, which was highly virulent on all but one of the host lines. Neither geographic origin nor morphological characteristics such as pycnidial diameter, spore size, colony color, or radial growth of each isolate were related to virulence. This study provided evidence that the population of *A. rabiei* in the Palouse is composed of diverse virulence forms. Local breeding efforts to develop blight-resistant chickpea cultivars may need to incorporate broad-spectrum or multigenic resistance to be successful.

Chickpea (*Cicer arietinum* L.) is a native Asian pulse plant grown especially in dryland areas of Asia (17). More recently, it has also been grown in the western United States and Canada. Chickpea production in the Palouse area of northern Idaho and eastern Washington began in 1983.

Ascochyta blight, a disease of chickpea caused by the fungus *Ascochyta rabiei* (Pass.) Labrousse, is of major importance in areas where cool, cloudy, humid weather (15–25 C and >150 mm of annual rainfall) occurs during the growing season (13). In the Palouse, the preferred kabuli-type cultivars are blight susceptible and have sustained severe yield losses. In 1988 and 1989, chickpea culture was curtailed in several northern Idaho counties in an attempt to reduce reservoirs of the blight pathogen.

A. rabiei is seedborne and also persists on host plants and infested crop residues. The disease is best controlled with resistant cultivars but, in their absence, the destruction or removal of infested crop residues and the use of clean seed (10) are recommended controls.

In 1988, *Mycosphaerella rabiei* Kovachevski, the teleomorph of *A. rabiei*, was observed in the Palouse (9). The presence of this sexual stage may be a source of genetic variability. *M. rabiei*, therefore, may be contributing new local virulence forms of *A. rabiei* that may play an important role in the breakdown of host resistance.

Previous studies of *A. rabiei* isolates on differential chickpea lines indicated that the pathogen is heterogeneous relative to virulence (5,6,8,11). New races of the pathogen have been reported to overcome the blight resistance in some chickpea cultivars (1,7).

In testing the virulence of several Indian isolates of *A. rabiei*, Vir and Grewal (18) reported two physiologic races and a biotype. Bedi and Aujla (3) also reported several races of *A. rabiei* in Punjab, India. Gowen et al (6) and Kaiser (8) observed differences in virulence among isolates of *A. rabiei* from southern and western Asia on different chickpea cultivars. In Pakistan, several races of the pathogen have been tentatively identified in the Punjab (12), whereas at the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria, six races of *A. rabiei* have been described (16). *A. rabiei* is also reported to vary in morphology as well as virulence (6,7,12,16).

An understanding of the virulence spectrum of *A. rabiei* in the Palouse is needed to determine if the local pathogen population is heterogeneous or homogeneous. Such information should yield clues about its origin and about its potential for control with resistant cultivars. Such information would facilitate screening chickpea lines for resistance and supplement local efforts to develop durable blight-resistant chickpea cultivars.

The primary objective of this study was to sample the population of *A. rabiei* in the Palouse and determine if different virulence forms exist. In additional tests, we attempted to determine if isolates of similar virulence were unique morphologically or in geographic distribution.

MATERIALS AND METHODS

Collection and purification of *A. rabiei* isolates. Beginning in 1988, a survey and collection of blighted chickpea plants and seed and infested residues was conducted. Chickpea seeds bearing symptoms of *A. rabiei* infection were surface-disinfested in 0.5% NaOCl/5% ethyl alcohol for 10 min. Infected chickpea leaves and stems were cut into segments approximately 1 cm long and surface-disinfested in NaOCl solution for 2 min. Surface-disinfested plant parts were dried on sterile blotting paper, placed on acidified water agar plus streptomycin (20 g of Difco Bacto agar, 1,000 ml of water, 25 mg of streptomycin sulfate, and 2.5 ml of 2% lactic acid) in disposable sterile petri plates, and incubated at 22–23 C for 10 days under alternate 12-hr periods of darkness and fluorescent light (10 $\mu E \cdot m^{-2} \cdot s^{-1}$).

Preliminary isolates of *A. rabiei* were purified by streaking a loopful of conidia (from a single pycnidium crushed in sterile distilled water) on plates of potato-dextrose agar (PDA) (200 g of Difco

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potato extract, 20 g of dextrose, 15 g of Difco Bacto agar, 1,000 ml of distilled water, and 25 mg of streptomycin sulfate). After incubation as described earlier for 3–5 days, isolated colonies that developed from single spores (checked microscopically) were transferred to chickpea seed meal agar (CPA) (40 g of chickpea seed meal, 18 g of Bacto agar, 20 g of dextrose, and 1,000 ml of distilled water) in petri plates. A total of 39 single-spore isolates of *A. rabiei* from each of 39 different locations were obtained in this manner. Each was maintained in a stock culture on CPA for 14 days at 22 C and held at 3 C in the dark for the duration of the study.

Virulence tests. ICARDA races 3 and 6 of *A. rabiei* (provided by M. P. Haware via M. C. Saxena, ICARDA, Syria) were used as standards for comparison with local Palouse isolates. In addition, a widely recognized set of 14 differential chickpea lines (from M. P. Haware, ICARDA, Syria), along with one local cultivar (UC-5), was used as standard differential hosts (Table 1). Three seedlings of each of the 15 host lines were grown in Sunshine Mix No. 1 (Fisons Horticulture, Inc., Vancouver, B.C.) in 10-cm-diameter pots in the greenhouse at 20–25 C with a 12- to 14-hr natural photoperiod. Host plants and *A. rabiei* cultures were initiated so that both seedlings and inoculum were ready for use after 10–15 days.

Inoculum of each purified *A. rabiei* isolate was developed on freshly prepared CPA. Each CPA plate was streaked with an excised 5-mm disk of the original stock culture and incubated at 22–23 C under alternate 12-hr fluorescent light (10 $\mu E \cdot m^{-2} \cdot s^{-1}$) and dark periods. After 10–15 days, spores were washed from pycnidia with sterile distilled water, counted with a hemacytometer, and adjusted to 200,000 spores per milliliter (2).

The capacity of the incubation chamber permitted up to eight isolates to be tested for virulence during a given 2-wk period. A set of three seedlings of each differential host line was prepared for inoculation with each isolate. Each seedling was carefully and uniformly sprayed to runoff with 5 ml of spore suspension with a hand sprayer. The sprayer was carefully rinsed with 95% ethanol and dried between each batch of inoculum. Each inoculated set of differential hosts was isolated in a translucent plastic-covered chamber in the greenhouse for 14 days. Relative humidity was maintained at 80–100% with humidifiers, and temperature ranged from 20 to 25 C accompanied by a 12- to 14-hr natural photoperiod (8,15).

Determination of virulence groups. A nine-point disease rating scale (Table 2) modified from Reddy and Nene (14) was used to evaluate blight development on each seedling at 10 and 14 days after inoculation. Virulence forms were designated according to the spectrum of disease reactions each isolate induced on the differential host lines. For the purpose of this study, the nine-point rating scale was divided into two virulence classes. Isolates were considered weakly virulent on a given host line if after 14 days the mean disease rating of the three inoculated seedlings ranged from 1.0 to 5.9. Isolates were considered highly virulent on a given host line if after 14 days the mean disease rating of the three inoculated seedlings ranged from 6.0 to 9.0. Isolates that produced identical spectra of weak and high virulence on all the differential hosts were grouped within the same virulence form.

In an initial test of all 39 isolates, disease reactions were rated and virulence forms were tentatively identified. These disease ratings and virulence designations were reexamined in two additional tests with 17 of the 39 isolates. The 17 selected isolates represented the diversity of virulence observed in the initial test of all 39 isolates and included at least one isolate of each of the virulence forms described in Table 1. All virulence spectra presented herein were developed with disease ratings collected 14 days after inoculation.

Isolate morphology vs. virulence. In another study, colony diameter and color, pycnidial diameter, and conidial size were compared within and among the apparent virulence forms. For this study, 20-day-old cultures of *A. rabiei* on both CPA and PDA were used.

Colony color and diameter were noted on five plates of medium inoculated with a 3-mm-diameter agar disk cut from the margin of an original stock culture of each isolate. These cultures were incubated at 22–23 C for 20 days in alternate 12-hr periods of darkness and fluorescent light (10 $\mu E \cdot m^{-2} \cdot s^{-1}$). For pycnidial diameter and conidial size, 5-mm-diameter disks were cut from the margin of the original culture of each isolate and streaked on the surface of each medium. These cultures were incubated as described

Table 1. Virulence characteristics[x] of 39 Palouse isolates and races 3 and 6 of *Ascochyta rabiei* on 15 differential chickpea genotypes

Chickpea genotype	Virulence form[y]											r3[z]	r6[z]
	A	B	C	D	E	F	G	H	I	J	K		
ILC-72	W	W	W	H	W	W	W	W	W	W	W	W	W
ILC-194	W	W	W	W	W	W	H	W	W	W	W	W	H
ILC-202	W	W	W	W	W	W	H	W	W	W	W	W	H
ILC-215	W	W	W	W	W	W	W	W	W	W	W	W	H
ILC-249	W	H	H	W	W	W	W	W	W	W	W	W	H
ILC-482	W	W	W	H	H	W	W	W	W	W	W	W	H
ILC-1929	W	H	H	H	H	H	H	H	H	H	H	H	H
ILC-2506	W	H	H	W	W	W	W	W	W	W	W	W	H
ILC-3279	W	W	W	W	W	W	W	W	W	W	W	W	H
ICC-1903	W	H	W	H	W	W	H	W	W	W	H	W	H
ICC-3996	W	W	W	W	W	W	H	W	W	W	W	W	H
ICC-9189	W	W	W	W	W	W	H	W	H	W	W	W	H
F-85-111	W	W	H	W	W	W	W	W	W	W	W	W	H
F-85-84	W	W	W	H	W	H	W	W	W	W	W	W	H
UC-5	H	H	H	H	H	H	H	H	H	W	H	H	H

[x]Ascochyta blight rated on a modified 1–9 scale according to Reddy and Nene (14). Isolates causing mean disease ratings of 1.0–5.9 and 6.0–9.0 were considered weakly virulent (W) and highly virulent (H), respectively.
[y]Total isolates comprising each virulence form are A, 7; B, 7; C, 3; D, 4; E, 1; F, 3; G, 2; H, 7; I, 2; J, 2; and K, 1.
[z]r3 = International Center for Agricultural Research in the Dry Areas (ICARDA) race 3, r6 = ICARDA race 6.

Table 2. Rating scale[z] for Ascochyta blight on chickpea seedlings

Disease rating	General appearance of diseased seedlings
1	No symptoms
2	Lesions few, small, inconspicuous, up to 2 mm in size occasionally present on some plant parts
3	Lesions few, scattered, larger, conspicuous, up to 5 mm but restricted in size
4	Lesions obvious on some or all plant parts, may exceed 5 mm in size, defoliation initiated
5	Lesions common, unrestricted in size, obvious on all plants/parts, defoliation and breaking and drying of branches slight to moderate
6	Lesions as in 5, defoliation, broken, dry branches common, some plants killed
7	Lesions as in 5, defoliation, broken, dry branches very common, up to 25% of the plants killed
8	Symptoms as in 7 but up to 50% of the plants killed
9	Symptoms as in 7 but up to 100% of the plants killed

[z]Modified from Reddy and Nene (14).

earlier. Pycnidial diameter and conidial length and width were recorded for 50 pycnidia and 50 conidia from each of two plates of each medium using a micrometer at ×100 and ×400 under a compound microscope. Mean values for each morphological characteristic were compared using Fisher's LSD, $P = 0.05$.

RESULTS AND DISCUSSION

In agreement with earlier studies (4), symptoms of Ascochyta blight appeared on the differential chickpea lines in our tests within 7 days after inoculation. Developing symptoms were similar but slightly more definitive 14 days after inoculation compared with 10 days after inoculation. The most virulent isolates killed host lines within 10–13 days. All three virulence tests of the 17 representative isolates resulted in the same spectra of weakly or highly virulent designations for each isolate/host combination.

Disease rating scale. Rating blight symptoms on the inoculated seedlings with the nine-point scale was relatively uncomplicated. On the other hand, when this scale of general disease appearance was supplemented with counts of buds killed, stems with lesions, and branches or stems broken, as proposed by Reddy et al (15), inconsistencies arose. In our tests, the percentage of stems with lesions was not closely tied to overall disease severity. Normally, more than 50% of the buds were killed on each seedling with a disease rating of 3 or above (Table 2). Only 10–20% of the stems bore lesions when disease was rated 3–5.

Virulence forms of *A. rabiei*. The 39 local isolates of *A. rabiei* and ICARDA races 3 and 6 yielded very different patterns of virulence on the differential host lines (Table 1). Dividing the disease ratings into weakly virulent and highly virulent classes distinguished 11 virulence forms (A–K) among the 39 Palouse isolates (Table 1).

Although the isolates in this study differed in virulence, we did not designate the different virulence forms as races because uniform techniques for race identification in *A. rabiei* are not available. As in previous studies (1,5–8,11), we used sets of differential hosts and a disease rating scale divided into severity categories. However, in each of these studies, the host set, disease rating scale, and severity categories were not consistent. Thus, there is strong evidence from several independent studies in addition to our own that races of *A. rabiei* exist, but an accepted system of race nomenclature or enumeration does not. Therefore, the virulence forms we identified qualify for designation only as Palouse races A–K, for comparison, for example, with ICARDA races 3 and 6.

In our test of Palouse isolates, seven isolates were grouped into virulence form A because each was uniquely highly virulent on chickpea line UC-5 and weakly virulent on all other genotypes. Two other isolates (virulence form J) were highly virulent only on host line ILC-1929. Two groups of seven isolates with unique virulence spectra were distinguished as virulence forms B and H. Virulence forms A, B, and H may be the most prevalent in the Palouse because they accounted for 21 of the 39 isolates. Two isolates (virulence forms E and K) displayed unique virulence spectra. Except for two isolates (virulence form J), all isolates were highly virulent on local cultivar UC-5. None of the isolates was weakly or highly virulent on all host lines.

The seven isolates in virulence form H resembled ICARDA race 3. These isolates were highly virulent only on UC-5 and ILC-1929 and were collected near Genesee, ID, and Pullman, WA. Because Ascochyta blight in the Palouse was originally observed in test plots in these two locations, race 3 may have been introduced with seed obtained originally from ICARDA. ICARDA race 6, unlike any local isolate, was highly virulent on all the differential host lines except ILC-72.

Most isolates comprising each virulence form had a similar geographic origin. However, some isolates within the same virulence form were collected at widely separated sites in Washington and Idaho. Such dislocation may result from concurrent but separate development of the virulence form, shipment of infected seed, or other transport of infested material.

Virulence within the local population of *A. rabiei* appears remarkably variable considering the few years of the pathogen's documented existence in the Palouse. Perhaps the local occurrence of its teleomorph, *M. rabiei*, contributes to this variability. Recently, it was also noted that *A. rabiei* can persist locally on hosts other than chickpea (9). This characteristic is further evidence of the pathogen's diversity. The local breeding effort to develop blight-resistant chickpea cultivars may need to incorporate broad-spectrum or multigenic resistance to be successful.

Morphological characteristics. When the isolates of each virulence form were compared for characteristics such as spore size, colony color, and radial growth in vitro, no definitive relationships to virulence were found. Only virulence forms A and B significantly differed in pycnidial diameter (218 vs. 171 μm, respectively, on PDA, $P = 0.05$). Differences in pycnidial diameter among all isolates were more pronounced on PDA than on CPA. Pycnidia ranged from 81 to 268 μm, and pycnidiospores ranged from 8.8 to 14.4 × 5.6 to 5.9 μm on both PDA and CPA. Colony color was mostly dark green brown and/or black and more variable on PDA than on CPA. In other studies (7,12), different races of *A. rabiei* were reported to have distinct cultural characteristics.

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