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Identification of Cercospora leaf spot resistance among fenugreek accessions and characterization of the pathogen

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Abstract

Cercospora leaf spot is a destructive and widespread disease of fenugreek (*Trigonella foenum-graecum* L.). To identify resistant germplasms and to morphologically characterize the disease pathogen on fenugreek plants, 48 accessions adapted to western Canada were screened by artificially inoculating plants with 1×10^4 spores ml^{-1} conidial suspensions of *C. traversiana* in growth cabinets. Out of these, nine accessions were characterized as resistant (R) or moderately resistant (MR) and the rest were susceptible. A more detailed study with 20 selected (9 resistant and 11 other) accessions using the artificial inoculation method revealed that the accessions were significantly ($p \leq 0.01$) different in their reaction to the disease. In the later test only two (L3717 and PI138687) had R and two (L3698 and F86) had MR ratings. An ANOVA for the agronomic traits measured indicated that the interaction effect of accession and treatment (with or without inoculation) was significant and the R or MR rated accessions were less affected by inoculation than the rest. The western Canada adapted accessions recovered from the fungal inoculation and produced a reasonable seed and biomass yield. Although further studies are warranted to verify the findings, the disease symptoms described, the disease effect on agronomic traits observed, and morphology of the pathogen may serve as a reference for future studies involving plant reaction to *C. traversiana*.

Keywords: Fenugreek, *Cercospora traversiana*, *Trigonella foenum-graecum*, cercospora leaf spot.
Abbreviations: ANOVA_ Analysis of variance; CABI_Centre for Agriculture and Biosciences International; ml_milliliter; PDA_ Potato-Dextrose Agar; RCBD_Randomized Complete Block Design; SEM_Scanning Electron Microscopy.

Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is an annual legume crop belonging to the family Fabaceae which is often cultivated in regions of India, the Mediterranean region and North Africa (Acharya et al., 2011, 2010; Petropoulos, 2002). Fenugreek seed is used as a spice, is one of the main ingredients in curry powder (Mary, 2009; Srinivasan, 2006) and also has a long history of use as a medicinal herb, being used extensively in both Indian Ayurvedic and traditional Chinese medicine (Tiran, 2003). A number of studies have revealed that fenugreek seeds contain several highly desirable biological compounds such as galactomannan, diosgenin, 4-hydroxyisoleucine and alkaloids that have specific health benefits (Bawadi et al., 2009; Acharya et al., 2007; Raju and Bird, 2006; Amin et al., 2005; McCue and Shetty, 2003). Fenugreek seedlings also are used as vegetables in parts of India. Ancient literature has highlighted its use as a forage crop for animals (Acharya et al., 2006) and it is a favorite fodder crop currently used in Egypt, India, Turkey and the Mediterranean region (McCormick et al., 2009). In recent years fenugreek is being developed as a forage crop for Canadian beef and dairy industries. Fenugreek is affected by many fungal diseases. Cercospora leaf spot caused by *Cercospora traversiana* is considered one of the most serious, destructive and widespread diseases of this crop (Acharya et al., 2010; Petropoulos, 2002; Ryley, 1989) and can cause considerable economic losses (Zimmer, 1984; Leppik, 1959). The disease is prevalent in countries where fenugreek is cultivated extensively (Singh et al., 2011; Elwakil and Ghoneem, 2002; Petropoulos, 2002), but is also reported to infect fenugreek in countries such as Hungary (Voros and Nagy, 1972), Bulgaria (Bobev et al., 1999), Australia (Ryley, 1989) and Canada (Zimmer, 1984) where the crop was introduced in recent years. The pathogen *C. traversiana* is a member of the Dothideomycetes, and is a seed-borne fungus (Acharya et al., 2010; Elwakil and Ghoneem, 2002; Ryley, 1989). Several researchers have suggested that *C. traversiana* is the only species of the *Cercospora* that can infect fenugreek (Ryley, 1989; Cook, 1978). Leppik (1959) noted that the geographic centre of origin of *C. traversiana* is in southern Asia, where fenugreek is native, and that occurrence of the pathogen in other countries is likely due to transport of infected seeds. Traditional methods for management of plant pathogens include the use of chemicals and genetic resistance. However, continuous use of chemicals to control plant pathogens is not environmentally sound as these chemicals may create a negative impact on the surrounding environment (Iqbal et al., 2011; Nariani, 1960). The use of disease resistant/tolerant crop cultivars is regarded as an economical and durable method of controlling fungal diseases (Tivoli et al., 2006). Measures to control fungal foliar diseases have relied on identification of resistant/tolerant germplasm and development of resistant/tolerant cultivars through effective screening (Tivoli et al., 2006). However, literature on *C.*

traversiana resistance is scarce, and there is no literature on screening of fenugreek germplasm to identify resistant or tolerant germplasm of this crop to the fungal pathogen. Although cercospora leaf spot disease in fenugreek is not considered a yield limiting factor in Canada, the disease was observed in Morden, Manitoba, Canada and other locations in the prairie provinces during 1983 (Zimmer, 1984), with reductions in seed yield up to 80%. As fenugreek is gaining more recognition in Canada and other countries where it has been introduced, it is expected that the acreage of fenugreek will increase resulting in an increased potential for the pathogen to cause serious economic damage. Therefore, identification of tolerant genotypes (germplasm) of this self-pollinated crop will be useful for development of resistant cultivars for both areas where fenugreek is being introduced and in areas where cercospora leaf spot disease is prevalent.
The objectives of this study were:
i) To screen fenugreek world accessions for resistance/tolerance to *Cercospora traversiana*,
ii) To characterize the disease in detail as it presents itself in western Canada.

Results and Discussions

Disease development

Among the three treatments tested, spraying fenugreek accessions with a concentration of 1×10^4 spores/ml of *C. traversiana* was most effective in developing disease symptoms for the fungus. To confirm that the microorganism extracted from diseased fenugreek leaves were *C. traversiana*, spores were cultured on PDA plates, and then used to inoculate healthy fenugreek plants. The disease symptoms that developed on the newly inoculated plants were compared with the disease symptoms from the original infected plants. Disease symptoms were found to be similar in these two groups of plants, and the culture characteristics were also the same as the culture of *C. traversiana* that was obtained from Centre for Agriculture and Biosciences International (CABI), United Kingdom, confirming infection by *C. traversiana*. The pathogen *C. traversiana* is considered an exotic microorganism in Canada, necessitating artificial screening of fenugreek accessions where the plants were inoculated with a conidial spore suspension of the pathogen to observe a disease reaction, under controlled conditions in growth chambers. Such screening of plants in a controlled environment provided advantages such as uniform environmental conditions for screening the large number of accessions necessary for use in a breeding program (Sillero et al., 2006); and during periods when environmental conditions in the field were not conducive for plant growth or disease development. Moreover, testing under controlled conditions allowed epidemiological factors to be observed in detail which might have been affected by biotic or abiotic stresses under field conditions. Tivoli et al. (2006) also found growth cabinet experiments to be more suitable than field assessments, as the measure of inherent resistance levels in the plants was highly correlated to genetic resistance alleles without interference of other interacting macro and micro environmental factors often associated with field conditions.

In general, disease symptoms on fenugreek plants appeared within 10 days of inoculation with conidial suspensions. Cercospora leaf lesions initially presented as circular, sunken spots that were bleached in color, with narrow (1–2 mm) chlorotic halos on the leaf surfaces. The lesions tended to elongate rapidly as the infection progressed, producing gray necrotic areas on the leaves that were sharply defined, and often surrounded by a characteristic yellowish halo (Fig. 1). Lesion size was significantly larger on mature leaves, where sporulation was frequently evident with the appearance of a whitish, velvet-like layer. The development of more than one spot was followed rapidly by yellowing and withering of the leaves. Severely infected plants were found to have only a few leaves situated towards the apex of the plant, or no leaves at all. Stem and seed pod infections were also observed. Sunken and bleached lesions were observed on stems and petioles. In severely infected plants, the main stem became yellow and secondary branches were found to dry up. Disease symptoms on pods resulted in discoloration of infected areas, and severely infected areas on pods were shrunken and twisted. Disease symptoms were successfully produced on fenugreek plants by inoculating the plants with a conidial spore suspension of *C. traversiana*. Through repeated inoculations it was confirmed that the disease symptoms were due to infection by only *C. traversiana*. The disease symptoms observed on fenugreek plants in this study were similar to the disease symptoms caused by *C. traversiana* on fenugreek reported earlier (Acharya et al., 2010; Bobev et al., 1999; Ryley, 1989; Zimmer, 1984).

Screening of fenugreek accessions

The results of this study revealed that there was great variation in response to the spore inoculant among accessions. These accessions were categorized into six groups based on their disease severity. None of the genotypes tested was found to be highly resistant against *C. traversiana*. Among the fenugreek accessions evaluated in this study, 2 (4.2%) were found to be resistant, 7 (14.6%) were moderately resistant, 20 (41.7%) were moderately susceptible, 18 (37.5%) were susceptible, and 1(2.1%) were highly susceptible to *C. traversiana* (Table 2). After preliminary screening, 20 selected accessions were subjected to a final disease screening test against *C. traversiana* in a growth chamber. This test showed that the accessions tested, differed significantly ($p \leq 0.01$) from each other for Cercospora leaf spot severity (Table 3). None of the accessions included in the test were in the highly resistant category. Two accessions, L3717 and PI138687 were less affected by the pathogen and were categorized as resistant while L3698 was moderately resistant (Table 4). The accession L3721 was categorized as highly susceptible to *C. traversiana*. Among the locally adapted genotypes, F86 performed better against *C. traversiana* and was placed in the moderately resistant category. To the best of our knowledge, this is the first report on screening of fenugreek world accessions for tolerance to Cercospora leaf spot disease. Partial resistance rather than complete resistance against a fungal disease observed in fenugreek is a common phenomenon in other legume crops. In lentil (*Lens culinaris*) and lupine (*Lupinus perennis*) partial resistance only has been reported for anthracnose (Tivoli et al., 2006; Yang et al., 2004; Buchwaldt et al., 1996, 2003; Bernier et al., 1992), whereas in pea (*Pisum sativum*) and chickpea (*Cicer arietinum*) only partial resistance against ascochyta blight has been observed (Kraft et al., 1998; Tivoli and Onfroy, 1997; Malhotra et al., 1996; Knappe and Hoppe, 1995). Observation of partial resistance in this study may have been due to the fact that our collection did not include highly resistant accessions or because our artificial inoculation method was too harsh on the plants. It is also possible that resistance for cercospora leaf spot disease is quantitatively inherited and that multiple genes are needed to impart resistance (Chattopadhyay et al., 2010) and so absolute resistance was not observed. Qualitative resistance is

Table 1. Fenugreek accessions categorized into six disease severity groups (Iqbal et al., 2011) based on the results of the primary and final disease screenings when inoculated with *Cercospora traversiana* pathogen in growth cabinets.

Resistant category	Disease severity	Number of Genotypes	Accessions
Highly resistant	0	0	--
Resistant	1	2	L3717, PI138687
Moderately resistant	2	7	L3690, L3696, L3698, L3701, L3715, PI269994, F86
Moderately susceptible	3	20	L3172, L3177, L3691, L3693, L3694, L3695, L3706, L3707, L3708, L3709, L3713, L3714, L3720, PI195691, PI577711, PI57713, Amber, F70, F80, Tristar
Susceptible	4	18	L3308, L3312, L3697, L3699, L3700, L3703, L3704, L3705, L3711, L3716, L3718, L3719, NGC2001, PI199264, PI211636, Quatro, X92-23-3, ZT-5
Highly susceptible	5	1	L3721

Fig 1. *Cercospora traversiana* fungal structures on sharply defined grey necrotic area on a diseased fenugreek leave (a characteristic symptom for cercospora leaf spot disease) (magnification 20X) under a compound microscope.

usually monogenic in nature, typically inherited in a simple Mendelian fashion in host plants, whereas quantitative resistance is based on the assumption that there is multiple gene control that collectively confers divergent levels of resistance. The resistance pattern observed in the fenugreek accessions tested suggests that the resistance response may be quantitative, although further work (including field work) is required to confirm the resistance response of this crop.

Impact of the disease on agronomic traits

Agronomic traits such as plant height, pod number per plant, seed weight per plant, and dry biomass weight per plant were measured for the 20 fenugreek accessions included in the final test. For the purpose, each accession treated with *C. traversiana* and their non-infected counterparts were utilized to find out the percent loss for the traits tested due to pathogenic infestation. An ANOVA on data generated for the agronomic traits showed that the accessions were significantly ($p \leq 0.01$) different from each other (Table 5). The treated plants were also significantly ($p \leq 0.01$) different from the control. The interaction effect of accession and treatment was also found to be statistically significant ($p \leq 0.01$). Cercospora leaf spot affected plant height, pod number per plant, seed weight per plant, and biomass weight per plant in fenugreek plants infected by spraying with a culture of *C. traversiana*. For plant height, a reduction of 0.8% to 63.8% was observed as a result of cercospora leaf spot disease (Table 5). The accessions L3717 and PI138687 were found to be less affected by the pathogen for this trait, whereas L3700, L3697 and L3721 were found to be affected most by the inoculation. A range of 22.1% to 100.0% loss in pod number was observed among the fenugreek accessions due to inoculation (Table 5). Accessions L3717, PI138687 and L3698 performed well for this trait under disease pressure. In contrast, accessions L3697 and L3721 were affected most by the pathogen and produced no pods at all. For seed weight per plant, a 30.3% to 100.0% loss was observed due to cercospora leaf spot disease (Table 5). Accessions PI138687, L3698 and L3717 were found to be the least affected, whereas L3697, L3721 and L3700 were most affected by this pathogen. For biomass weight per plant, an 8.9% to 90.5% loss was observed due to the disease (Table 5). Accessions PI138687 and L3717 performed well for this trait, whereas L3697, L3700 and L3721 were found to be the most affected. The results show that the fenugreek world accessions tested varied greatly in their reaction to *C. traversiana* for the agronomic traits tested. For the agronomic traits observed, resistant types were affected less than susceptible types. Among the locally adapted genotypes, it was interesting to note that although Amber, F70 and Tristar showed a moderate susceptibility reaction to *C. traversiana*, they overcame the disease stress towards maturity, and performed relatively well in comparison to other genotypes as observed

Table 2. ANOVA on disease severity index of 20 fenugreek accessions used in the final disease screening test conducted in a growth cabinet.

Disease Severity		
Source of variation	DF	Mean Square
Accession	19	6.018**
Residual	140	0.405
Coefficient of variation (%)	10.05	

* Denotes significance at $p \leq 0.01$.

earlier (Ryley, 1989). This observation on locally adapted genotypes may have been due to their exposure to low levels of pathogen interaction during many years of testing over a wide range of environments in western Canada and can be considered field level resistance from a crop producer prospective.

Pathogen morphology

Fenugreek seeds collected from diseased plants were used to evaluate the seed-borne nature of the microorganism. *Cercospora traversiana* colonies were recovered from all contaminated seeds, confirming that there is seed-borne inheritance of the pathogen. In this study, *C. traversiana* colonies were seen as cottony white and slightly raised on the upper side of the colony, and the underside of the colonies was olivaceous black with narrow sectors of pale olivaceous grey. The colonies were circular, 46±4 mm in diameter, with irregular margins. Fungal growth on the diseased leaves is shown in Figure 1. Conidiophores of *C. traversiana* were dark, paler towards the tip, unbranched, and rarely geniculate or septate. These conidiophores developed in groups of 3 to 12 conidiophores per fascicle, with a length ranging from 17.6 to 28.8 µm and a width ranging from 1.78 to 3.01 µm (Figure 2). The conidia were hyaline, acicular, straight or slightly curved, with a rounded apex, a truncate base and multicellular, with a length ranging from 2.3 to 2.8 µm and a width ranging from 1.2 to 1.9 µm. This study confirmed that seed-borne contamination by *C. traversiana* occurs as observed in other studies (Elwakil and Ghoneem, 2002; Ryley, 1989; Zimmer, 1984). The morphology and shapes of different structures of the fungus reported in this study also was found to be in agreement with previous studies (Ryley, 1989; Zimmer, 1984; Leppik, 1959). The only difference in the fungi observed in the present study was in the size of the conidia and conidiophores. In this study, the sizes were very similar whereas the sizes of these structures were variable in previous studies. This may have happened due to the differential culture environment and nature of the substrate used to grow the pathogen (Ryley, 1989).

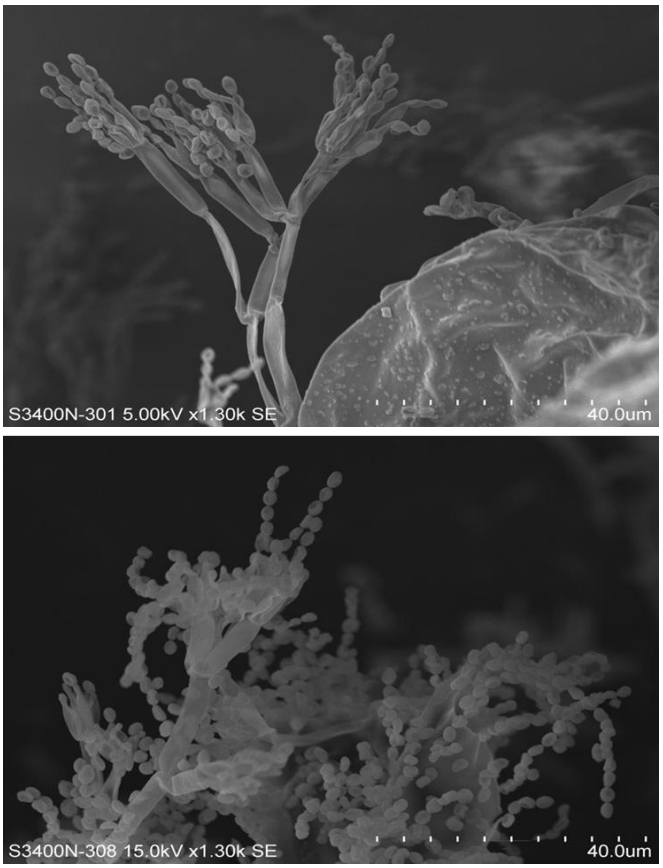

Fig 2. Scanning Electron Micrograph (SEM) of *Cercospora traversiana* fungal structures on diseased fenugreek leaves showing chains of conidia,characterized by rounded apex and truncate base, on unbranched conidiophores and conidia (A and B) under an electron microscope (magnification at 1.3×103x).

Discussion

The pathogen *C. traversiana* is considered an exotic microorganism in Canada, necessitating artificial screening of fenugreek accessions where the plants were inoculated with a conidial spore suspension of the pathogen to observe a disease reaction, under controlled conditions in growth chambers. Such screening of plants in a controlled environment provided advantages such as uniform environmental conditions for screening the large number of accessions necessary for use in a breeding program (Sillero et al. 2006); and during periods when environmental conditions in the field were not conducive for plant growth or disease development. Moreover, testing under controlled conditions allowed epidemiological factors to be observed in detail which might have been affected by biotic or abiotic stresses under field conditions. Tivoli et al. (2006) also found growth cabinet experiments to be more suitable than field assessments, as the measure of inherent resistance levels in the plants was highly correlated to genetic resistance alleles without interference of other interacting macro and micro environmental factors often associated with field conditions. Disease symptoms were successfully produced on fenugreek plants by inoculating the plants with a conidial spore suspension of *C. traversiana*. Through repeated inoculations it was confirmed that the disease symptoms were due to infection by only *C. traversiana*. The disease symptoms observed on fenugreek plants in this study were similar to the disease symptoms caused by *C. traversiana* on fenugreek reported earlier (Acharya et al., 2010; Bobev et al., 1999; Ryley 1989; Zimmer 1984). Partial resistance rather than complete resistance against a fungal disease observed in fenugreek is a common phenomenon in other legume crops. In lentil (*Lens culinaris*) and lupine (*Lupinus perennis*) partial resistance only has been reported for anthracnose (Tivoli et al., 2006; Yang et al., 2004; Buchwaldt et al., 1996, 2003;

Table 3. Mean disease severity of 20 fenugreek accessions used in final disease screening trial using *Cercospora traversiana* inoculum.

Accession	Disease severity	Resistant category	Accession	Disease severity	Resistant category
Amber	2.625^{def}	MS	L3704	4.000^{ab}	S
F70	2.625^{def}	MS	L3705	3.750^{abcd}	S
F80	3.250^{bcdef}	MS	L3707	3.250^{bcdef}	MS
F86	2.250^{fgh}	MR	L3713	3.500^{abcde}	MS
L3312	3.875^{abc}	S	L3716	3.500^{abcde}	MS
L3693	3.250^{bcdef}	MS	L3717	1.250^{h}	R
L3697	3.875^{abc}	S	L3720	3.500^{abced}	MS
L3698	2.500^{efg}	MR	L3721	4.500^{a}	HS
L3699	4.000^{ab}	S	PI138687	1.375^{gh}	R
L3700	3.875^{abc}	S	Tristar	2.750^{cdef}	MS

R=Resistant; MR=Moderately resistant; MS=Moderately susceptible; S=Susceptible; HS=Highly susceptible
Means sharing same superscripts are not significantly different from each other (Tukey's Honestly Significant Difference (HSD) at $p < 0.05$).

Fig 3. Showing (A) Controlled fenugreekplant (healthy plant); (B) Moderately susceptible fenugreekplant; and (C) Highly susceptible fenugreek plant when infected with C. traversiana

Bernier et al., 1992), whereas in pea (*Pisum sativum*) and chickpea (*Cicer arietinum*) only partial resistance against ascochyta blight has been observed (Kraft et al., 1998; Tivoli and Onfroy 1997; Malhotra et al., 1996; Knappe and Hoppe 1995). Observation of partial resistance in this study may have been due to the fact that our collection did not include highly resistant accessions or because our artificial inoculation method was too harsh on the plants. It is also possible that resistance for cercospora leaf spot disease is quantitatively inherited and that multiple genes are needed to impart resistance (Chattopadhyay et al., 2010) and so absolute resistance was not observed. Qualitative resistance is usually monogenic in nature, typically inherited in a simple Mendelian fashion in host plants, whereas quantitative resistance is based on the assumption that there is multiple gene control that collectively confers divergent levels of resistance. The resistance pattern observed in the fenugreek accessions tested suggests that the resistance response may be quantitative, although further work (including field work) is required to confirm the resistance response of this crop. The results show that the fenugreek world accessions tested varied greatly in their reaction to *C. traversiana* for the agronomic traits tested. For the agronomic traits observed, resistant types were affected less than susceptible types. Among the locally adapted genotypes, it was interesting to note that although Amber, F70 and Tristar showed a moderate susceptibility reaction to *C. traversiana*, they overcame the disease stress towards maturity, and performed relatively well in comparison to other genotypes as observed earlier (Ryley 1989). This observation on locally adapted genotypes may have been due to their exposure to low levels of pathogen interaction during many years of testing over a wide range of environments in western Canada and can be considered field level resistance from a crop producer prospective. This study confirmed that seed-borne contamination by *C. traversiana* occurs as observed in other studies (Elwakil and Ghoneem 2002; Ryley 1989; Zimmer 1984). The morphology and shapes of different structures of the fungus reported in this study also was found to be in agreement with previous studies (Ryley 1989; Zimmer 1984; Leppik 1959). The only difference in the fungi observed in the present study was in the size of the conidia and conidiophores. In this study, the sizes were very similar whereas the sizes of these structures were variable in previous studies.

Table 4. ANOVA on data generated using *Cercospora traversiana* treated and untreated plants and their interaction on plant height, pod number per plant, seed weight per plant and dry biomass weight per plant observed on 20 fenugreek accessions.

Source of variation	DF	Mean Square			
		Height (cm)	Pod number	Seed weight (g)	Dry biomass weight (g)
Accession	19	52.65**	445.42**	3.26**	31.33**
Treatment	1	3165.76**	33636.00**	338.62**	2328.14**
Accession×Treatment	19	37.78**	221.80**	1.62**	2.53**
Replication	7	5.18	35.62	0.62	1.85
CV (%)		7.49	15.51	19.25	10.72

** Denotes significance at $p \leq 0.01$.

Table 5. Mean agronomic performance of 20 fenugreek accessions with *Cercospora traversiana* treatment compared to untreated control (in parenthesis).

Accessions	Height (cm)		Pod number		Seed yield (g)		Biomass weight (g)	
Amber	33.35^{abcd}	(73.9)	12.50^{bcd}	(44.1)	0.91^{bc}	(34.4)	5.13^{abcde}	(64.3)
F70	32.63^{abcd}	(73.9)	14.88^{bc}	(52.6)	0.73^{bcde}	(23.3)	5.51^{abcd}	(64.4)
F80	24.76^{def}	(59.8)	7.00^{cde}	(22.1)	0.34^{cdef}	(11.6)	3.39^{cdef}	(40.7)
F86	34.62^{abcd}	(73.6)	12.75^{bcd}	(25.5)	0.81^{bcd}	(29.3)	5.63^{abcd}	(62.1)
L3312	29.94^{bcde}	(66.7)	1.50^{e}	(5.5)	0.12^{f}	(4.2)	1.74^{f}	(17.9)
L3693	25.19^{def}	(55.6)	3.50^{e}	(19.6)	0.27^{def}	(10.4)	3.03^{cdef}	(32.8)
L3697	15.64^{f}	(37.5)	0.0^{e}	(0.0)	0.0^{f}	(0.0)	0.79^{f}	(9.5)
L3698	41.50^{abc}	(87.4)	18.86^{ab}	(70.9)	1.97^{a}	(68.8)	8.18^{a}	(65.4)
L3699	23.39^{def}	(51.8)	0.50^{e}	(1.0)	0.03^{f}	(0.8)	1.24^{f}	(12.4)
L3700	15.84^{f}	(36.2)	0.88^{e}	(3.4)	0.11^{f}	(5.5)	0.90^{f}	(9.5)
L3704	27.76^{def}	(61.2)	2.50^{e}	(8.5)	0.09^{f}	(3.2)	2.53^{ef}	(24.1)
L3705	28.80^{cde}	(74.6)	4.13^{e}	(16.0)	0.30^{def}	(1.3)	3.23^{cdef}	(36.4)
L3707	33.55^{abcd}	(65.7)	7.13^{cde}	(22.7)	0.47^{cdef}	(17.7)	4.99^{bcde}	(48.8)
L3713	26.06^{def}	(57.4)	4.88^{de}	(16.6)	0.23^{ef}	(9.7)	3.41^{cdef}	(34.7)
L3716	27.55^{def}	(57.9)	1.34^{e}	(6.1)	0.19^{ef}	(8.2)	2.76^{def}	(31.4)
L3717	44.93^{a}	(99.2)	24.36^{a}	(77.9)	1.88^{a}	(68.1)	8.01^{ab}	(85.7)
L3720	28.29^{def}	(54.7)	3.63^{e}	(11.4)	0.29^{def}	(11.1)	3.12^{cdef}	(31.9)
L3721	18.69^{ef}	(40.6)	0.0^{e}	(0.0)	0.0^{f}	(0.0)	0.81^{f}	(10.2)
PI138687	42.74^{ab}	(96.0)	24.63^{a}	(71.6)	1.83^{a}	(69.7)	7.53^{ab}	(91.1)
Tristar	31.85^{bcd}	(71.1)	17.50^{ab}	(52.1)	1.14^{b}	(31.5)	5.94^{abc}	(63.8)

Means with same superscripts within a column were not significantly different from each other (Tukey's Honestly Significant Difference (HSD) at $p < 0.05$).

Table 6. The fenugreek accessions, their corresponding sources from which seed was obtained and origin.

Accessions	Source	Origin	Accessions	Source	Origin
AMBER	AAFC, Morden, MB	Unknown	L3707	Gujarat	India
Tristar	AAFC, Lethbridge, AB	Shiraz, Iran	L3708	Hyderabad	India
F70	CDC South, AB	Turkey	L3709	Mumbai	India
F80	CDC South, AB	India	L3710	Varanasi	India
F86	CDC South, AB	Afghanistan	L3711	Lucknow	India
L3068	AAFC, Lethbridge, AB	India	L3712	Pushkar	India
L3172	India	India	L3713	Bhopal	India
L3177	India	India	L3714	Chennai	India
L3308	CDC South, AB	Unknown	L3715	Imphal	India
L3312	CDC South, AB	Unknown	L3716	Gauhati	India
L3375	China	China	L3717	Bangalore	India
L3690	Gujrat	India	L3718	Bhubaneshwar	India
L3691	Hyderabad	India	L3719	Srinagar	India
L3692	Chennai	India	L3720	Rajasthan	India
L3693	Rajasthan	India	L3721	Rajasthan	India
L3694	Lucknow	India	NGC 2001	Grocery store, Canada	Unknown
L3695	New Deli	India	PI138687	PGRC, Canada	Shiraz, Iran
L3696	Guwahati	India	PI143504	PGRC, Canada	Hamadan, Iran
L3697	Amritsar	India	PI195691	PGRC, Canada	Ethiopia
L3698	Madhya Pradesh	India	PI199264	PGRC, Canada	Greece
L3699	Bangalore	India	PI211636	PGRC, Canada	Afghanistan
L3700	Kidderpore	India	PI269994	PGRC, Canada	Pakistan
L3701	Mumbai	India	PI577711	PGRC, Canada	Meknes, Morocco
L3702	Bhubaneshwar	India	PI577713	PGRC, Canada	Madrid, Spain
L3703	Rajasthan	India	PI229626	CDC North, AB	Unknown
L3704	Amritsar	India	QUATRO	PGRC, Canada	Unknown
L3705	New Delhi	India	X92-23-3	PGRC, Canada	Unknown
L3706	Kulkata	India	ZT-5	PGRC, Canada	Unknown

AAFC=Agriculture and Agri-Food Canada; AB=Alberta; CDC=Crop Development Centre; PGRC=Plant Gene Resources of Canada

This may have happened due to the differential culture environment and nature of the substrate used to grow the pathogen (Ryley, 1989). This study identified some resistant (L3717 and PI138687) and some moderately resistant (F86 and L3698) accessions, which may be useful in breeding programs aimed at development of cercospora leaf spot resistant cultivars. The two resistant accessions performed well agronomically and so can be used for development of cercospora leaf spot resistant fenugreek cultivars without fear of losing agronomic performance. Out of the four resistant and moderately resistant accessions observed, two (L3717 and L3698) were from India, one (F86) from Afghanistan and one from (PI138687) Iran. Since south-east Asia is the center of origin for fenugreek, a more extensive germplasm collection from these areas may include more resistant types. In this study, disease symptoms, disease effect on some important plant traits, and morphology of the pathogen were characterized; these traits may serve as a reference for future studies regarding fenugreek and its reaction to *C. traversiana*.

Materials and Methods

Fenugreek accessions tested

Screening of available cultivars and germplasm for presence of funtional resistant/tolerant gene(s) constitutes the basis of identifying resistant sources to plant pathogenic diseases (Ashfaq et al., 2007; Tivoli et al., 2006; Buchwaldt et al., 1996). In this study, a total of 53 fenugreek accessions (Table 6) were evaluated for cercospora leaf spot development after artificial inoculation with *C. traversiana* spores in a growth chamber. These 53 accessions were selected from our collections for their adaptation to western Canada growing conditions and included Canadian cultivars (Amber and Tristar), and three genotypes (F70, F80 and F86) that were selected for improved forage and seed yield performance in western Canada.

Culture of pathogenic fungi and preparation of inocula

A live pure culture of *Cercospora traversiana* isolated from *Trigonella foenum-graecum* L. was obtained from CABI, United Kingdom (IMI # 318080) and cultured on PDA. Plates containing the cultures were incubated at $25^{o}\pm2^{o}$C in darkness for 30 days to promote sporulation (Ryley, 1989).

Inocula were prepared from 30-day old fungal colonies growing on PDA plates kept at $25^{o}\pm2^{o}$C in darkness. Sterile distilled water (5 ml) was added to each plate and conidia were removed by brushing the colony's surface with a fine camel hair brush. The suspension was filtered through a double layer of cheese cloth and then adjusted to 1×10^{4} spores/ml (Ryley, 1989).

Growth chamber experiment

All 53 accessions were included in a screening experiment using a growth chamber at the Agriculture and Agri-Food Canada Lethbridge Research Centre, Lethbridge, Alberta. The plants were grown in a growth chamber set at 23±2 ^{o}C and a 16/8 h day/night photoperiod cycle following a Randomized Complete Block Design (RCBD)(Sillero et al., 2006). Before all the accessions were inoculated an effective spore concentration for use in a spray solution was determined by using two accessions of different origin. One accession of Indian origin and one of Iranian origin were inoculated separately with three concentrations (0.5×10^{4} spores/ml, 1×10^{4} spores/ml and 1.5×10^{4} spores/ml). The number of conidia in the spray suspension was estimated using a haemocytometer as described in Reddy et al. 1987. A conidial concentration of 1×10^{4} spores/ml in the spray suspension was effective in producing symptoms of cercospora leaf spot disease in developing fenugreek plants.

Among the 53 accessions included in the test, five repeatedly showed very poor germination ability, and for this reason they were removed from the experiment. For each accession eight plants were grown in the growth chamber. Out of these, four 21-day old plants were inoculated with *C. traversiana* conidial suspension spray and four uninoculated plants were kept as controls. Disease severity in each fenugreek accession was measured by comparing treated plants with control plants of the same accession. For cercospora leaf spot of fenugreek, it has been reported that high temperature and a high level of relative humidity is critical for development of the disease (Acharya et al., 2010; Petropoulos, 2002; Ryley, 1989). To maintain a high relative humidity for successful infection, a "mini-dome technique" developed by Chen and Muehlbauer (2003) was used. Twenty-one day old seedlings were inoculated with a conidial suspension by spraying them until run-off from the plant was observed; the seedlings were covered immediately with inverted translucent polythene bags to form mini-domes. The plastic bags were removed after 5 days. Disease development was evaluated 10 days after inoculation. The inoculated plants were scored as % of affected leaves. Fenugreek leaves are relatively smaller than most of the leguminous species. The *C. traversiana* inoculum was able to generate high levels of leaf infection on susceptible fenugreek plants and so it is relatively easy to visually score for the level of infection. At first of the infection stage, small olive-black like dots appears on the adaxial surface of leaves, which rapidly coalesce together and cover 50% or more of the leaf surface, ultimately causing shedding off of the infected leaves. Thus % leaf loss due to infection was a good indication of leaf infection caused by *C. traversiana* (Figure 3). Scores based on % infected leaves were done rapidly and, were found to be reproducible (Sillero et al., 2006). The percentage of leaves affected by cercospora leaf spot were assessed visually on a scale of 0 (highly resistant) to 5 (highly susceptible) as described in Iqbal et al. 2011. This scale considers 0 = 0% plant leaves affected (highly resistant=HR), 1 = 1-15% plant leaves affected (resistant=R), 2 = 16-40% plant leaves affected (moderately resistant=MR), 3 = 41-65% plant leaves affected (moderately susceptible=MS), 4 = 66-90% plant leaves affected (susceptible=S) and 5 = 91-100% plant leaves affected (highly susceptible=HS) (Figure 1). Visual assessment of disease severity on a given plant can be different depending on the evaluator, as each evaluator has a subjective perception of the percent plant leaves affected by the disease. To overcome this, each plant was rated by two people separately and the averages of these two ratings were used as a final disease score for the plant. Four top accessions exhibiting cercospora leaf spot resistance and 11 accessions with MS or S scores but with good growth from the primary disease trials were selected for a final trial. In addition to the above 15 accessions this trial included five Canadian adapted genotypes (Amber, Tristar, F70, F80 and F86). In spite of our best efforts to control procedural variability in the primary disease trials, some plant to plant variability was observed within accessions. Therefore, in the final disease trial, number of replicates was increased to eight to improve chances of detecting statistical differences in cercospora leaf spot resistance among the accessions. In this case, eight plants were inoculated with *C. traversiana* inoculum and eight plants were kept as controls for each genotype. The

inoculation and disease severity rating process were the same as described for the primary disease trial. The plants in both categories (treated and control) were allowed to grow and mature after disease severity ratings were determined. Data on selected agronomic traits such as plant height, number of pods/plant, seed weight/plant and biomass weight/plant were observed from treated and control plants for each accession.

Evaluation of the seed-borne nature of Cercospora traversiana

Fenugreek seeds from diseased pods and from healthy pods were used to evaluate the seed-borne nature of the microorganism. Five seeds from each type were soaked in 95% ethanol for 40 seconds and then washed in two changes of sterile distilled water (Ryley, 1989). The seeds were then partly submerged in separate PDA plates, sealed with parafilm™ and kept at $25^{o}\pm2^{o}$C in darkness for 10 days. The same procedure was repeated in three consecutive weeks.

Morphology of Cercospora traversiana

The pathogen that grew on infected fenugreek leaves was examined with a compound microscope, and the shape and structure of the microorganism was evaluated under an electron microscope (HITACHI S-3400N). Scanning Electron Microscopy (SEM) images were taken to measure the size of the conidia, conidiophores and mycelia of *C. traversiana*. Fifteen randomly selected SEM images were used to measure these structures.

Statistical analysis

In the final screening test a Randomized Complete Block Design (RCBD) with eight replications were applied. ANOVA was performed on the disease scores gathered from the eight treated plants from each accession using SAS PROC MIXED (SAS Institute, Cary, NC) analysis. The data were subjected to Square Root Transformation before an ANOVA was done on the data. The probability level for ANOVA was set at 1%. Treatment mean comparisons were made using Tukey's Honestly Significant Difference test and the probability level was set at 5% level.

Conclusions

This study identified some resistant (L3717 and PI138687) and some moderately resistant (F86 and L3698) accessions, which may be useful in breeding programs aimed at development of Cercospora leaf spot resistant cultivars. The two resistant accessions performed well and so can be used for development of Cercospora leaf spot resistant fenugreek cultivars without fear of losing agronomic performance. Out of the four resistant and moderately resistant accessions observed, two (L3717 and L3698) were from India, one (F86) from Afghanistan and one from (PI138687) Iran. Since south-east Asia is the center of origin for fenugreek, a more extensive germplasm collection from these areas may be useful for disease resistant cultivar development. In this study, disease symptoms, disease effect on some important plant traits, and morphology of the pathogen were characterized; these traits may serve as a reference for future studies regarding fenugreek and its reaction to *C. traversiana*. Since all inoculated accessions developed disease symptoms, and only their level of reaction varied, it is postulated that the resistance to Cercospora leaf spot in fenugreek is quantitatively inherited.

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