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RESEARCH ARTICLE

# Sources of resistance to ascochyta blight in wild species of lentil (*Lens culinaris* Medik.)

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**Abstract** Cultivated lentil (*Lens culinaris* Medik. subsp. *culinaris*) has a relatively narrow genetic base and many commercial cultivars are susceptible to ascochyta blight caused by *Ascochyta lentis* Vassilievsky. A total of 375 accessions of six wild species of lentil received from ICARDA and 18 cultivated genotypes were screened for resistance to *A. lentis* under both field and greenhouse conditions in Saskatoon, Canada. A mixture of three monoconidial isolates of *A. lentis* was used as an inoculum and the level of infection rated using the Horsfall-Barratt scale (0–11). Accessions with resistance to *A. lentis* were observed in all wild species except for *L. culinaris* subsp. *tomentosa* (Ladiz.) Ferguson et al. showing no resistant accessions. Several consistently resistant accessions were found among entries of *L. ervoides* (Brign.) Grande and *L. nigricans*, (M. Bieb.) Godr., both of which belong to the secondary gene pool and a few in *L. culinaris* subsp. *orientalis* (Boiss.) Ponert and *L. culinaris* subsp. *odemensis* (Ladiz.) Ferguson et al. belonging to the primary gene pool. Some accessions of *L. ervoides* exhibited lower disease ratings and AUDPC values than the resistant control cv. 'Indianhead.' Thirteen accessions, previously reported as resistant to Syrian isolates of *A. lentis* were also resistant to the Canadian isolates; some also had resistance to anthracnose. The highest frequency of resistance was found in accessions of *L. ervoides* which originated from Syria and Turkey. These wild accessions represent a useful and untapped source for improving disease resistance in lentil.

**Keywords** Ascochyta blight · *Lens culinaris* Medik. · Resistance

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## Introduction

Canadian lentil production accounts for 26% of global production and 39% of global exports (Food and Agriculture Organization STAT 2007). Global lentil consumption is projected to grow by 2.5% per year to reach 5 million tonnes by 2020 (McNeil et al. 2007). Ascochyta blight, caused by *Ascochyta lentis* Vassilievsky (teleomorph: *Didymella lentis* WJ Kaiser, BC Wang, and JD Rogers), is a widespread foliar disease (Muehlbauer and Chen 2007) that causes extensive crop losses in lentil crops in the Canadian prairies. Ascochyta blight was first observed in Canada in 1978 (Morrall and Sheppard 1981). Its occurrence and severity is weather-dependent, and environmental conditions that favor high yield also favor the disease. Cool, moist weather is ideal for ascochyta blight development, particularly when these conditions occur in July and August, coinciding with the reproductive and seed-filling stages of the

lentil crop. Yield losses of up to 40% and seed discoloration have been reported from foliar infection of susceptible cultivars in Canada (Gossen and Morrall 1983) and in Australia (Brouwer et al. 1995). Yield loss may increase if late maturing cultivars are sown, and if swathing is used as a pre-harvest treatment during periods when the environment favors disease development (Morrall 1997). Seed quality losses are also economically important when the fungus causes seed staining, as reported in New Zealand (Cromey et al. 1987) and Canada (Morrall and Sheppard 1981).

Tivoli et al. (2006) suggested control measures for ascochyta blight pathogens using an integrated pest management approach involving combinations of cultural management, chemical control, and genetic resistance. Resistance breeding is important for developing a durable and sustainable strategy for managing problems with ascochyta blight, and various sources of resistance have been reported in lentil germplasm (Slinkard et al. 1983; Ahmad et al. 1997).

Isolates of *A. lentis* exhibit variability in growth, sporulation, morphology, and the degree of virulence, making breeding for resistance complex (Kaiser et al. 1994b). Nasir and Bretag (1997) found six distinct pathotypes based on reactions of six lentil lines. Ahmed et al. (1996) found that *A. lentis* isolates collected in 1991 were more virulent than isolates collected in 1978 and 1985. Repeated epidemics of ascochyta blight promoted changes in the population to more virulent strains. As a result, 50% yield loss of cv. Laird was observed (Morrall 1997). Similar studies have shown resistance can become ineffective over time, as first observed in ILL-5588 in Australia (Nasir and Bretag 1997) and in Canada (S. Banniza, unpublished data). ILL-5588 was extensively used as a source of resistance for ascochyta blight at the Crop Development Centre (CDC) in Canada and in Australia, but it was subsequently reclassified as susceptible. Even though several sources of resistance have been deployed over time, epidemics of ascochyta blight continue to occur in regions where lentil is produced (Porta-Puglia et al. 1994). In the Canadian province of Saskatchewan, for example, levels of ascochyta blight infection in commercial seed samples of lentil have increased from year to year (Morrall et al. 2004).

A narrow genetic base of the crop and loss of genes for higher productivity, as well as biotic and abiotic stresses were identified as an underlying cause for limited genetic advances in yield of lentil (Ferguson et al. 1998; Ford et al. 1999; Gupta and Sharma 2006, 2007). The intraspecific variability in the cultivated species of lentil maintained at ICARDA, USDA and Australia were low compared to the interspecific variation using markers. This low level of variability, according to Abo-elwafa et al. (1995), Tanksley and McCouch (1997), and Ferguson et al. (1998), was the "genetic bottleneck" imposed on cultivated species during domestication and through modern plant breeding. Previous results demonstrated that 112 out of 114 cultivated accessions showed identical cpDNA/RFLP patterns compared to the diversity within individual wild species, which supported this conclusion (Mayer and Soltis 1994). According to Rao et al. (2003) and Tanksley and McCouch (1997) a narrow range of variability for resistance and creation of a more virulent biotype due to selection pressure necessitates the need to incorporate a broad spectrum of potentially useful genes for resistance from wild species.

The genus *Lens* comprises seven taxa in four species, namely; *L. culinaris* Medikus subspecies [*culinaris*, *orientalis* (Boiss.) Ponert, *tomentosa* (Ladiz.) M.E. Ferguson et al. and *odemensis* (Ladiz.) M.E. Ferguson et al.], *L. ervoides* (Brign.) Grande *L. nigricans* (M. Bieb.) Godr. and *L. lamottei* Czefr. (Ferguson et al. 2000). *L. ervoides* and *L. nigricans* are considered to be in the secondary gene pool (Ladizinsky et al. 1984; Muehlbauer and McPhee 2005).

Wild species have been used to develop ascochyta blight [*Ascochyta rabiei*, teleomorph: *Didymella rabiei var.* Arx] resistance in chickpea (Singh and Reddy 1993; Stamigna et al. 1998; Collard et al. 2001). A high level of resistance to lentil ascochyta blight (*Ascochyta lentis*) and to two races of anthracnose, caused by *Colletotrichum truncatum* (Schwein.) Andrus et W.D. Moore, were reported in wild lentil species by Bayaa et al. (1994) and Tullu et al. (2006), respectively, of which *L. ervoides* had the highest frequency of resistance to these diseases. With the aid of ovule and embryo rescue techniques (Cohen et al. 1984), we were able to facilitate embryo development of the hybrid from the cross of *L. culinaris* subsp. *culinaris* cv. Eston and *L. ervoides* (PI 72815) accession which was later on advanced to $F_2$ plants and then to $F_{8:9}$ recombinant inbreds lines (RILs) (unpublished data).

The objective of this study was to evaluate accessions of wild species of *Lens* for resistance to a mixture of Canadian *A. lentis* isolates under field and greenhouse conditions. The long-term goal is to expand the number of useful genes available for resistance breeding to ascochyta blight through introgression from wild lentil species to the cultivated lentil.

## Materials and methods

### Sources of *Lens* germplasm

Four hundred accessions of wild *Lens* species were received from the International Center for Agricultural Research in the Dry Areas (ICARDA). These accessions were originally collected from diverse geographic regions (Cyprus, Spain, France, Iran, Italy, Jordan, Syria, Turkey, former Soviet Union, and Yugoslavia), ranging from latitude 30°–45°N, longitude 3°W–59°E, and from sea level to 2,500 m (Barulina 1930; Cubero 1981; Bayaa et al. 1994). Among a total of 400 accessions, 375 were selected for planting based on seed availability. Control genotypes of *L. culinaris* subsp. *culinaris* included both susceptible cultivars (Chilean, Pardina, and Eston) and resistant lines (cv. Indianhead, cv. CDC Redberry and germplasm lines ILL 7537, ILL 5588, PI 320937 and 1156-2-17A), each of which was planted 5–8 times in each replication (Table 1).

### Screening for *A. lentis* resistance under field conditions

Field experiments were conducted at the Preston Farm of the University of Saskatchewan in Saskatoon, Saskatchewan, Canada, in the summer of 2004. The soil type was dark brown and chernozemic, with clay loam texture (Nleya et al. 2001). Prior to planting, the seeds were placed in a freezer at −20°C for 1 day and then scarified using sandpaper to improve imbibition. Twenty to 40 seeds of each accession were planted manually in plots of two 1 m

**Table 1** Mean rating of ascochyta blight infection (Horsfall-Barratt 1945) for lentil controls and other cultivated germplasm under greenhouse and field (Preston Farm, Saskatoon) environments

| *Lens culinaris* subsp. *culinaris* germplasm | Horsfall-Barratt score (0–11) | | Estimated % disease[a] | Cumulative AUDPC[b] | AUDPC as % of Eston |
|---|---|---|---|---|---|
| | Field | Green-house | | | |
| Indianhead[c] | 2.0 | 3.0 | 5.0 | 58.0 | 17.0 |
| CDC Redberry[c] | 4.0 | 4.0 | 18.5 | 83.5 | 24.0 |
| IPK 1156 | 4.0 | – | 18.5 | 99.8 | 29.0 |
| ILL 7537[c] | 3.0 | 4.0 | 9.4 | 112.0 | 32.0 |
| IPK 1254 | 4.0 | – | 18.5 | 114.0 | 33.0 |
| IPK 102 | 4.0 | – | 18.5 | 116.0 | 33.0 |
| IPK 969 | 4.0 | – | 18.5 | 122.0 | 35.0 |
| IPK 153 | 4.0 | – | 18.5 | 132.0 | 38.0 |
| PI 320937 | 4.0 | 4.0 | 18.5 | 138.0 | 39.0 |
| CDC Robin[c] | 4.0 | 3.0 | 18.5 | 112.0 | – |
| 1156-2-17A[c] | 4.0 | 2.0 | 18.5 | 112.0 | – |
| IPK 214 | 5.0 | – | 37.5 | 191.0 | 55.0 |
| ILL 1704 | 7.0 | 5.0 | 81.3 | 173.0 | 50.0 |
| IPK 181 | 6.0 | – | 62.5 | 234.0 | 67.0 |
| CDC Milestone | 8.5 | – | 93.0 | 207.0 | 59.0 |
| ILL 5588 | 7.0 | 7.0 | 81.3 | 272.0 | 78.0 |
| IPK 899 | 7.0 | – | 81.3 | 282.0 | 80.0 |
| Chilean[d] | 8.0 | 7.0 | 90.6 | 270.0 | 77.0 |
| Pardina[d] | 10.0 | 8.0 | 97.7 | 306.0 | 88.0 |
| Richlea[d] | 8.0 | 7.0 | 90.6 | – | – |
| Eston[d] | 10.0 | 9.0 | 97.7 | 350.0 | 100.0 |

[a] Based on field HB data

[b] Calculated from HB rating data of three separate dates with 10–12 days interval

[c] Resistant control

[d] Susceptible control

rows, with 30 cm between rows, with 2 replications in a randomized complete block design. Accessions that did not emerge within 10 days were replanted.

Lentil plant debris infected with *A. lentis* was collected in 2003. To initiate infection, the debris was chopped into small pieces and spread across the experimental plots, 7–10 days after emergence. To increase the disease pressure, sterilized wheat seeds inoculated with a mixture of three Saskatchewan isolates (3C-2, AL-5, and AL-6), selected based on their higher aggressiveness (S. Banniza, unpublished data), were manually spread at about 10 g $m^{-2}$ between the rows in the field at 5–6 weeks after emergence. However, as pigeons were evidently consuming the infected seeds soon after application, a second inoculation was performed by manually rubbing the emerged plants with the colonized wheat seeds. Manual applications were repeated until a noticeable level of infection was detected on the susceptible control lines. Disease symptoms were visually scored three times at 10–12 day intervals after the third manual application with colonized wheat seeds using the Horsfall-Barratt (HB) scale of 0–11 (0 = no infection; 11 = 100% infection) (Horsfall and Barratt 1945). Accessions were classified as resistant (HB score ≤4) or susceptible (HB score >5) based on the reactions of the resistant and susceptible controls. Scores were also converted to percent infection, and the area under the disease progress curve (AUDPC) calculated as described by Shaner and Finney (1977).

### Greenhouse experimental methods

Depending on seed availability, accessions of wild lentil species tested in the field were also evaluated in the greenhouse for their reaction to *A. lentis*. Resistant controls included the cultivars 'Indianhead', CDC Robin and germplasm accessions ILL-5588, ILL-1704, ILL-7537, 1156-2-17A, and susceptible controls included accessions cultivars Chilean, Pardina and Richlea (Andrahennadi 1994; Tar'an et al. 2003). Plants were inoculated 3 weeks after emergence by spraying 1.5–2.0 ml conidial suspension per plant with a mixture of three isolates of *A. lentis* (3C-2, AL-5, and AL-6) at a concentration of $2.5 \times 10^5$ conidia $ml^{-1}$. Immediately after inoculation, the plants were transferred to a plastic-covered humidity chamber, providing 100% relative humidity. After 48 h incubation, the plants were transferred to a greenhouse bench equipped with overhead misting nozzles that were activated for 10 s every 30 min to maintain leaf wetness. This experiment was repeated once. Plants were assessed for the level of infection at 10, 22, and 32 days after inoculation using the HB scale. Accessions were also classified as resistant (HB score ≤4) or susceptible (HB score >5).

## Results

### Disease reaction of *Lens* species under field conditions

The 2004 growing season was characterized by below normal maximum (18.9°C) and minimum (6.6°C) mean air temperatures during the entire growing season (May to September), while the total precipitation was ~26% above normal (235.8 mm). The weather conditions were adequate for stand establishment, with average temperatures of 10°C and 36 mm rainfall in May, 15°C and ~87 mm rainfall in June, and >19°C for most of the month of July with a rainfall amount of ~75 mm (Fig. 1). The distribution of precipitation after emergence was satisfactory, with intermittent warm and sunny days up to the middle of May. The spread of infected plant debris and colonized wheat seeds did not induce the desired level of infection at the initial stage in the

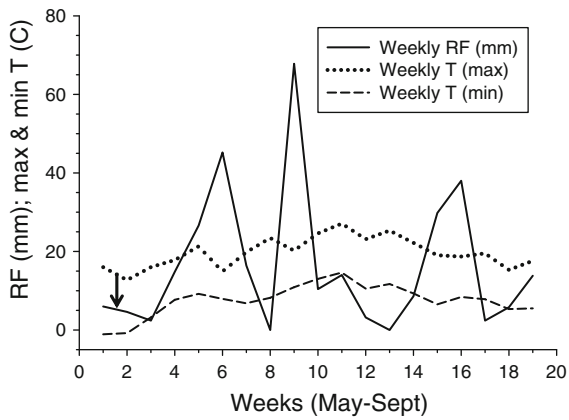


**Fig. 1** Rainfall (*RF*, mm), maximum and minimum temperature [*T* max (°C) and *T* min (°C)] during the growing season (May to August) in 2004 (*arrow* indicates seeding date; long term average rainfall for the month of May, June, July and August was 47, 61, 60, 39 mm, respectively.)

susceptible control lines, probably due to the low minimum temperature (2–10°C). Two weeks later, the temperature had increased (mean maximum 21°C and minimum 10°C; Fig. 1), and adequate infection occurred when *A. lentis* seed inoculum was manually rubbed against plants. After the second manual inoculation was applied in early July, the disease progressed well due to adequate moisture and higher temperatures, resulting in a rapid development of ascochyta infection until the end of July. The susceptible control lines reacted consistently to *A. lentis* infection, indicating the disease incidence was sufficient and the disease rating technique was valid. The mean HB score for ascochyta blight (AB) infection in the field for cv. Eston and common Pardina was 10, while common Chilean scored 8.

Of the 375 accessions planted at Preston Farm, Saskatoon, 212 had good stand establishment in both replications, 163 had good growth in only one replication [*L. ervoides* (55), *L. lamottei* (6), *L. nigrican*s (17), *L. culinaris* subsp. *odemensis* (20), *L. culinaris* subsp. *tomentosa* (3) and *L. culinaris* subsp. *orientalis* (62)], and the remainder did not germinate. Following artificial inoculation, AB symptoms were evident on susceptible germplasm by the first week after inoculation. None of the accessions tested revealed complete immunity to *A. lentis* that could have indicated non-host resistance. The distribution of mean scores (mean of both replicates) of AB reaction of 212 accessions of different species to a mixture of isolates of *A. lentis* revealed resistance in 67% of accessions in *L. ervoides*, 58% of *L. nigricans*, 15% of *L. culinaris* subsp. *orientalis*, 14% (3 out of 21) of *L. culinaris* subsp. *odemensis* (Fig. 2). Two accessions of *L. lamottei* were resistant. No resistance was observed in the five accessions of *L. tomentosa*. The mean AB reaction ranged from 2.7 to 3.2 in *L. ervoides* and *L. nigricans* to 6.0–7.6 in *L. culinaris* subsp. *orientalis* and 8.0 in *L. culinaris* subsp. *tomentosa*. Twenty *L. ervoides*, 12 *L. nigricans*, 3 *L. c.* subsp. *odemensis* L., 2 *L. lamottei,* and 14 *L. c.* subsp. *orientalis* accessions had infection levels comparable to the mean AB severity score (3.7) of the resistant control lines (Table 1). Except for a few number of *L. ervoides* and *L. nigricans* accessions, the resistant control Indianhead had the lowest score, followed by ILL 7537 with a score of 3.0, and CDC Redberry and PI 320937 with scores of 4.0 in the field with a similar trend in the greenhouse.

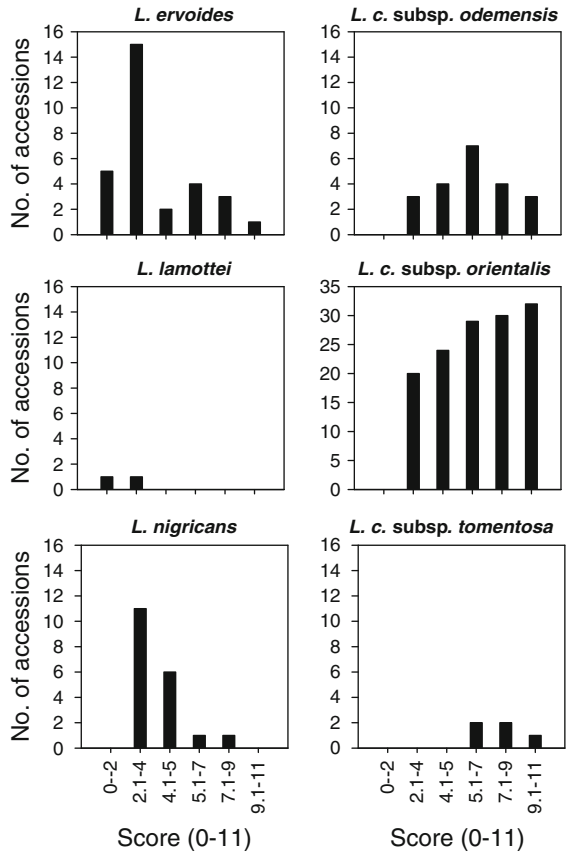


**Fig. 2** Distribution of field disease reaction to a mixture of *Ascochyta lentis* isolates in six wild *Lens* species at Preston Farm, Saskatoon. (*L. c.* represents *Lens culinaris*)

The susceptible controls, Pardina and Eston, had severity ratings of 10 whereas Chilean had 8 (Table 1). The ratings of susceptible controls were higher in the field than in the greenhouse.

Disease progress curves for the ascochyta blight infection (mean of replicates) of the resistant accessions, grouped by their respective species, are shown in Fig. 3. For all assessments, the AUDPC values were calculated directly from the HB infection data, based on assessed values after seeding. Overall, values of AUDPC for the resistant and susceptible lines showed similar trends compared to disease severity ratings. Among the resistant lines, accessions IG 72570, IG-72571, IG-72576, IG-72654, IG 72665, IG 72657, IG-72862, IG-72910, IG-72921 from *L. ervoides*; accessions IG 72553, IG-72560, IG-72561, IG 72633, IG 72634, IG-116032 from *L. nigricans*; accessions IG 72623, IG-116029 from *L. culinaris* subsp. *odemensis*; and accessions

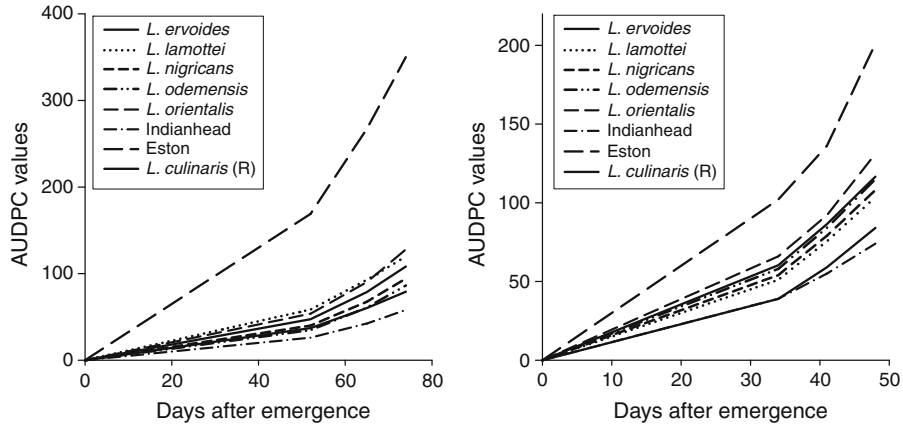


**Fig. 3** Mean AUDPC of ascochyta blight infection for resistant accessions of six lentil species and a susceptible control cultivar Eston assessed on three occasions after emergence under field (Preston Farm, Saskatoon, Canada) (*left*) and greenhouse (*right*) conditions. cv. Indianhead is the best resistant control among *L. culinaris* subsp. *culinaris* lines. (*L. culinaris*, *L. orientalis* and *L. odemensis* are subsp. of *L. culinaris* Medik.)

IG-72611, IG 72618, IG-72670, IG-72711 from *L. culinaris* subsp. *orientalis* exhibited good podding, earliness, and standability traits under field conditions. In comparison, the resistant accessions had the lowest infection level (13–40%) expressed as percent of the Eston (AUPDC value of 350).

### Disease reaction of *Lens* species under greenhouse conditions

A total of 240 accessions were tested for reaction to the mixture of *A. lentis* isolates. The susceptible controls (Pardina, Richlea, and Eston) were heavily infected with scores of 7–8.5 on the HB scale; notably, these values were lower than corresponding field results. The resistant controls Indianhead, CDC Robin, and 1156-2-17A had infection rates below that recorded for the most susceptible control Eston. Eston had a much faster rate of disease development resulting in a high AUDPC value of 202 (Fig. 3). Mean scores of infection for resistance to AB were different among species. Thirty-five percent of all accessions evaluated survived the ascochyta infection. Similar to the field results, considerable AB resistance was evident in accessions of *L. ervoides* (29%), *L. culinaris* subsp. *orientalis* (36%), *L. culinaris* subsp. *odemensis* (37%), and *L. nigricans* (39%). Only one accession of *L. lamottei* was tested, and it was found to be resistant. Among the resistant accessions, low AUDPC values were evident in *L. ervoides*, *L. nigricans*, *L. culinaris* subsp. *odemensis*, *L. culinaris* subsp. *orientalis* and *L. lamottei*, compared to an AUDPC value for Eston (Fig. 3). High AUDPC values (up to 98% of Eston) were also observed in the other susceptible controls, including Pardina. Wild lentil accessions with HB disease ratings between 1 and 5 in both field and greenhouse experiments are shown in Table 2. Most accessions with resistant reactions in the field were also resistant when inoculated under controlled environmental conditions in the greenhouse, although field resistance ratings were in general slightly higher. Overall, with the exception of *L. culinaris* subsp. *tomentosa*, which showed relatively little or no resistance, AB resistance was evident in all species, including the cultigen, *L. culinaris* subsp. *culinaris*.

## Discussion

Ascochyta blight symptoms on the susceptible controls were clearly evident in the field, indicating the effectiveness of the inoculation procedures. Isolates of *A. lentis* infected all species of *Lens* tested in these experiments indicating that the host range of this pathogen comprises the entire genus. Most resistant accessions showed resistance in both the field and the greenhouse (Table 2). Some accessions showed inconsistent reactions, possibly caused by heterogeneity among individual plants within an accession. Unlike the resistance to anthracnose in lentil, caused by *Colletotrichum truncatum* (Tullu et al. 2006), and

**Table 2** Mean disease rating based on a Horsfall-Barratt scale (0–11) of accessions for lentil species when inoculated by a mixture of isolates of *Ascochyta lentis* in the field and greenhouse (GH)

| IG number | HB score (0–11) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Field[a] | GH | IG number | Field | GH | IG number | Field | GH |
| *Lens nigricans*[b] | | | | | | | | |
| 72557[b] | 2.0 | 2.0 | 116025 | 3.0 | 3.5 | 72789 | 3.5 | 4.0 |
| 72714 | 2.0 | 3.0 | 72791 | 3.0 | 3.5 | 116035[b] | 3.5 | 3.5 |
| 72795 | 2.0 | 3.5 | 116024 | 3.0 | 3.0 | 72549 | 4.0 | 4.0 |
| 72850 | 2.0 | 5.0 | 116030[b] | 3.0 | 3.0 | 72713[c] | 4.0 | 5.0 |
| 72560 | 2.5 | 2.0 | 116031 | 3.0 | 3.5 | 72554 | 4.5 | 3.0 |
| 72633[c] | 2.5 | 3.5 | 116037 | 3.0 | 3.0 | 72745 | 4.5 | 4.0 |
| 116032 | 2.5 | 3.5 | 72536 | 3.5 | 4.5 | 72539 | 5.0 | 5.0 |
| 72553 | 3.0 | 3.5 | 72542 | 3.5 | 3.0 | 72546 | 5.0 | 3.0 |
| 72555[b] | 3.0 | 3.5 | 72556 | 3.5 | 3.0 | 72744 | 5.0 | 4.0 |
| 72634 | 3.0 | 5.5 | 72561 | 3.5 | 2.5 | 116018 | 5.0 | 5.0 |
| *Lens ervoides* | | | | | | | | |
| 72846[b, c] | 1.0 | 3.0 | 72784[c] | 2.0 | – | 72646[b, c] | 3.0 | 3.0 |
| 72565[b] | 1.5 | 2.5 | 72826[c] | 2.0 | – | 72799[b] | 3.0 | 5.5 |
| 72665[b, c] | 1.5 | 4.5 | 72792[b, c] | 2.0 | – | 72707[b, c] | 3.0 | 2.0 |
| 72564[b, c] | 2.0 | – | 72576[b] | 2.0 | 5.0 | 72862 | 3.0 | 5.5 |
| 72567[b] | 2.0 | – | 72914 | 2.0 | 5.0 | 107445[b] | 3.0 | 3.5 |
| 72570[b] | 2.0 | 5.0 | 72921 | 2.0 | – | 72582[b] | 3.5 | 7.0 |
| 72576[b] | 2.0 | 5.0 | 72910 | 2.5 | 2.5 | 72652[b, c] | 4.0 | 8.0 |
| 72575[b] | 2.0 | – | 72566[b] | 2.5 | – | 72590 | 4.0 | 4.0 |
| 72654[b] | 2.0 | 6.0 | 72578 | 2.5 | 5.0 | 72708[b, c] | 4.0 | 5.0 |
| 72657[c] | 2.0 | 7.5 | 72924[b] | 2.5 | – | 72815[b] | 4.0 | 4.5 |
| 72729[c] | 2.0 | 7.5 | 72571[b] | 3.0 | 4.0 | 72859 | 4.0 | 4.5 |
| 72730[b, c] | 2.0 | 5.0 | 72577[b] | 3.0 | – | 72659[b, c] | 5.0 | 4.0 |
| 72731[c] | 2.0 | – | 72579[b] | 3.0 | 4.5 | 72664[b, c] | 5.0 | – |
| 72783 | 2.0 | 6.0 | 72841[c] | 3.0 | 5.0 | | | |
| *Lens culinaris* subsp. *orientalis* | | | | | | | | |
| 72838[c] | 2.0 | 5.0 | 72905 | 3.5 | 3.0 | 72711 | 4.5 | – |
| 72847 | 2.0 | 5.0 | 72611[c] | 4.0 | 4.0 | 72704[c] | 5.0 | – |
| 116028 | 2.0 | 5.0 | 72622 | 4.0 | 4.0 | 72715 | 5.0 | 5.0 |
| 72666 | 2.5 | 3.0 | 72642 | 4.0 | 3.0 | 72771[c] | 5.0 | 3.0 |
| 72670 | 3.0 | 2.0 | 72740 | 4.0 | 2.5 | 72677 | 5.0 | 2.0 |
| 72618 | 3.0 | – | 72829 | 4.0 | 5.0 | 72529[c] | 5.0 | 5.0 |
| 72869 | 3.0 | – | 72907 | 4.0 | 5.0 | 72592[c] | 5.0 | 5.0 |
| 116042 | 3.0 | – | 72732 | 4.5 | 5.0 | 72600[c] | 5.0 | 5.0 |
| 72669[c] | 3.5 | 3.5 | 72893 | 4.5 | 2.5 | | | |
| *Lens lamottei* | | | | | | | | |
| 110811 | 2.0 | – | 110813 | 4.0 | – | 110812 | – | 4.0 |
| *L. culinaris* subsp. *odemensis* | | | | | | | | |
| 72543[c] | 2.0 | 4.0 | 72606 | 3.0 | 4.0 | 72744 | 5.0 | 5.0 |
| 116008 | 2.0 | 5.0 | 107447 | 4.0 | – | 72698 | 5.0 | 3.0 |
| 72623 | 2.5 | 5.0 | 72606 | 5.0 | 3.0 | 72745 | 5.0 | 5.0 |

**Table 2** continued

| IG number | HB score (0–11) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Field[a] | GH | IG number | Field | GH | IG number | Field | GH |
| 72777[c] | 2.5 | 9.0 | 72676 | 5.0 | 5.0 | 72695[c] | 5.0 | 2.5 |
| 116029 | 3.0 | 3.0 | 72693[c] | 5.0 | 3.5 | | | |
| *Lens culinaris* subsp. *culinaris* | | | | | | | | |
| Indianhead[b] | 2.0 | 2.0 | PI320937[b] | 4.0 | 4.0 | ILL 5588 | 7.0 | 6.0 |
| CDC Redberry[b] | 4.0 | 2.5 | ILL 7537 | 4.0 | 4.0 | Pardina | 10.0 | 8.0 |
| | | | | | | Eston | 10.0 | 8.0 |

*GH* greenhouse data from two replications

[a] Field data either from one or two replications

[b] Field resistance to anthracnose (Tullu et al. 2006)

[c] Resistance to Syrian isolates of ascochyta blight (Bayaa et al. 1999)

to fusarium wilt in chickpea, caused by races 0 and 5 of *Fusarium oxysporum* f. sp. *ciceris* (Kaiser et al. 1994a), resistance to Canadian isolates of *A. lentis* was found in most of the species including *L. culinaris* subsp. *culinaris* in the current study.

Based on the mean AUDPC values of resistant accessions in each species, the rate of infection in the susceptible control cv. Eston (Fig. 3) was much higher than in the wild species. The resistant control cv. Indianhead, consistently maintained the highest resistance throughout the three inoculation regimes, with AUDPC values lower than the mean scores of resistant accessions of the wild species evaluated. Individual accessions of *L. ervoides*, and *L. nigricans* from the secondary gene pool, and *L. culinaris* subsp. *orientalis* and *L. culinaris* subsp. *odemensis* from the primary gene pool maintained resistance to ascochyta blight throughout the extended inoculation period (Table 2), even though the environment was conducive for rapid disease development (Fig. 1). Although the ranking of accessions was slightly different using AUDPC values vs. the HB rating scale, categorization of accessions as resistant and susceptible using the rating scale was direct and did not require additional data manipulation. The use of AUDPC values could be useful in breeding experiments because the progression of ascochyta blight in individual genotypes can be noted and monitored (Tekeoglu et al. 2000).

Tangible progress in plant breeding will result from evaluating and exploiting the genetic variation available in the different gene pools. A higher frequency of ascochyta blight resistance was evident in *L. ervoides* and *L. nigricans* (58–67%), followed by *L. culinaris* subsp. *odemensis*, *L. culinaris* subsp. *orientalis*, and *L. lamottei* (14–15%). In contrast, all accessions of *L. tomentosa* were susceptible although the mean AUDPC value (206) was less than the susceptible control Eston (350). Overall, 30 and 36% of the total wild accessions were resistant in the field and the greenhouse environments, respectively. Among the resistant accessions reported by Bayaa et al. (1994), 17 of 36 *L. ervoides*, 2of 3 *L. nigricans*, and 4 of 24 *L. culinaris* subsp. *orientalis* were also resistant to the mixture of Canadian AB isolates used in this study. The occurrence of higher level of resistance in disproportionately larger number of accessions of *L. ervoides* and *L. nigricans* when challenged with *A lentis* isolates collected from infected cultivated lentil fields could indicate a possible divergence of pathogen populations as described by Frenkel et al. (2007, 2008) in the chickpea pathogen, *A. rabiei*, where significant differences in pathogen aggressiveness were detected between isolates collected from domesticated and wild plants grown in sympatric distribution. Variable proportions of resistant accessions in different species of *Lens* screened in the current study could also be due to duplication in accessions of the source collections. Further investigations to characterize *A. lentis* isolated from *L. ervoides* and *L. nigricans*, and to determine their aggressiveness and host specialization on wild and domesticated lentil plants are required.

Apart from resistance in the host and conducive environmental conditions, the severity of disease is also influenced by the virulence of the pathogen. *A. lentis* populations are highly variable and genetically diverse (Ahmed and Morrall 1996; Nasir and Bretag 1997) which can contribute to the development more virulent pathotypes (Watson 1970). An increased appearance in the frequency of more virulent isolates of *A. lentis* in lentil may have contributed to the decline in production of lentil cultivar 'Laird' (Morrall 1997) and more recently, in germplasm accession ILL-5588 (cv. Northfield), previously reported to be resistant to ascochyta blight in Australia (Ford et al. 1999; Ye et al. 2001), and Saskatchewan, Canada (S. Banniza, unpublished data), indicating that regular monitoring of pathogen populations and the search for new resistance genes remains important. Further, development of lentil cultivars with multiple genes for resistance to major diseases should help to develop management strategies in the future, requiring allelism studies to identify and pyramid novel genes for resistance. For combined resistance to the two distinctly different pathogens *C. truncatum* and *A. lentis*, 13 accessions from *L. ervoides* and 3 accessions of *L. nigricans* (Table 2), most of which are from Middle Eastern countries, could be utilized.

The main goal of our research was to identify sources of resistance from wild species to ascochyta blight. However, adaptability (visual early vigor, earliness, podding ability, and standability) of accessions to the growing season of the Canadian Prairies characterized by short, but warm summers and frequent strong winds become critical for sustainable production. For this purpose, *L. ervoides* accessions IG 72665, IG 72570, IG 72576, IG 72654, IG 72657, IG 72921, IG 72910, IG 72571, IG 72862, IG 72578, IG 72571 and *L. nigricans* IG 72560, IG 72633, IG 72553, IG 72634, IG 116032, IG 72561, appear to be promising. However, information is lacking on the extent to which resistance traits are incorporated in cultivar development in pulse crops such as lentil ((Hoisington et al. 1999; Muehlbauer et al. 2006; Hajjar and Hodgkin 2007). With the aid of embryo rescue, we were successful in obtaining a fertile hybrid between the cultigen (*L. culinaris* subsp. *culinaris*) and *L. ervoides* to develop recombinant inbred populations (RILs) and to transfer resistance to anthracnose to the cultivated background (Fiala et al. 2009).

Development of fine mapping and identification of associations among candidate genes, in conjunction with phenotypic data and DNA sequences of lentil genomic regions of interest, are critical to achieving a better understanding of the molecular architecture of resistance to ascochyta blight, anthracnose, and other diseases. A lentil genomics initiative to improve disease resistance, nutritional quality, and other traits of commercial importance is underway in the lentil breeding program at the Crop Development Centre, University of Saskatchewan, Canada.

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