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ORIGINAL PAPER

Screening lentil germplasm for stemphylium blight resistance

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Abstract Stemphylium blight is an important fungal disease of lentil caused by *Stemphylium botryosum*. It occurs in all major lentil growing regions of the world, including Bangladesh, India, Nepal, Syria, USA and Canada. As lentil production in Australia is increasing, stemphylium blight is considered a potential threat to the industry. However, information on this pathogen and resistance levels in Australian lentil germplasm is limited. Five fungicides were tested for efficacy against seven isolates of *Stemphylium botryosum*. Mancozeb and thiram were the most effective in inhibiting the growth of the pathogen under in-vitro conditions. To prevent a disease outbreak, it is prudent to screen germplasm to eliminate stemphylium blight susceptible lines from the breeding program prior to release. Therefore, a screening method for evaluating stemphylium blight resistance in lentil was developed for use under controlled environment conditions. Using this method, stemphylium blight disease ratings were provided on all current Australian lentil varieties. In addition, screening of advanced breeding lines showed variation in resistance/susceptibility for this disease and allowed selection of resistant lines for stemphylium blight resistance breeding. Seven International lentil varieties were tested against Australian isolates of *S. botryosum* and showed varying levels of resistance. Three hundred lentil accessions obtained from the International Centre for Agricultural Research in the Dry Areas (ICARDA) were evaluated and six accessions were identified with higher resistance than the resistant check ILL6002 that could be used in lentil breeding for stemphylium blight resistance.

Keywords Breeding · Disease resistance · Fungicide efficacy · Minimal medium · Phenotyping method · *Stemphylium botryosum* · Variety

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Introduction

Lentil (*Lens culinaris subsp. culinaris*) is one of the most important pulse crops in Australia, grown for human food, animal feed, and for its rotational benefits to cropping systems (McMurray et al. 2006). Pulses are an important food for millions of people in developing countries, where it is an indispensable source of proteins and it also contains the amino acid lysine, which is generally deficient in food grains (Iqbal et al. 2006). The growing population in South Asia and the Middle East is providing a demand driven export opportunity for countries like Australia. In Australia, lentils are mainly grown in medium to low rainfall, winter cropping areas of South Australia, Victoria and New South Wales, where its cropping area has increased from 165,000 to 230,000 ha during the last 5 years, to produce an average of 284,000 t annually (Pulse Australia 2015). To mitigate the risk of a disease outbreak, it is necessary to monitor for diseases that might retard lentil production. Fungal diseases, such as ascochyta blight and botrytis grey mould can seriously reduce lentil production, but stemphylium blight caused by *Stemphylium spp.* Wallr. teleomorph *Pleospora tarda* Simmons, (Simmons 1969) is another common disease of lentil, which under conducive conditions may cause significant yield losses in Australia (Murray and Brennan 2012). Stemphylium blight has caused major yield losses to lentil production in South

Asia and North America (Morrall et al. 2006) where it was initially considered a minor disease (Bakr and Zahid 1986; Bayaa and Erskine 1998).

Significant diversity is present in the *Stemphylium* genus which was erected based on the type species *Stemphylium botryosum* Wallr. (Wallroth 1833). Species within this genus vary from approximately 30 to 150 (Inderbitzin et al. 2009) and more new species have been recently reported (Pei et al. 2011; Arzanlou et al. 2012) based on both morphological observations and molecular phylogenetic data. Many of these species are saprophytic, growing on dead plants and cellulose materials (Simmons 1969; Ellis 1971), but *S. botryosum*, *S. solani* and *S. vesicarium* are recognised plant pathogens that cause disease in many agricultural and horticultural crops (Wang et al. 2010). These pathogens have demonstrated adaptability to different environments ranging from the humid tropics to subtropical regions in the Americas, Asia and Oceania countries and infects plants in 43 genera (Booth and Pirozynski 1967; du Toit and Derie 2001; Blancard 2012). *Stemphylium botryosum* has also shown host-specificity with *forma specialis* designations made, such as *S. botryosum* f. sp. *lactucae* for isolates infecting lettuce (Padhi and Snyder 1954; Raid et al. 1997), *S. botryosum* f. sp. *lycopersici* for isolates on tomato (Rotem and Bashi 1977), and *S. botryosum* f. sp. *spinacia* for isolates on spinach (Koike et al. 2001). While in alfalfa, two biotypes were suggested from cool-temperature and warm-temperature isolates as a result of the symptoms displayed (Cowling et al. 1981). No such speciality for *S. botryosum* is known in pulses.

Some studies on stemphylium blight epidemiology and management were conducted to understand the disease and its associated risk in lentil (Ahmed 1989; Bakr and Ahmed 1992; Salam et al. 2016). Plant debris as well as infected seed are important sources of *S. botryosum* inoculum (Taylor et al. 2007). Infected seed causes transmission of the disease from region to region and also serves as a source of initial inoculum in the season (Agarwal and Sinclair 1996). The conidia and ascospores produced on infected plant debris are responsible for the primary infection and spread of the disease (Mwakutuya 2006). The secondary infection occurs by airborne conidia that develop in successive generations on distinct conidiophores on the leaf surface (Mwakutuya and Banniza 2010). Lentil are most susceptible to the disease in the last third of the growing season and epidemics are favoured by warm temperatures between 25 and 30 °C and wet conditions of more than 85% humidity for 48 h (Mwakutuya and Banniza 2010). Symptoms consist of small, light beige lesions that enlarge and coalesce, spreading to infect entire leaves and shoots. Leaf drop and loss in biomass can significantly affect photosynthetic capacity at the crucial pod-filling stage resulting in poor yield and seed quality (Bakr and Zahid 1986).

Landraces and wild species are an important source of tolerant germplasm for biotic and abiotic stresses. Podder et al. (2013) investigated stemphylium blight resistance in accessions from seven species of *Lens* and reported the greatest susceptibility in *L. culinaris* accessions and the highest frequency of resistance was found in *L. lamottei* and *L. ervoides*. It may be possible to identify stemphylium blight resistance in landraces and wild lentil accessions that could be used to develop new commercial varieties. Studies on the genetics of resistance to stemphylium blight in lentil are limited too. Kumar (2007) suggested quantitative inheritance of stemphylium blight resistance in the lentil cross Barimasur-4 × CDC Milestone. Similarly, Saha et al. (2010) reported one and three quantitative trait loci (QTLs) from an ILL6002 x ILL5888 RIL in two cropping years with one QTL common in both years. They identified a tightly linked marker to this QTL that could be used in marker-assisted selection. In Bangladesh, resistance studies have helped to breed and develop better stemphylium blight resistant varieties, but those varieties may not be resistant against all pathotypes. For instance, ILL8006, also known as Barimasur-4 was released in Bangladesh as a stemphylium blight resistant variety (Sarker et al. 1999). The same variety when screened under controlled environment conditions, showed moderate resistance within Canadian studies (Banniza et al. 2005). Therefore it is important to screen against pathotypes present in each production zone.

In Western Australia, re-emergence of grey leaf spot disease in lupins caused by *S. botryosum* was reported, following the release of susceptible varieties which suggested that regular screening for resistance to grey leaf spot should have been part of the lupin breeding program (Thomas et al. 2011). To prevent a similar situation occurring in lentil, it may be necessary to screen the lentil breeding material and evaluate the stemphylium blight resistance levels before varieties are released. The release of new varieties that are susceptible could result in severe outbreaks of stemphylium blight. Therefore, the main objective of this study was to establish a phenotypic screening method to investigate disease development and identify resistant genotypes. Commercial varieties, breeding lines and landraces of *L. culinaris* were screened and their disease ratings are provided. We have also tested the efficacy of fungicides under in-vitro conditions against *S. botryosum*

Materials and methods

Isolates and inoculum production

The isolates were collected from seed of different lentil varieties or breeding lines namely 96-047 L, Northfield, Cumra, CIPAL 404, 99H011, CIPAL 416 and Nugget at the Department of Economic Development, Jobs, Transport and Resources (DEDJTR) Horsham, from field trials in 2006 (Trevor Bretag, pers. comm.). These isolated have been lodged with the VPRI numbers from 42,502 to 42,508 respectively and stored in the Herbarium, DEDJTR, Bundoora.

Preparation of culture media for spore production

To identify the most suitable media for spore production, seven types of culture media were used. They were 1) Malt extract agar (MEA: 20 g malt extract, 1 g proteose peptone, 20 g sucrose and 15 g Difco Technical agar); 2) Potato dextrose agar (PDA: 39 g Difco PDA); 3) V8 juice agar (V8: 200 mL of Campbell's V8 juice, 4 g calcium carbonate and 15 g Difco Technical agar); 4) V8 + PDA (150 mL V8 juice agar, 10 g PDA, 10 g Difco Technical agar); 5) Coon's medium (Coons: 1.2 g magnesium sulphate, 2.7 g potassium dihydrogen phosphate and 20 g Difco Technical agar); 6) Nutrient agar (NA: 20 g Difco Technical agar, 4 g dextrose, and 2 g potassium nitrite); 7) Water agar (WA: 20 g Difco Technical agar). All ingredients were added into deionised water to make a final volume of 1 L, then autoclaved at 121 °C and 15 psi for 30 min.

Approximately 1 cm^2 blocks/discs were cut from each isolate of one month old culture of *S. botryosum* grown on the same media and placed in the middle of 90 mm Petri dishes. Three replicates were grown for each isolate. The culture plates were incubated at 22 °C under cool white fluorescent light (44 μmol/m2/s) for a photoperiod of 20 h light/day. After four weeks incubation, the spores were harvested by adding 10 mL of water to each Petri dish to create a spore suspension. The suspension was then filtered twice through cheese cloth and each sample was counted three times using a haemocytometer (Blaubrand®, Sigma-Aldrich) to estimate spore numbers.

Fungicide efficacy to *S. botryosum*

Five fungicides were tested for their efficacy against *S. botryosum*. Fungicides (Thiraflo® (active constituent thiram 600 mg/L), Penncozeb® (active constituent mancozeb 750 mg/L), Unite® 720 (active constituent chlorothalonil 720 mg/L), Spin Flo® (active constituent carbendazim 500 mg/L), and thiabendazole (catalogue no. T8904, Sigma-Aldrich, 99.9% pure powder) were used. Efficacy was tested by determining the reduction in growth of the isolates when grown on a minimal media containing the most effective concentration of the fungicide compared to a control that did not have any fungicide. All isolates were grown on a fungicide concentration of 25 μg/mL which was found to be the most effective (data not shown). The isolates were then grown for two weeks and colony radius was recorded by measuring the growth from the middle of the colony to the farthest point of growth at 3 points and the mean calculated. To calculate the fungicide effect, the growth of the isolate on fungicide plates was subtracted from the control plates.

The minimal media contained 10 mM glutamate sodium salt, 2 g/L sodium nitrate, 1 g/L potassium dihydrogen phosphate (K_2HPO_4), 15 g/L agar and 10 mL/L 100× Trace chemical stock, made up to 1 L with deionised water. The 100× micronutrient solution was prepared separately by mixing 50 g/L potassium chloride (KCl), 50 g/L magnesium sulphate heptahydrate ($MgSO_4.7H_2O$), 1 g/L zinc sulphate heptahydrate ($ZnSO_4.7H_2O$), 1 g/L ferrous sulphate heptahydrate ($FeSO_4.7H_2O$), 0.25 g/L copper(II) sulphate pentahydrate ($CuSO_4.5H_2O$) and was kept at 4 °C in a bottle covered with aluminium foil and added to the minimal media just before autoclaving at 121 °C and 15 psi for 30 min. Thiraflo®, Penncozeb®, Unite® 720 and Spin Flo® were dissolved in water while thiabendazole was dissolved in dimethyl sulfoxide (DMSO) to make a stock solution of 10 mg/mL, which was then stored at −20 °C until use. For thiabendazole fungicides effects, control plates had DMSO in the same concentration as that of the fungicide treatments to determine any effect of DMSO on the growth of isolates.

Plant material, growing conditions and inoculation

To evaluate the level of stemphylium blight resistance within Australian commercial varieties and lentil breeding material, seed was procured from the Pulse Breeding Australia's (PBA) lentil breeding program, based at DEDJTR, Horsham. Stage 3 lentil breeding lines, 12 commercial varieties and nine advanced breeding lines were tested. The Stage 3 breeding lines comprised of 110 lines were screened in a separate trial to the commercial and advanced breeding lines. ILL6002 (Saha et al. 2010) was used as the resistant check variety in this and all subsequent experiments.

Ten seeds of each test line were sown in 20 cm diameter, 4.3 L pots filled with potting media and thinned to six seedlings after 2 weeks. The potting media consisted of 1000 L composted pine bark (Bio Gro, Mount Gambier, SA) containing 1 kg Floranid® N32, 1 kg 8–9 month Osmocote®, 1 kg 3–4 month Osmocote®, 225 g MicroMax® Complete, 225 g Quality® FeEDDHA Chelate (6% Fe), 30 kg agricultural lime, and 2 kg Saturaid®. Two replications were arranged in randomized complete block design (RCBD) within a glasshouse at 22 ± 4 °C with natural daylight for 5 weeks. Five weeks after sowing, when most plants had just started to set flower buds, the plants were moved from the glasshouse in the same order to a growth chamber with benches enclosed by plastic tents (235 × 75 × 105 cm). The growth chamber was maintained at 25 ± 2 °C with a 12 h photoperiod provided by cool-white fluorescent lights (c. 100 μmol/m2/s). After 24 h, the plants were inoculated with a spore suspension containing a mixture of all seven isolates each at 10×10^4 spores/mL in deionized water with 0.02% *v*/v Tween® 20 (Sigma, Saint Louis, MO, USA) as a surfactant. The spore suspension was sprayed by hand using an atomized spray bottle at the finest setting until run-off. Two hours after inoculation, a fogging unit using reverse osmosis water (Ionmax Ultrasonic Cool Mist Humidifier ION60) was turned on to maintain ≥90% humidity in the tents for the first 48 h and then foggers were turned on for 2 h each day until disease scoring. Symptoms were scored two weeks post-inoculation on a 1–9 scale as described in Table 1, where 1

Table 1 Disease scale for stemphylium blight disease caused by *S. botryosum* in lentil

Rating	Score	Description
R	1	No infection or tiny non-spreading lesions
R-MR	2	Few chlorotic lesions, leaf drying affecting ≤5% leaves
MR	3	Expanding lesions on 6 to 15% of leaves turning necrotic
MR-MS	4	16 to 30% leaves infected and defoliation
MS	5	31 to 45% leaves infected and defoliation
MS-S	6	46 to 60% leaves infected and defoliation
S	7	61 to 80% leaves infected, defoliation and stem lesions
S-VS	8	81 to 90% leaves infected, stem lesions and plant left with terminal leaves only
VS	9	Whole plant death

R resistant, *MR* moderately resistant, *MS* moderately resistant, *S* susceptible, *VS* very susceptible

was no infection or tiny non-spreading lesions and 9 was 100% plant death. Plants were scored individually and an average score was given for all plants per pot.

To determine the resistance of lentil varieties released overseas to Australian isolates of *S. botryosum*, a trial was conducted using lentil varieties from Canada (CDC Eston, CDC Glamis and CDC Milestone), an ICARDA lines ILL5588 (syn. Northfield) and ILL8006 (syn. Barimasur-4) and the Argentinian variety Precoz (a very-early maturing variety). The seed of these lentil varieties were obtained from the PBA lentil breeding program, DEDJTR, Horsham. These varieties were screened against the Australian isolates, then the results were compared to their previously recorded resistance against *S. botryosum* isolates from Canada (Banniza et al. 2005). The experiment was randomized with three replicates, conducted and scored under the same conditions as described above.

To identify new sources of resistance, an un-replicated screening of 300 diverse *Lens culinaris* ICARDA accessions was undertaken in the glasshouse in the conditions described above. These landraces were obtained from the Australian Grains Genebank (AGG), DEDJTR, Horsham. After the initial screen, 250 susceptible lines were eliminated. A second trial was then conducted with the 40 most resistant and 10 most susceptible accessions. The trial contained five plants per pot per accession arranged in a randomized complete block design in the glasshouse and then the experimental design and pot arrangement were retained in the growth chamber for inoculation.

Statistical analysis

All statistical analyses were performed using GenStat® 16th edition (VSN International, UK). Least significant difference (*LSD*) was calculated at $P = 0.05$. Multiple comparisons of genotypes were performed using Bonferroni test at $P = 0.05$.

Results

Selection of media for maximum spore production

Spore production of isolates varied significantly ($P < 0.001$) on different culture media (Fig. 1), whereas on a given medium all isolates showed similar growth. The highest spore production was obtained on PDA with an average of 125 × 10^4 conidia/mL followed by V8 + PDA media (94 × 10^4 conidia/mL) and V8A (88 × 10^4 conidia/mL). The least number of spores were formed on

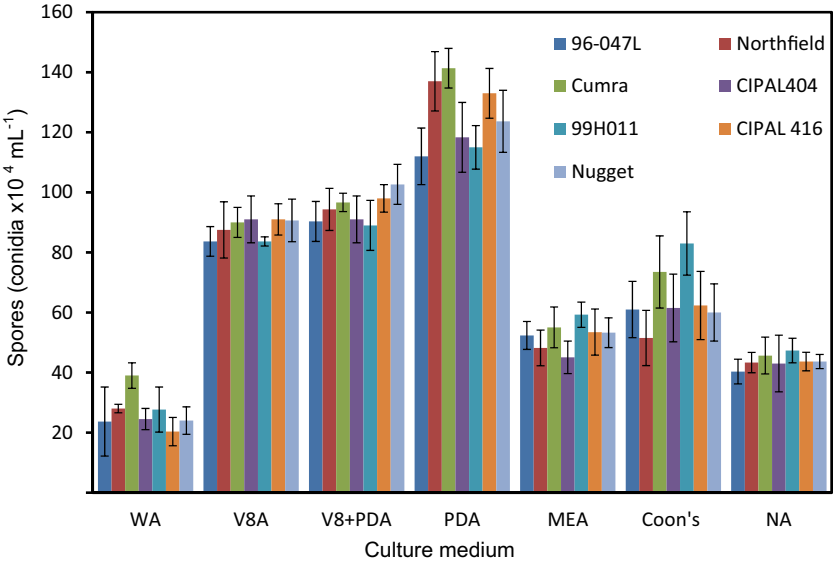

Fig. 1 Spore production by seven *S. botryosum* isolates from different lentil lines on seven different media. Error bars are ± LSD of the mean ($n = 9$). Please refer to material and methods for the media details

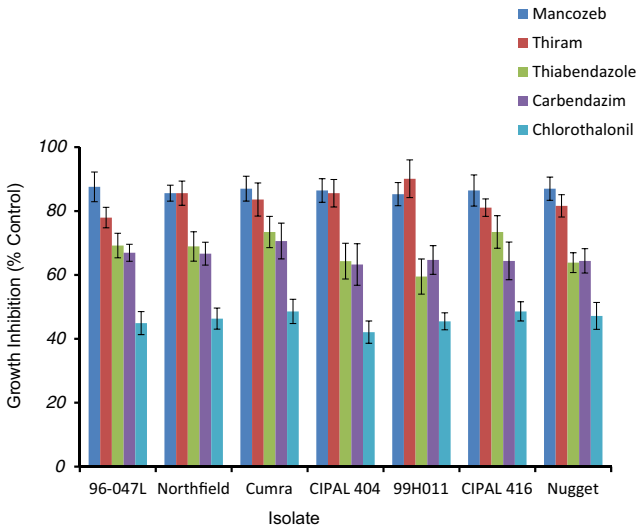

Fig. 2 Ability of 5 fungicides to inhibit growth of seven *S. botryosum* isolates. Error bars represents ±LSD of the mean (n = 9)

WA. As the highest number of spores were formed on PDA medium, thereafter it was used for spore production.

Efficacy of fungicides to restrict *S. botryosum* isolates radial growth

A fungicidal concentration of 25 μg/mL reduced the growth of the Cumra isolate by up to 50% therefore this concentration was selected for testing all isolates. All fungicides significantly ($P < 0.001$) inhibited mycelial radial growth of all isolates compared to the control (Fig. 2). Mancozeb and thiram had a similar efficacy and reduced mycelial radial growth by 77–90%, followed by thiabendazole and carbendazim which showed a reduction of 50–73%. The least effective fungicide was chlorothalonil which only reduced mycelial growth by 42–48%. These results indicate that there are fungicide options available to control *S. botryosum* growth.

Phenotyping lentil for stemphylium blight resistance

Disease symptoms first appear on leaves after 6–7 days on the lower part of the plant and progressed upwards, from older to younger leaves. The lesions began as pale yellow, pin-head size dots on leaflets that spread, resulting in chlorosis of the whole leaf (Fig. 3a-c). Chlorotic leaves then became withered and dried showing early senescence (Fig. 3d). Leaf fall progressed in susceptible plants, such that two weeks post inoculation, the plants were completely dried or defoliated and with only apical green leaves left (Fig. 3e). In severe cases necrotic lesions were also observed on stems. Within 3–4 weeks after infection, the pathogen sporulated and diseased leaves took on a grey to black appearance.

The commercial varieties and advanced breeding lines showed variability in resistance to stemphylium blight, with disease scores from 3.7 to 8.4 (Table 2). The varieties PBA Ace, PBA Herald XT, PBA Bounty, PBA Jumbo showed a disease reaction similar to the resistant check ILL6002, while the advanced breeding line CIPAL1301 showed significantly ($P = 0.05$) better resistance than ILL6002. In contrast, PBA Flash and Nugget were susceptible to stemphylium blight.

Resistance levels within the 110 breeding lines, showed a normal distribution of disease scores (Fig. 4). A substantial number of the breeding lines (37 lines) exhibited an intermediate reaction with disease scores within 4–5. These continuous and normally distributed disease scores suggest that resistance for the disease is under quantitative genetic control.

Within the trial of the international lentil varieties, the early maturing, Argentinean variety Precoz had the lowest disease score at 2.8 (Fig. 5). ILL6002 and CDC Eston were the next

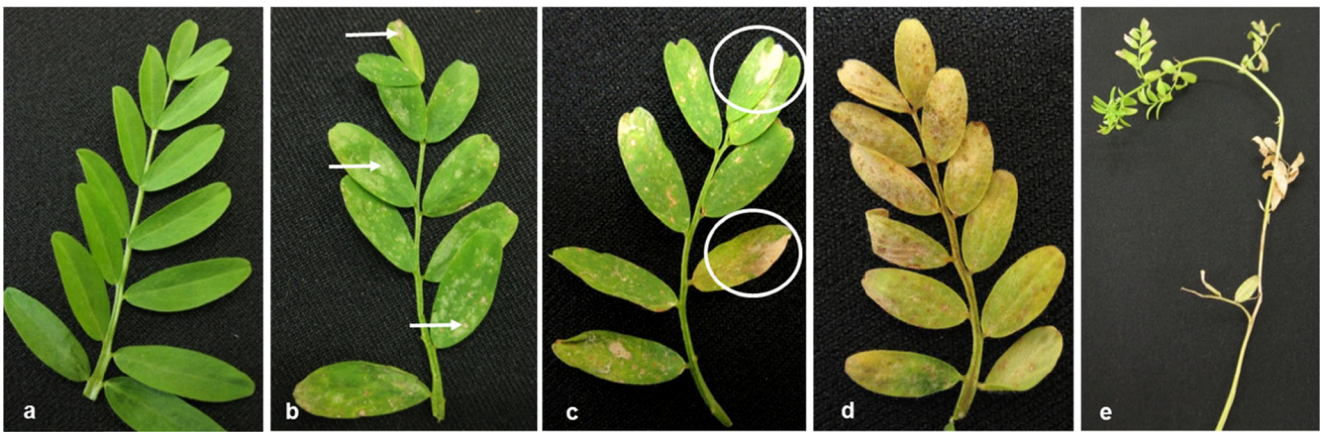

Fig. 3 Stemphylium blight symptoms on lentil (**a**) uninfected leaf, (**b**) appearance of tiny lesions shown by arrow heads on leaflets, (**c**) spreading of chlorotic lesions, (**d**) whole leaf becoming withered and dry, (**e**) early senescence and defoliation of the lower leaves

Table 2 Disease ratings of commercial lentil varieties and advanced breeding lines for stemphylium blight inoculated with *S. botryosum* under controlled environment conditions

Test line	Mean disease score (1–9)*
CIPAL1301	3.7
PBA Ace	4.2
PBA Herald	4.4
PBA Bounty	4.5
ILL6002**	4.7
CIPAL1104	5.0
PBA Jumbo	5.1
CIPAL1204	5.2
CIPAL1303	5.2
CIPAL1207	5.3
Northfield	5.4
Boomer	5.5
PBA Jumbo 2	5.7
PBA Bolt	6.0
PBA Hurricane	6.0
CIPAL0901	6.2
Nipper	6.2
PBA Blitz	6.5
ILL8006	7.1
CIPAL1302	7.2
CIPAL1001	7.4
PBA Flash	7.7
Indian head	8.2
Nugget	8.4
LSD (P = 0.05)	0.40

*1 no disease, 9 plant dead

**Resistant check

most resistant lines at 4.6 and 4.9, and were not significantly different (P = 0.05) from each other. Next were Northfield and CDC Glamis with disease scores of 5.6 and 6.1, respectively. ILL8006 and CDC Milestone were most susceptible but non-significantly different (P = 0.05) from each other with diseases scores of 7.0 and 7.7, respectively.

Preliminary screening of 300 lentil accessions produced disease scores that ranged from 2 to 9 (data not shown). The 40 most resistant accessions were identified that showed disease scores of ≤5, corresponding to <45% of the plant affected with symptoms. In the second trial, the screening of the segregating set of 50 accessions identified six accessions that had significantly (P = 0.05) better disease scores than the resistance check ILL6002, while another 19 accessions showed disease scores that were not significantly different (Table 3). The six most resistant accessions were ILL6408, ILL0133, ILL0379, with ILL0426, ILL0427 & ILL0215 the most resistant. ILL0131 showed highly susceptible symptoms of leaf drying and defoliation as compared to the most resistant landrace ILL0426 (Figs. 6a and b), while the resistant check ILL6002 showed a moderately resistant response where 30–40% leaves were symptomatic (Fig. 6c).

Discussion

This is the first evaluation of Australian lentil germplasm for resistance to stemphylium blight. Considerable variation in resistance/susceptibility to stemphylium blight was observed in the Australian varieties and breeding lines tested. As a consequence the Australian lentil breeding program has benefited from the development of a screening method that can identify susceptible lines which could be eliminated. Landraces with better resistance than current varieties were identified and will be utilised as parents to enhance resistance in new varieties.

Efficacy of fungicides against isolates of *S. botryosum* in-vitro showed that the fungicides mancozeb and thiram were more effective than thiabendazole, carbendazim and chlorothalonil. Mancozeb and thiram belong to the M3 group of fungicides (dithiocarbamates) and also contains sulphur. Propineb, a fungicide from the same chemical group has been successfully used to control stemphylium blight in Bangladesh (Bakr and Ahmed 1992). Similarly, a Canadian study showed sulphur was an effective fungicide for stemphylium blight control on lentil whereas chlorothalonil, a Group B2 (nitrile) fungicide, was least effective in controlling stemphylium blight (Banniza et al. 2005), which concurs with the results of this

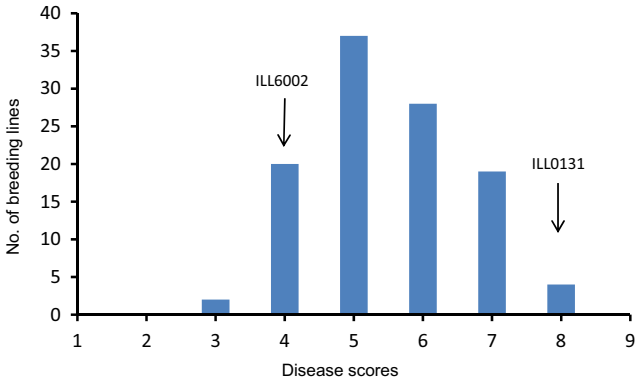

Fig. 4 Frequency distribution of stemphylium blight disease scores in 110 Australian lentil breeding lines. Arrows show mean disease scores of resistant check (ILL6002) and susceptible check (ILL0131)

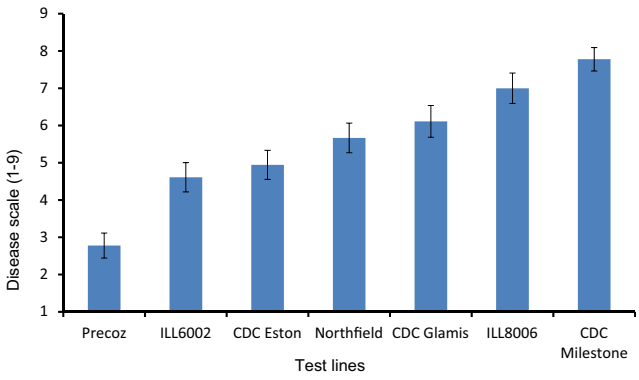

Fig. 5 Resistance of international varieties and two ICARDA accessions of lentil against Australian isolates of *S. botryosum*. Error bars represents ±*LSD* of the mean (n = 24). Least significant difference at (P = 0.05) was 0.62

Table 3 Disease severity of stemphylium blight across diverse lentil landraces inoculated with *S. botryosum* under controlled conditions

Test Line	Mean disease score (1–9)*
ILL0426	1.6[a]
ILL0427	1.8[a]
ILL0215	2.5[ab]
ILL6408	2.5[ab]
ILL0133	2.6[ab]
ILL0379	2.6[ab]
ILL0365	2.9[abc]
ILL0192	3.2[abcd]
ILL8093	3.5[bcde]
ILL0360	3.6[bcde]
ILL0420	4b[cdef]
ILL0065	4.3[cdefg]
ILL0374	4.3[cdefg]
ILL6002**	4.3[cdefg]
ILL0406	4.5[cdefgh]
ILL0241	4.8[defghi]
ILL0251	5.0[efghij]
ILL0283	5.3[fghijk]
ILL0317	5.3[fghijk]
ILL0381	5.3[fghijk]
ILL0300	5.4[fghijkl]
ILL0431	5.4[fghijkl]
ILL0367	5.6[fghijklm]
ILL0097	5.7[ghijklmn]
ILL0270	5.7[ghijklmn]
ILL0135	6.1[hijklmno]
ILL0166	6.2[ijklmno]
ILL0318	6.2[ijklmno]
ILL0085	6.3[ijklmno]
ILL0220	6.3[ijklmno]
ILL0207	6.4[ijklmno]
ILL0211	6.4[ijklmno]
ILL0180	6.6[jklmnop]
ILL0181	6.6[jklmnop]
ILL0233	6.6[jklmnop]
ILL0272	6.6[jklmnop]
ILL0216	6.7[klmnop]
ILL0157	6.8[klmnopq]
ILL0222	6.8[klmnopq]
ILL0294	6.8[klmnopq]
ILL0321	6.8[klmnopq]
ILL0238	6.9[klmnopq]
ILL0174	7.0[lmnopq]
ILL0221	7.1[mnopq]
ILL0240	7.1[mnopq]
ILL0326	7.2[mnopq]
ILL0040	7.3[nopq]
ILL0269	7.3[nopq]
ILL0179	7.5[opq]
ILL0279	7.6[opq]
ILL0320	7.6[opq]
ILL0118	8.1[pq]
ILL0131	8.4[q]
LSD ($P = 0.05$)	0.76

Different superscript letters show significance difference in genotypes calculated by Bonferroni test ($P = 0.05$)

*1 no disease, 9 plant dead

**Resistant check

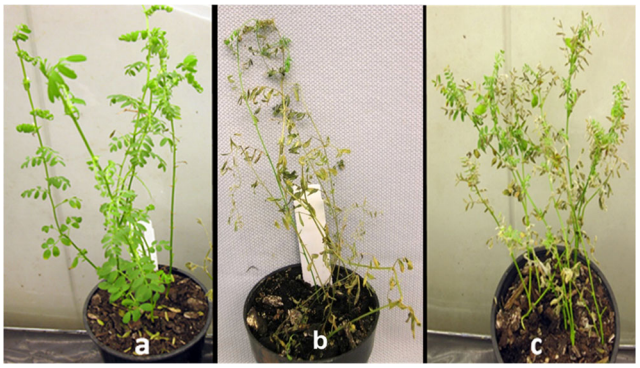

Fig. 6 Differential responses of the lentil genotypes to *S. botryosum* (**a**) A resistant reaction of ILL0426, (**b**) susceptible reaction of ILL0131, (**c**) intermediate reaction of ILL6002

study. Mancozeb and thiram appear to be good options to control *S. botryosum* but further studies are needed to test the efficacy of these fungicides on lentil under field conditions.

The disease screening of the international lentil varieties confirmed they have similar levels of resistance to the Australian and Canadian isolates of *S. botryosum*. We compared our results with Canadian studies where the same varieties were tested with the Canadian isolates of *S. botryosum* (Banniza et al. 2005). This confirmed that the resistance genes present in the Canadian varieties are also effective against Australian isolates. ILL8006, which is also known as Barimasur-4, a stemphylium blight resistant variety released in Bangladesh in 1999 (Sarker et al. 1999), showed moderate resistance to the Canadian isolates (Banniza et al. 2005), whereas it was moderately susceptible to the Australian isolates. The reasons of susceptibility of ILL8006 to Australian isolates is not known and further studies are required to confirm if there is pathogenic variation within local or international populations of *S. botryosum.*

ICARDA has had significant impact on the Australian lentil breeding program, providing a large number of accessions (Brennan et al. 2002), which are presently housed at the AGG, Horsham. Five out of six landraces identified resistant to stemphylium blight in this study were originally collected from costal drylands in Chile, where lentils are grown in sandy to heavy clay soils with an annual rainfall average of 900 mm (Muehlbauer and Kaiser 1994). Reports from North America such as North Dakota, Saskatchewan and Montana specified that lentil are most susceptible to stemphylium blight when rain occurs late in the season, from late bloom through to late pod-filling stage (Chen et al. 2013). The reason for stemphylium blight resistance in these landraces might be due to adaptability to high moisture conditions at post vegetative stages. Utilization of these landraces could provide resistance genes for stemphylium blight into the Australian lentil breeding program.

Our disease screening protocol has been utilized by the Australian lentil breeding program to develop stemphylium blight resistant varieties. Also the elimination of highly susceptible lines prior to release will help minimise the likelihood of stemphylium blight outbreak in the field. This protocol will allow rapid identification of stemphylium blight resistance in early generation and parental lines. Understanding the disease symptoms within controlled environment trials will help in the identification of the disease in the field. It will also help in

distinguishing stemphylium blight from other necrophytic fungi such as botrytis grey mould and sclerotinia which are also favoured by similar environmental conditions. Surveillance for stemphylium blight is necessary to monitor the distribution and importance of *S. botryosum* in lentil growing regions. Further epidemiology studies are needed to fully understand the ongoing risks associated with this disease in Australia.

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