ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2013) **11**(1); 26–35
doi:10.1017/S1479262112000329

Screening of wild and cultivated lentil germplasm for resistance to stemphylium blight

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Received 17 July 2012; Accepted 13 September 2012 – First published online 31 October 2012

Abstract

Lens culinaris Medik. ssp. *culinaris* is the only cultivated species in the genus *Lens*. Intensive selection pressure to develop new cultivars, a narrow genetic base, co-evolution of pathogens to partially resistant cultivars and other factors have accelerated susceptibility to different fungal diseases in this species. Few sources for resistance to stemphylium blight (SB) caused by *Stemphylium botryosum* Wallr. exist among commercial lentil cultivars. A total of 70 accessions were selected from seven species of the genus *Lens* to screen for SB resistance. The *L. culinaris* accessions were screened in four different environments, and the accessions of *Lens ervoides*, *L. culinaris* ssp. *orientalis, Lens tomentosus, Lens nigricans*, *Lens odemensis* and *Lens lamottei* in growth chamber or greenhouse experiments to identify resistance sources for potential use in lentil breeding. A highly aggressive isolate of SB was used as an inoculum to screen them under controlled conditions. Lentil cultivars 'Eston' (resistant) and 'CDC Glamis' (susceptible) were used as checks with consistent results in all experiments. Most of the *L. culinaris* accessions were susceptible to SB, whereas more than 70% of the wild lentil accessions had disease severity scores equal to or significantly lower than that of the SB-resistant check 'Eston'. Some wild species accessions previously identified with resistance to anthracnose (*Colletotrichum truncatum*) and ascochyta blight (*Ascochyta lentis*) were also highly resistant to SB. The highest frequency of resistance to SB was found in *L. lamottei* followed by *L. ervoides* of the secondary gene pool. These sources can potentially be used to develop new commercial cultivars with multiple or single disease resistance.

Keywords: *Lens culinaris*; lentil; stemphylium blight; *Stemphylium botryosum*; wild *Lens*

Introduction

Pulse crops are used with cereals in human diets around the world. Cultivated lentil (*Lens culinaris* Medik. ssp. *culinaris*) is believed to have been domesticated and consumed since pre-historic times (Sandhu and Sarvjeet, 2007). World lentil production in 2010 was estimated at 4.58 Mt on about 4.18 M ha with an average yield of 1094 kg/ha (FAOSTAT, 2010).

Lentil production is often biotically constrained by fungal diseases which cause yield instability and reduced seed quality. Stemphylium blight (SB) caused by *Stemphylium botryosum* Wallr. is a devastating disease of lentil in Bangladesh, Nepal and north-eastern India (Bakr and Ahmed, 1992; Chen *et al*., 2009). Reports of SB exist from Canada, Syria, Egypt, Hungary and the USA (Bayaa and Erskine, 1998; Morrall *et al*., 2006; Chen *et al*., 2009). It can cause yield losses above 80% in south Asia (Bakr and Ahmed, 1992). Results from commercial seed tests revealed high levels of SB in samples of lentil from central and northern districts of Saskatchewan, Canada (Morrall *et al*., 2006). No fungicides are registered

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specifically for the control of SB on lentil in Canada, and little is known about the efficacy of fungicides on this pathogen.

Use of disease-resistant cultivars is considered the most economical approach to SB management. Two studies have been conducted on genetic control of SB resistance in lentil, both indicating that resistance to SB is quantitatively inherited (Kumar, 2007; Saha, 2009). Sarker *et al.* (1999) reported on the development of cultivar BARIMasur-4 with resistance to SB, first released in Bangladesh in 1996 through a collaboration of the Bangladesh Agricultural Research Institute (BARI) and the International Centre for Agricultural Research in the Dry Areas (ICARDA) in Syria (Sarker *et al.*, 2004) followed by three more SB-resistant cultivars. Few sources of resistance to SB exist among cultivars in other lentil production regions (A. Vandenberg, unpublished results). Tullu *et al.* (2011) reported potential sources of resistance to SB from the secondary and tertiary gene pools of lentil. Preliminary evidence showed high levels of resistance to SB in lines derived from hybrids between cultivated lentil and *Lens ervoides*. These were originally developed for potential introgression of resistance to ascochyta blight (caused by *Ascochyta lentis* Vassiljevsky) and anthracnose (caused by *Colletotrichum truncatum* (Schwein.) Andrus & Moore) (Tullu *et al.*, 2006, 2010, 2011; Fiala *et al.*, 2009).

Development of improved resistance to SB in lentil will help reduce yield loss and stabilize production without raising input costs. Interspecific hybridization in *Lens* for introgression of genes from the wild gene pool is recognized as a valuable breeding tool for widening the genetic base. Evidence exists for resistance in the wild gene pool to diseases and abiotic stresses (Bayaa *et al.*, 1994; Gupta and Sharma, 2006; Tullu *et al.*, 2010). No reports exist on systematic screening of wild lentil germplasm for SB resistance. We hypothesized that resistance to SB existed in wild lentil species and that it would be possible to identify superior sources of SB resistance for transfer to the cultivated species through the introgression of resistance genes.

Materials and methods

Selection of parents from Lens culinaris *and wild* Lens *species*

Fourteen *L. culinaris* genotypes representing a range of adaptations were selected as a baseline for characterizing the SB reaction of cultivated lentil and were evaluated in a growth chamber (Supplementary Table S1, available online only at http://journals.cambridge.org). Ten of the 14 genotypes were subsequently characterized in the greenhouse and field at the University of Saskatchewan during 2011. Nine of the 14 were also characterized for the SB reaction in the field during the winter season of 2011–2012 at the Pulses Research Centre (PRC) of the BARI, Ishurdi, located in north-eastern Bangladesh (24°8′0″N, 89°5′0″E). The group included four Canadian lentil cultivars, international germplasm accessions and parents of recombinant inbred lines (RILs) developed at the Crop Development Centre (CDC) of the University of Saskatchewan. For some accessions, preliminary results on their SB reaction from earlier studies under controlled condition or in the field were available (Supplementary Table S1, available online only at http://journals.cambridge.org), whereas others were selected either on the basis of their performance against SB in foreign environments, or as a parent of newly developed RILs. Canadian cultivars 'Eston', a small-seeded green lentil (Slinkard and Bhatty, 1981), and 'CDC Glamis', a large-seeded green lentil (Vandenberg *et al.*, 2002a, b), classified as resistant and susceptible checks, respectively, on the basis of their previous performance, were included in all experiments as controls.

A total of 56 wild species accessions were selected for the screening of SB reactions under growth chamber and greenhouse conditions, with priority given to accessions previously screened for resistance to anthracnose and ascochyta blight. Fifty-one accessions, designated with the 'IG' prefix, were obtained from the ICARDA and originated from diverse geographic areas of the globe with a high genetic variability at the intra and interspecies level (Tullu, *et al.*, 2006, 2010). Four accessions (designated with the 'PI' prefix) were selected from among 73 wild accessions provided by the Western Regional Plant Introduction Station, USDA-ARS, Pullman, Washington, USA (Tullu *et al.*, 2006). One accession, L-01-827A, was developed by single plant selection from the *Lens orientalis* accession IG 72847 and was categorized as *L. ervoides* based on morphological similarity with this species (Fiala *et al.*, 2009). The accessions selected from *L. ervoides*, *L. culinaris* ssp. *orientalis* and *Lens tomentosus* were evaluated for SB resistance in growth chambers in separate experiments to accommodate a variation in growth habit and flowering date. The accessions of *Lens nigricans*, *Lens odemensis* and *Lens lamottei* were evaluated in the greenhouse in separate experiments.

Experiments under controlled conditions

Experiments under controlled conditions were conducted in growth chambers (Conviron Winnipeg, Manitoba) and in greenhouses of the University of Saskatchewan. Each experiment was conducted twice. Day length was set for 16 h at 20°C and 8 h darkness at

https://doi.org/10.1017/S1479262112000329 Published online by Cambridge University Press

15°C in growth chambers, where a cluster of 34 fluorescent tubes (PHILIPS-Silhouette high output, F54T5/835/HO/A/EA, 49 W, 3500K, Alto collection Holland) provided light with approximately 400–500 $\mu E/m^2 s$ light intensity during day time. In the greenhouse, the photoperiod was maintained at 18 h day and 6 h night at 20–22°C/16–18°C (day/night) temperature. High-pressure sodium lights were used to provide approximately 300–1100 $\mu mol/m^2 s^1$ photosynthetically active radiation in the light period. Six scarified seeds of each entry were planted in a 10 cm plastic pot with soil-less media (Sunshine 4 mixture; Sun Grow Horticulture, Vancouver, British Columbia, Canada) and thinned to four plants per pot after germination. Four replicate pots per accession were used for each experiment in a randomized complete block design. For screening wild species, *L. culinaris* checks were planted 1 week later to synchronize the correct growth stage, pre-flowering to flowering, for inoculation. A soluble mixture of N, P and K (20:20:20) at 2 g/l water was applied once per week after emergence. Insects were controlled in the growth chamber as required using various control and repellent measures. Prior to inoculation, each pot was wrapped with transparent plastic to increase humidity around the plants.

Inoculation of experiments under controlled conditions

An aggressive isolate of *S. botryosum* (SB-19) was selected on the basis of previous screening of several isolates (S. Banniza, unpublished results). As sporulation of *S. botryosum* is very poor (Chowdhury *et al.*, 1996), mycelial suspensions were used based on a protocol previously developed in the CDC (S. Banniza, unpublished results). Stemphylium cultures were grown in 90 mm sterile Petri dishes containing V8-PDA medium (150 ml V8 Original Blend Vegetable Cocktail [Campbell Co., Canada], 10 g Difco™ Potato Dextrose Agar, 10 g Difco™ Agar, Granulated [both Becton Dickinson and Co., Sparks, MD, USA], 3 g $CaCO_3$ [EMD Chemicals, Inc., Darmstadt, Germany] and 850 ml distilled water) and incubated for 2 weeks at 27°C. Erlenmeyer flasks containing 500 ml modified Richard's medium (10 g sucrose [Fisher Scientific, USA], 10 g KNO_3 [EMD, USA], 5 g KH_2PO_4 [VWR, USA], 2.5 g $MgSO_4.7H_2O$ [EM Science, Germany], 0.02 g $FeCl_3$ [EMD, USA], 150 ml V-8 juice, 850 ml distilled water, pH = 6.0) were inoculated with ten mycelial plugs from the growing edge of colonies and were incubated in a rotary shaker at 110 rpm for 10 d at 20°C. The liquid culture was vacuum-filtered, dried under sterile conditions, ground with a coffee grinder for 1 min and kept at 4°C in Petri dishes. The ground mycelium was blended with distilled water (1 g mycelium/l water) and 0.01% agar to maintain mycelial suspension. The suspension was diluted to obtain a spectrophotometer reading of approximately 1 when read at a wavelength of 600 nm which was equivalent to approximately 9200 colony-forming units/ml suspension based on colonies counted from dilutions plated out on tap-water agar (data not presented). Two drops of Tween® 20 were added to mycelial suspensions to reduce the surface tension of water, and each plant was

Table 1. Percentage stemphylium blight severity and disease reaction of *Lens culinaris* genotypes in four different environments[a]

	Growth chamber		Greenhouse		Field, Saskatoon, Canada		Field, Ishurdi, Bangladesh	
Genotype	Mean DS %	Std. error	Mean DS %	Std. error	Mean DS %	Std. error	Mean DS %	Std. error
CDC Glamis	54 (S)	3.1	62 (S)	1.9	63 (S)	2.5	48 (S)	3.3
CDC Milestone	38 (I)	1.8	33 (R)	1.5	38 (I)	2.5	35 (I)	7.0
CDC Robin	40 (I)	2.5	41 (I)	3.0	45 (I)	4.0	38 (S)	2.5
Eston	24 (R)	1.4	29 (R)	1.3	25 (R)	4.0	23 (R)	6.2
ILL 1704	24 (R)	1.0	24 (R)	1.9	35 (I)	4.0	30 (R)	2.8
ILL 4605-2	48 (I)	2.0	39 (I)	2.2	55 (S)	4.0	20 (R)	2.8
ILL 5588	53 (S)	2.1	44 (I)	3.0	58 (S)	3.3	–	–
ILL 5888	54 (S)	3.1	69 (VS)	1.1	60 (S)	2.8	68 (VS)	2.5
ILL 7537	43 (I)	1.4	45 (I)	2.0	–	–	–	–
ILL 8006	25 (R)	1.7	30 (R)	2.5	53 (S)	2.5	15 (R)	0.0
ILL 8008	46 (I)	1.5	39 (I)	2.7	–	–	–	–
ILL 8009	57 (S)	2.5	50 (I)	2.5	–	–	–	–
VIR 421	53 (S)	2.0	53 (I)	1.6	–	–	–	–
PI 320937	60 (VS)	2.0	50 (I)	1.6	60 (S)	2.8	48 (S)	2.5

[a] Disease reactions: DS, Disease severity; S, similar to CDC Glamis; I, intermediate between Eston and CDC Glamis; R, similar to Eston; VS, more susceptible than CDC Glamis.

https://doi.org/10.1017/S1479262112000329 Published online by Cambridge University Press

sprayed with 2 ml of this suspension using an air-brush (Badger Airbrush model TC 20) at 138 kPa. Plants were incubated in an incubation chamber at 100% humidity and 20°C for 48 h. Plants for growth chamber experiments remained in the growth chamber where two humidifiers (VICKS Ultrasonic Humidifiers; Fabrique Paz Canada, Inc., 510, Milton, Ontario) were engaged for 10 min every 2 h to ensure leaf wetness until final scoring. For greenhouse experiments, plants were moved from the incubation chamber to misting benches in the greenhouse where they were misted for 60 s every hour from 6 am to 11 pm. Plants were evaluated for SB severity 15 and 21 d after inoculation.

Field experiments at the University of Saskatchewan, Saskatoon, 2011

A field evaluation was conducted in the summer of 2011 in fields with a dark brown, clay loam soil texture with a good water-holding capacity at the University of Saskatchewan in Saskatoon (52°36′N, 106°6′W). The average maximum and minimum air temperature of Saskatoon in 2011 (May–August) was 18.4 and 10.9°C respectively (Environment Canada, 2012). As in experiments under controlled conditions, 'CDC Glamis' and 'Eston' were used as susceptible and resistant checks, respectively. Hill plots of four rows of 30 cm length with 30 cm spacing

Table 2. Disease severity (DS) percentage for the accessions of *Lens ervoides* and *Lens nigricans* inoculated with *Stemphylium botryosum* isolate SB-19, and reactions to ascochyta blight and anthracnose in previous studies

			Stemphylium blight[a]				
Accessions	Origin	ILWL no.	Mean DS %	Std. error	Disease reaction	Ascochyta blight disease reaction	Anthracnose disease reaction
L. ervoides							
IG 72 646	Syria	123	23	3.6	R	R[b]	R[c]
IG 72 651	Syria	128	28	2.8	R	R[d]	R[c]
IG 72 654	Syria	131	30	4.0	R	R[b]	R[c]
IG 72 799	Turkey	276	24	1.4	R	R[b]	R[c]
IG 72 803	Turkey	280	22	3.4	VR	–	R[c]
IG 72 815	Turkey	292	25	1.4	R	R[b,e]	R[c]
IG 116033	Turkey	461	16	3.7	VR	–	R[c]
IG 107435	Syria	406	44	5.7	I	S[b]	R[c]
IG 107441	Syria	413	36	1.9	R	S[b]	R[c]
L 01-827A[f]	Canada	–	22	2.8	VR	R[f]	R[c]
Eston[g]	Canada	–	25	4.3	R	S[b]	S[i]
CDC Glamis[g]	Canada	–	56	3.4	S	R[h]	S[h]
L. nigricans							
IG 72 539	France	16	40	1.7	R	MR[b]	–
IG 72 547	Unknown	24	25	4.1	R	R[b]	–
IG 72 548	Turkey	25	23	4.4	VR	–	–
IG 72 549	Unknown	26	35	3.4	R	–	–
IG 72 550	Ukraine	27	33	5.2	R	–	MR[c]
IG 72 551	Unknown	28	14	1.5	VR		MR[c]
IG 72 553	Spain	30	22	3.0	VR	R[b]	–
IG 72 557	Ukraine	34	25	3.0	VR	R[b]	R[c]
IG 72 560	Turkey	37	32	2.4	R	R[b]	–
IG 72 633	Turkey	110	38	1.5	R	R[b]	
IG 72 713	Turkey	190	21	1.0	VR	R[b,d]	MR[c]
IG 72 795	Turkey	272	27	2.0	R	R[b]	–
IG 72 843	Turkey	320	18	0.7	VR	–	–
IG 116018	Turkey	446	29	3.3	R	MR[b]	–
IG 116024	Turkey	452	49	1.9	I	R[b]	
IG 136636	Unknown	–	42	2.1	R	–	–
IG 136641	Unknown	–	49	2.8	I	–	–
IG 136645	Unknown	–	24	4.2	VR	–	–
Eston[g]	Canada	–	34	2.1	R	S[b]	S[i]
CDC Glamis[g]	Canada	–	71	1.8	S	R[h]	S[h]

[a] For stemphylium blight: R, similar to Eston; VR, more resistant than Eston; I, intermediate between Eston and CDC Glamis; S, similar to CDC Glamis; MR, moderately resistant. [b] Tullu *et al.* (2010). [c] Tullu *et al.* (2006). [d] Bayaa *et al.* (1994). [e] E. Sari, University of Saskatchewan, Canada, personal communication. [f] Fiala *et al.* (2009). [g] *L. culinaris* controls. [h] Vandenberg *et al.* (2002a, b). [i] Armstrong-Cho *et al.* (2012).

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between the rows were established in a randomized complete block design. A total of 20 seeds were planted in each hill. The outer rows of each hill were planted with the resistant and susceptible checks, respectively. The two centre hill rows were one of the ten genotypes.

Spreader plants consisting of a random mixture of susceptible cultivars 'CDC Glamis', ILL 5588, VIR 421 and ILL 4605 were developed at six plants per 4 l pot in a polyhouse complex in July 2011 and were inoculated as described before. One pot of six spreader plants was transplanted after every third hill in each block. Following irrigation after transplanting, each block was covered with a perforated green polyethylene low tunnel to maintain higher levels of humidity. Temperature and humidity were monitored with a Hobo data logger (Onset Computer Corp., MA, USA) and plots were irrigated twice. Plants were evaluated for SB severity at 65 and 80 d after planting or at 21 and 35 d after transplanting spreader plants.

Field experiments at the PRC, Ishurdi, Bangladesh, 2011–2012

In Bangladesh, field experiments were conducted at the PRC of BARI at Ishurdi. A completely randomized block design experiment was established with four replicates. A highly susceptible local cultivar BARIMasur-1 (ILL 5888) and the resistant cultivar BARIMasur-4 (ILL 8006) were used as susceptible and resistant checks, respectively. The two Canadian cultivars 'Eston' and 'CDC Glamis' were also used as resistant and susceptible checks, respectively. Each line was planted in a 1 m row plot with a row of ILL 5888 planted adjacent at 30 cm distance. Each block was surrounded by ILL 5888 that served as spreader plants. Plant debris collected from highly infected plants from the 2010–2011 field season was crushed and spread in plots at the vegetative to pre-flowering stage as a source of inoculum. Disease severity was assessed 120 d after seeding.

Disease scoring and statistical analysis

Plants in all experiments were evaluated using a 0–10 scale, where 0 = 0% disease severity (DS), 1 = 1–10% DS, and so forth, up to 10 = 91–100% DS. All statistical analyses were conducted with SAS version 9.2 (SAS institute, Inc., Cary, NC, USA). Data were transformed into percentage data using the midpoint of each disease class, and tested for homogeneity of variance with Levene's test for homogeneity. Data analysis was done using the mixed model procedure where accessions

Table 3. Disease severity (DS) percentage for the accessions of *Lens culinaris* ssp. *orientalis* and *Lens tomentosus* inoculated with *Stemphylium botryosum* isolate SB-19, and reactions to ascochyta blight and anthracnose in previous studies

			Stemphylium blight[a]				
Accessions	Origin	ILWL no.	Mean DS (%)	Std. error	Disease reaction	Ascochyta blight disease reaction	Anthracnose disease reaction
L. culinaris ssp. *orientalis*							
IG 72 622	Turkey	99	13	1.7	R	R[b]	–
IG 72 642	Syria	119	24	5.2	R	R[b]	–
IG 72 829	Turkey	306	10	1.4	VR	R[b]	–
IG 72 611	Turkey	88	13	1.3	R	R[b,c]	–
IG 72 592	Turkey	69	12	1.7	R	R[b,c]	–
IG 72 907	Tajikistan	384	15	1.9	R	S[b]	–
IG 72 905	Tajikistan	382	26	4.6	R	R[b]	–
IG 110824	Lebanon	421	20	3.0	R	–	–
PI 572385	Turkey	–	13	1.4	R	S	–
PI 572390	Turkey	–	48	5.0	S	S	–
PI 572379	Turkey	–	36	4.0	I	S	–
PI 572375	Israel	–	43	3.3	S	S	–
Eston[d]	Canada	–	21	1.7	R	S[b]	S[e]
CDC Glamis[d]	Canada	–	53	2.3	S	R[f]	S[f]
L. tomentosus							
IG 72 613	Turkey	90	36	9.7	I	–	–
IG 72 643	Turkey	120	11	1.4	VR	–	–
Eston[d]	Canada	–	22	1.8	R	S[b]	S[e]
CDC Glamis[d]	Canada	–	55	2.3	S	R[f]	S[f]

[a] For stemphylium blight: R, similar to Eston; VR, more resistant than Eston; S, similar to CDC Glamis; I, intermediate between Eston and CDC Glamis. [b] Tullu *et al.* (2010). [c] Bayaa *et al.* (1994). [d] *L. culinaris* controls. [e] Armstrong-Cho *et al.* (2012). [f] Vandenberg *et al.* (2002a, b).

https://doi.org/10.1017/S1479262112000329 Published online by Cambridge University Press

were considered as a fixed factor and replicates as a random factor. Means were separated using Fisher's least significant difference. After analysis, genotypes were categorized into five groups: genotypes more resistant to 'Eston' were considered as very resistant (VR), those equal to 'Eston' resistant (R); genotypes with DS similar to CDC Glamis were susceptible (S), those with significantly more SB very susceptible (VS); genotypes with DS higher than 'Eston' and lower than 'CDC Glamis' were considered as intermediate (I).

Results

Disease severity ratings of Lens culinaris *genotypes*

Disease severity in the experiments under controlled and field conditions ranged from 25% (ILL 8006) to 54% (PI 320937) in the growth chamber, 24% (ILL 1704) to 69% (ILL 5888) in the greenhouse, 15% (ILL 8006) to 67% (ILL 5888) in the field at the PRC, Ishurdi, Bangladesh, and from 25% ('Eston') to 62.50% ('CDC Glamis') in the field at Saskatoon (Table 1). Significant differences in SB severity were observed among the genotypes under controlled and field conditions. A significant difference for disease severity was observed between both the resistant and susceptible checks in all experiments. None of the genotypes showed VR disease reaction from all environments. Most, but not all, of the *L. culinaris* accessions showed consistency in disease reactions under the different test conditions. For example, 'Eston' showed a consistent resistant reaction in all environments with a DS score ranging from 23 to 29%. Variable results were also observed, e.g. the genotype ILL 1704 had similar levels of resistance compared with 'Eston' in all tests with the exception of the field experiment at Saskatoon where disease severity was significantly higher for ILL 1704 than for 'Eston' (Table 1). The highly susceptible line ILL 5888 from Bangladesh showed VS reaction in the greenhouse and under field conditions in Bangladesh, and S in the other two environments. The highest number of intermediate reactions was observed under greenhouse conditions, whereas most of those genotypes would display a susceptible reaction under the other test-

Table 4. Disease severity (DS) percentage for the accessions of *Lens lamottei* and *Lens odemensis* inoculated with *Stemphylium botryosum* isolate SB-19, and reactions to ascochyta blight and anthracnose in previous studies[a]

			Stemphylium blight				
Accessions	Origin	ILWL no.	Mean DS (%)	Std. error	Disease reaction	Ascochyta blight disease reaction	Anthracnose disease reaction
L. odemensis							
IG 72 543	Palestine	20	28	2.4	R	R[b]	–
IG 72 606	Turkey	83	20	1.6	VR	R[b]	–
IG 72 623	Turkey	100	30	2.2	R	R[b]	–
IG 72 639	Syria	116	37	2.6	I	–	–
IG 72 676	Syria	153	43	2.5	I	MR[b]	–
IG 72 693	Syria	170	52	2.5	I	R[b]	–
IG 72 745	Turkey	222	24	2.1	R	MR[b]	–
IG 72 777	Syria	254	25	4.5	R	R[b]	–
IG 116008	Turkey	436	33	1.7	R	MR[b]	–
Lens culinaris controls							
Eston	Canada	–	32	1.1	R	S[b]	S[c]
CDC Glamis	Canada	–	63	1.2	S	R[d]	S[d]
L. lamottei							
IG 72 537	France	14	14	1.0	VR	–	–
IG 72 552	Spain	29	14	2.4	VR	–	MR– race 1[e] S – race 0[e]
IG 110809	Spain	428	16	2.6	VR	–	R[e]
IG 110810	Spain	429	22	3.1	R	–	MR[e]
IG 110813	Spain	432	21	3.1	R	R[b]	R – race 1[e] S – race 0[e]
L. culinaris controls							
Eston	Canada	–	26	1.1	R	S[b]	S[c]
CDC Glamis	Canada	–	62	2.1	S	R[d]	S[d]

[a] R, similar to Eston; VR, more resistant than Eston; I, intermediate between Eston and CDC Glamis; MR, moderately resistant; S, similar to CDC Glamis. [b] Tullu *et al.* (2010). [c] Armstrong-Cho *et al.* (2012). [d] Vandenberg *et al.* (2002a, b). [e] Tullu *et al.* (2006).

https://doi.org/10.1017/S1479262112000329 Published online by Cambridge University Press

ing conditions. Maximum susceptible reactions in genotypes were observed in the field at Saskatoon followed by tests in the growth chamber.

Disease severity of wild Lens *species*

Typical symptom development 3–4 d after inoculation on susceptible *L. culinaris* checks confirmed the virulence of isolate SB-19. Mean DS values for 'Eston' (28%) and 'CDC Glamis' (62%) were consistent and significantly different from each other in all experiments. Among the 56 accessions from the six wild species, a high number of accessions were found with resistance to SB as good as or better than the resistant check 'Eston' (Tables 2–4). No wild species accessions had higher DS than the susceptible check 'CDC Glamis'.

Among *L. ervoides,* accessions L-01-827, IG 116033 and IG 72 803 had significantly lower DS than the resistant check 'Eston' and were classified as VR, whereas accessions IG 72 651, IG 72 815, IG 72 646, IG 72 799, IG 72 654 and IG 107441 had resistant (R) reactions similar to 'Eston' (Table 2). The remaining accession IG 107435 had DS significantly higher than 'Eston' and lower than 'CDC Glamis', and thus was considered as intermediate. Among the 18 accessions from *L. nigricans,* seven fell into the VR category, and nine in the R category (Table 2). The remaining two accessions displayed intermediate reactions, and none of the accessions showed higher DS than 'CDC Glamis'.

One accession (IG 72 829) out of 12 from *L. c.* ssp. *orientalis* showed VR disease reactions, and two accessions (PI 572375 and PI 572390) were susceptible to SB with a similar DS score as 'CDC Glamis' (Table 3). Among the remaining nine accessions, eight were resistant and one was intermediate in its reaction to SB. The two *L. tomentosus* accessions IG 72 643 and IG 72 613 had R and I reactions, respectively (Table 3). Three accessions out of nine from *L. odemensis* (IG72639, IG 72 676 and IG 42 693) had intermediate scores to SB, five accessions showed resistance to SB and only one accession (IG 72 606) had significantly lower DS than 'Eston'. Among the five *L. lamottei* lines, IG 72 552, IG 72 537 and IG 110809 were VR and the remaining two were resistant to SB (Table 4).

The highest percentage of resistant accessions was found in *L. lamottei* (100%) followed by *L. ervoides* (90%) and *L. nigricans* (88%). The other three species, *L. c.* ssp. *orientalis L. odemensis* and *L. tomentosus*, had 75, 70 and 50% resistant accessions, respectively (Fig. 1).

Discussion

Detailed screening of *L. culinaris* genotypes and accessions of wild lentil species confirmed that resistance to SB in domesticated lentil is limited, but it occurs frequently in all wild species. The high frequency of resistance to SB in the wild lentil species is probably due to the much higher level of genetic variability in these populations compared with the cultivated species in which the gene pool probably has been narrowed through centuries of selection and breeding. A long history of co-evolution between *S. botryosum* and wild lentil species in the centre of origin most probably contributed to the high abundance of resistance in these wild populations, too. Indeed, *S. botryosum* has been reported from Syria (Hanounik, 1979), which is believed to be part of the centre of origin for cultivated lentil (Cubero *et al.*, 2009). Ten of the 56 wild species accessions originated

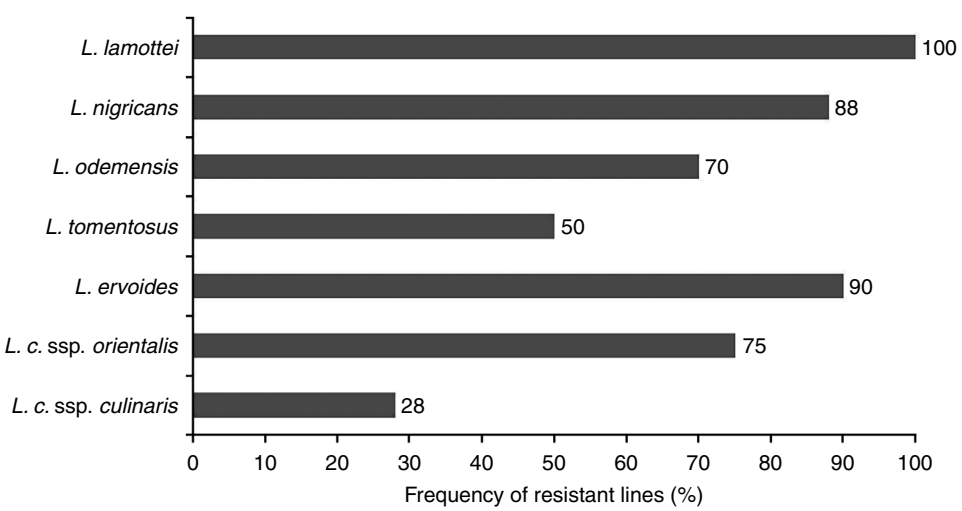

Fig. 1. Percentage of resistant accessions of the seven *Lens* species inoculated with *Stemphylium botryosum* isolate SB-19 under phytotron, greenhouse and field conditions.

https://doi.org/10.1017/S1479262112000329 Published online by Cambridge University Press

from Syria and another 22 from the geographic neighbour Turkey, and all of the *L. ervoides* accessions from these two countries had a higher frequency of resistance. Bayaa *et al*. (1994) and Tullu *et al*. (2006, 2010) reported similar results for ascochyta blight and anthracnose resistance. Almost all the accessions from *L. orientalis* and *L. odemensis*, which are considered as 'most likely candidates of the cultigens' (Cubero *et al*., 2009), showed highly resistant reactions to SB, and are of similar geographic origin as *L. ervoides*. These results support the hypothesis that species in the centre of origin are characterized by high levels of diversity, including disease resistance. Leppik (1970) reviewed sources of resistance with regard to their origin for a range of crop species and demonstrated that the primary or secondary centre of origin represented a valuable source of genotypic diversity for resistance to different biotic stresses.

Different morphological features among the different species compared with domesticated lentil may influence the ability of pathogens such as *S. botryosum* to invade the lentil. Highly significant differences between *L. culinaris* and other wild species have been reported by Hoffman *et al*. (1998) for number of leaflets, leaflet length and width, and leaf pubescence. A significant morphological and phenological variation between cultivated and wild *Lens* species was also observed by Ferguson and Robertson (1999), which was also related to different growth stages and disease development. For example, SB usually infects plants from flowering onwards (Kumar, 2007). Chowdhury *et al*. (1997) also reported on the role of epidermal hairs, the thickness of epidermis, the cortical layer depth and the number of stomata in disease resistance of cultivated lentil.

Some wild accessions had little infection, restricted to tiny lesions after a few days of inoculation, which did not expand further. Both susceptible and resistant accessions were found from all species except *L. lamottei* where all accessions were resistant to SB. The highest number of accessions with resistance to *C. truncatum*, both under greenhouse and field conditions, was found in *L. ervoides* (Tullu *et al*., 2006). The authors also identified good resistance to the more aggressive race 0 of *C. truncatum* among accessions of *L. ervoides* and *L. lamottei,* resistance that is not present in *L. culinaris*. Twenty-five wild lentil accessions among those reported as resistant to ascochyta blight by Tullu *et al*. (2010) were also identified as resistant to SB in this study (Tables 2–4). These results could be of significant importance for lentil improvement programmes because all three diseases are now a major concern for lentil cultivation in the northern temperate prairies of North America. Based on agroclimatic similarity, it can be expected that SB will also become a concern in northern temperate regions of Asia where lentil production is expanding. Availability of superior resistance to all three pathogens in the same germplasm will simplify the development of cultivars with triple resistance through single transfer of the resistance genes into cultivated lentil.

High humidity combined with high temperatures favour SB infection, and the disease is not considered to be a current major problem in the production of the cultivated species in the centre of origin. The entire world collection of accessions of all wild species combined is less than 600 (Tullu *et al*., 2010). The fact that all the wild species of lentil have a high frequency of resistance to SB may indicate that the centre of origin (Mediterranean basin) may have experienced climates more conducive to SB development in times prior to the domestication of the cultivated species.

Searching for SB resistance sources within cultivated lentil was the initial research goal related to breeding for resistance. The highest percentage of susceptibility to SB was found in *L. c*. ssp. *culinaris* under all conditions. Kumar (2007) reported very poor resistance to SB and inconsistent disease reactions in different environments among accessions of *L. culinaris*. In our experiments, consistent SB resistance was displayed by cultivar 'Eston', but this cultivar is highly susceptible to both ascochyta blight and anthracnose. Furthermore, it is desirable to use diverse sources of resistance in lentil breeding programmes to achieve durability of disease resistance. Several factors, such as higher selection pressure, poor management practices, narrow genetic base, rapid evolution of the pathogen population, changing climate and other environmental factors, can influence the breakdown of resistance in the cultivated species. Consequently, the high frequency of resistant accessions among wild accessions with higher resistance than in *L. culinaris* is very promising for future genetic improvement efforts.

Resistance sources from cultivated and wild species identified in this study can be used in intra and interspecific crosses to develop adapted cultivars with SB resistance. By strategically selecting accessions with multiple disease resistance for this purpose, resistance to SB can be incorporated into the lentil breeding programme to develop new cultivars with disease resistance to SB, ascochyta blight and anthracnose, as suggested by Tar'an *et al*. (2003). Research is underway at the CDC to develop lentil cultivars with genetic resistance to SB as part of a long-term strategy for reducing potential economic losses caused by the disease. Special techniques such as embryo rescue and grafting required to obtain fertile hybrids from distant or interspecies crosses (Fiala *et al*., 2009), tissue culture for multiplication of F_1 plants from rescued embryo (Shyamoli Saha, CDC, University of Saskatchewan,

https://doi.org/10.1017/S1479262112000329 Published online by Cambridge University Press

Canada, unpublished results), grafting to faba bean (Yuan *et al*., 2011) and reproduction using cutting for multiplication to achieve rapid disease screening (Vail *et al*., 2012) have been successfully established and have made it feasible to reliably transfer resistance from wild to cultivated lentil. The results from the present study contribute to scientific knowledge that will help with the development of an effective breeding strategy for SB resistance in lentil. Some wild species accessions having significantly lower DS (Tables 2–4) than 'Eston' can be given higher priority for future breeding efforts. Genetic characterization of sources of SB resistance across the genus *Lens* combined with the development and use of genomics-based marker-assisted selection techniques for breeding are important milestones for utilization. Future research will be required to determine whether the various species have the same genes for resistance to SB, and whether there is allelic variation for SB resistance within species.

Considering that all wild *Lens* species have good sources of resistance to SB, that the most accessible sources are in the primary gene pool consisting of *L. c*. ssp. *orientalis, L. tomentosus* and *L. odemensis*, and that these species are good sources of multiple resistance to fungal diseases, support should be given to efforts to collect and preserve these valuable sources of genetic diversity before further genetic erosion occurs.

Acknowledgements

The authors would like to acknowledge funding received from the Saskatchewan Pulse Growers and the Natural Sciences and Engineering Research Council of Canada, Industrial Research Chair Program. Technical assistance from research support staff of the following organizations is gratefully acknowledged: BARI, PRC, Ishurdi; breeding and pathology program staff for the field at greenhouse research at the CDC/Department of Plant Sciences, University of Saskatchewan.

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