Identification of Sources of *Sclerotinia sclerotiorum* Resistance in a Collection of Wild *Cicer* Germplasm

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Abstract

Sclerotinia sclerotiorum is an important fungal pathogen of chickpea (*Cicer arietinum* L.), and it can cause yield losses up to 100%. The wild progenitors are much more diverse than domesticated chickpea, and this study describes how this relates to *S. sclerotiorum* resistance. Initially, the pathogenicity of nine Australian *S. sclerotiorum* isolates was examined on three *Cicer* lines to develop a robust phenotyping assay, and significant differences in isolate aggressiveness were identified with six isolates being classed as highly aggressive and three as moderately aggressive. We identified two *S. sclerotiorum* isolates, CU8.20 and CU10.12, to be highly aggressive and moderately aggressive, respectively. A subsequent phenotyping assay was conducted using the two isolates to evaluate 86 wild *Cicer* accessions (*Cicer reticulatum* and *Cicer echinospermum*) and two *C. arietinum* varieties for resistance to *S. sclerotiorum*. A subset of 12 genotypes was further evaluated, and subsequently, two wild *Cicer* accessions with consistently

high levels of resistance to *S. sclerotiorum* were examined using the initially characterized nine isolates. Wild *Cicer* accessions Karab_084 and Deste_063 demonstrated consistent partial resistance to *S. sclerotiorum*. There were significant differences in responses to *S. sclerotiorum* across wild *Cicer* collection sites. The Cermik, Karabahce, and Destek sites' responses to the aggressive isolate CU8.20 ranged from resistant to susceptible, highlighting an interaction between isolate genotype and chickpea collection site for sclerotinia stem rot resistance. This is the first evidence of partial stem resistance identified in wild *Cicer* germplasm, which can be adopted in chickpea breeding programs to enhance *S. sclerotiorum* resistance in future chickpea varieties.

Keywords: *Cicer echinospermum*, *Cicer reticulatum*, crop wild relatives, disease resistance screening, *Sclerotinia sclerotiorum*, sclerotinia stem rot, wild chickpea

Chickpea is particularly important as a disease break crop in cereal production systems and for its ability to form a beneficial relationship with nitrogen-fixing Rhizobia bacteria (Siddique et al. 2013). Nutritionally, chickpea is high in protein, dietary fiber, and essential minerals and therefore, plays a critical role in the fight to reduce hunger and malnutrition in many developing countries (Jukanti et al. 2012; Xing et al. 2020).

Global chickpea production is threatened by various pathogens including *Ascochyta blight*, *Fusarium oxysporum* f. sp. *ciceri*, *Phytophthora medicaginis*, *Pratylenchus thornei*, *Botrytis cinerea*, and *Sclerotinia sclerotiorum*, causal agents of Ascochyta blight, Fusarium wilt, Phytophthora root rot, root lesion nematode disease, Botrytis gray mold, and Sclerotinia stem rot (SSR), respectively (Kukreja et al.

2018). Among other factors, the narrow genetic base of cultivated varieties makes chickpea very susceptible and vulnerable to these pathogens, as novel sources of resistance are difficult to identify in the domesticated chickpea gene pool (Thudi et al. 2016). While great efforts have been made to incorporate wild sources of resistance to *A. rabiei* (Collard et al. 2003a, b), *P. thornei* (Reen et al. 2019), and *P. medicaginis* (Knights et al. 2008) into chickpea varieties, little research efforts have been employed to improve resistance to *S. sclerotiorum*. In Australia, SSR can cause up to 100% yield loss in susceptible chickpea cultivars under favorable conditions (Fuhlbohmer et al. 2003; Pulse Australia 2020).

The phytopathogen *S. sclerotiorum* is an economically important necrotrophic fungus with a remarkably broad host range of >400 species, which includes wild plants, cereal, oilseed, and legume crops (Boland and Hall 1994). *S. sclerotiorum* is an important pathogen in Canada (Bardin and Huang 2001), America (Chen et al. 2006), India (Haware 1990), and Australia (Fuhlbohmer et al. 2003). Previous studies in Australia have demonstrated that the *S. sclerotiorum* population is diverse and unpredictable, and their responses depend on existing environmental conditions (Michael et al. 2020). In chickpea, SSR infects directly from sclerotia in the soil, where the mycelial hat penetrates host tissue through myceliogenic germination, causing stem whitening, wilting, and stem breakage (Chen et al. 2006). In Australia, incidences of the disease in legumes are increasing because of the large area of canola grown, resulting in inoculum (sclerotia) buildup in the soil (Brooks et al. 2018). Although previous reports have shown partial resistance to *S. sclerotiorum* in some members of the Leguminosae family (Kull et al. 2003; Leite et al. 2016; Webster et al. 2020), there is no published evidence of *S. sclerotiorum* resistance in chickpea.

Previous research has demonstrated *S. sclerotiorum* hosts such as model plants *Arabidopsis thaliana*, *Brassica napus*, and *Glycine max* manifest SSR symptoms ranging from high susceptibility to partial

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resistance to the pathogen, an indication of quantitative resistance responses (Chen and Wang 2005; Denton-Giles et al. 2018; Kim et al. 1999; Perchepped et al. 2010). Previous studies have reported mapped quantitative trait loci for resistance to *S. sclerotiorum* in legumes such as peanut (Liang et al. 2020), pea (Ashtari Mahini et al. 2020), common bean (Porto et al. 2019), and soybean (Boudhrioua et al. 2020).

Research has demonstrated that CWRs have the genetic potential to improve *S. sclerotiorum* resistance in other host crops. CWRs have been shown to harbor sources of resistance in other *S. sclerotiorum* hosts such as canola (Taylor et al. 2015) and sunflower (Qi et al. 2016). Previous studies have shown wild progenitors were used to improve the breeding of crops such as oat, rice, and tomatoes using their wild species *Avena sterilis*, *Oryza rufipogon*, and *Lycopersicon pimpinellifolium*, respectively (Barrantes et al. 2016; Ociepa 2019; Shakiba and Eizenga 2014). Therefore, understanding the disease resistance potential of CWRs is critical in sustaining global food security. Currently, there is no strong resistance to *S. sclerotiorum* in chickpea. Therefore, to improve the cultivated chickpea varieties, research that aims at the reintroduction of genes from the wild relatives is important (Abbo et al. 2003).

Currently, management of SSR in agricultural crops relies on timely, expensive fungicide applications. Therefore, the development of SSR-resistant genotypes is desirable. von Wettberg et al. (2018) used genotyping-by-sequencing (GBS) data to demonstrate that approximately 95% of the genetic diversity in the wild chickpea ancestor *C. reticulatum* has been lost in domesticated chickpea. Previous studies have demonstrated that wild *Cicer* species are a source of novel alleles for resistance to diverse chickpea diseases (Abbo et al. 2003; Muñoz et al. 2017; Reen et al. 2019).

Although crosses of other wild *Cicer* species have shown low efficacy, the annual species, *C. reticulatum* and *C. echinospermum*, are the only crop wild relatives (CWRs) that are readily cross-compatible with domesticated chickpea (Croser et al. 2003; Reen et al. 2019; von Wettberg et al. 2018). A collection mission to southeastern Turkey significantly increased the global collection of *C. reticulatum* and *C. echinospermum* accessions. This mission was particularly important, as these wild accessions are currently threatened by increasing urbanization and climate change (von Wettberg et al. 2018). Further, the authors showed that *C. reticulatum* and *C. echinospermum* each have intrinsic genetic differences and classified them into eight and four genetic population groups, respectively. This genetic diversity was largely related to the location of their collection sites. *C. reticulatum* accessions were located on sandstone in disturbed mixed pastures, while *C. echinospermum* accessions were found in open pastures in basaltic soils at a lower elevation (Coyne et al. 2020; von Wettberg et al. 2018). This germplasm collection has become of interest in the search for chickpea resistance to biotic and abiotic stresses and is a valuable resource for chickpea cultivar improvement (Coyne et al. 2020). Resistance to Ascochyta blight, pod-borer (*Helicoverpa armigera*) and root-lesion nematode have already been identified in this collection (Newman et al. 2021; Reen et al. 2019; von Wettberg et al. 2018).

Studies have demonstrated that crossing of chickpea using the wild annual *Cicer* spp. can improve the genetic base of cultivated varieties without compromising their yield or adaptation (Koseoglu et al. 2017; Singh and Ocampo 1997). Various crossing programs to elite cultivars have been successfully employed to introduce wild *Cicer* (*Cicer reticulatum* and *Cicer echinospermum*) sources of resistance to Ascochyta blight (Collard et al. 2001), Botrytis gray mold (Li et al. 2015), and Phytophthora root rot (Knights et al. 2008; Miranda 2019). These studies indicate that the wild collection harbor useful genes for diverse traits of benefit to the chickpea industry. An assay previously developed by Akem and Kabbabeh (1999) to screen chickpea cultivars in Pakistan for *S. sclerotiorum* resistance under controlled conditions did not identify any substantial resistance. Most Australian chickpea varieties are susceptible to *S. sclerotiorum* (Mwape et al., unpublished observations), and there is a lack of fungicides registered or under permit in Australia for *S. sclerotiorum* control (Pulse Australia 2020). Consequently, the chickpea industry is seeking solutions to this problem, thus evaluating the wild collection for resistance to *S. sclerotiorum* is of great interest.

To identify novel sources of resistance to *S. sclerotiorum* in wild chickpea germplasm, we adopted a stem inoculation strategy previously used to screen *B. napus* germplasm (Denton-Giles et al. 2018).

We used this assay to initially assess differences in the response of a representative sample of each *Cicer* spp. (*C. arietinum*, *C. echinospermum*, and *C. reticulatum*). The protocol was subsequently used to assess differences in aggressiveness of nine *S. sclerotiorum* isolates on the three *Cicer* spp. Subsequently, we used one highly aggressive and one moderately aggressive *S. sclerotiorum* isolate to characterize the resistance response of 19 *C. echinospermum* and 68 *C. reticulatum* wild accessions, in comparison with two popular Australian cultivars (PBA HatTrick XT and Kyabra) under greenhouse conditions. Correlations between wild chickpea geographical location origin and the level of *S. sclerotiorum* infection was determined. Additionally, *Cicer* spp. with partial stem resistance were identified and evaluated with multiple *S. sclerotiorum* isolates. Here, Karab_084 and Deste_063 showed consistently high levels of partial stem resistance relative to the most resistant chickpea control cultivar. This study is the first evidence of partial stem resistance in wild *Cicer* germplasm, which can be adopted in chickpea breeding programs to enhance SSR resistance in future chickpea varieties.

Materials and Methods

Sources of *S. sclerotiorum* isolates. This study aimed to examine the pathogenicity of nine *S. sclerotiorum* isolates, which are part of a world collection genotyped by Clarkson et al. (2017) and previously used to determine differences in the levels of aggressiveness between the isolates in *B. napus* (Denton-Giles et al. 2018). The *S. sclerotiorum* isolates were collected from *B. napus* (six isolates) and *Lupinus albus* (three isolates) from different Western Australia sites between 2013 and 2014 (Table 1). *S. sclerotiorum* is a broad host range pathogen, and isolates were selected as they belong to different intergenic spacer (IGS) and mycelial compatibility groups (MCGs) (Table 1).

Mature sclerotia were obtained from the Centre for Crop and Disease Management (CCDM), Curtin University, Western Australia. To produce inoculum from an actively growing culture, single sterile sclerotia were dissected and placed in a 9-mm Petri dish containing 39 g/liter of potato dextrose agar (PDA; Becton Dickinson) and incubated at 20°C for 5 to 7 days in the dark. The cultures were subcultured by using a sterile cork borer and forceps to transfer 5-mm agar plugs from the original plates onto fresh PDA plates, which were incubated for 2 days at 20°C to source actively growing mycelia for plant inoculation. Plants were inoculated with 5-mm mycelial plugs taken from the leading mycelial edge of the growing *S. sclerotiorum* culture.

Assessing *S. sclerotiorum* pathogenicity. The pathogenicity of nine *S. sclerotiorum* isolates (Table 1) was evaluated on *C. echinospermum* and *C. reticulatum* accessions S2Drd_065 and Besev_079, respectively, along with a domestic desi cultivar (PBA HatTrick). The *Cicer* accessions S2Drd_065 and Besev_075 were selected because the Chickpea Innovation Lab (University of California, Davis, CA) is generating high-quality genome sequences for these accessions as representatives for *C. echinospermum* and *C. reticulatum*, respectively. We chose PBA HatTrick as it is a popular current Australian *C. arietinum* cultivar. In all the experiments, the wild *Cicer* seeds were cut at the endosperm end with a scalpel blade to facilitate imbibition for germination. Seeds were sown into 4-liter pots with an all-purpose potting mix (UWA mix, Richgro, Perth, Australia). Plants from each accession/cultivar were mock-inoculated with PDA-only agar plugs, which served as the negative control that should not produce a stem lesion. At 4 weeks, plants were fertilized with 2 g of Nitrophoska perfert fertilizer (Incitec Pivot fertilizers, Victoria, Australia). Stem inoculation was conducted on 8-week-old plants following a stem inoculation assay previously described in *B. napus* by Denton-Giles et al. (2018). A 5-mm PDA plug with *S. sclerotiorum* mycelium from the leading edge of the culture was cut using a sterile cork borer, and a sterile metal spatula was used to transfer the plug onto Parafilm. The plug was placed upright on a strip of Parafilm and wrapped around the main stem. Measurements of stem lesion length were taken at 2, 4, 6, 8, 10, and 12 days post inoculation (dpi) to calculate the area under the disease progress curve (AUDPC) (Jeger and Viljanen-Rollinson 2001).

Screening wild *Cicer* accessions for *S. sclerotiorum* resistance. A set of 86 wild *Cicer* accessions (67 *C. reticulatum* and 19 *C. echinospermum*) and two domestic desi varieties (PBA HatTrick and Kyabra) (Table 2) were screened for *S. sclerotiorum* resistance using the

“highly aggressive” isolate CU8.20 and the “moderately aggressive” isolate CU10.12. Inoculation with the moderately aggressive isolate (CU10.12) was included in case all *Cicer* accessions were found to be equally susceptible to the highly aggressive (CU8.20) isolate. Seeds for the cultivars PBA HatTrick and Kyabra, as well as seeds of the wild accessions, were obtained from Australian Grains Gene Bank (AGG; Horsham, Victoria, Australia). The 86 wild accessions are part of a wild *Cicer* collection described and characterized by von Wettberg et al. (2018).

Validation of the partial stem resistance of wild *Cicer* accessions over time to *S. sclerotiorum*. Based on the stem assay results of 86 wild *Cicer* accessions, 11 wild accessions were further evaluated using the same highly aggressive isolate (CU8.20) to demonstrate reproducibility. The selected wild *Cicer* accessions demonstrated a range of stem lesion lengths in response to both isolates used in the previous experiment and included a reference *C. arietinum* cultivar, PBA HatTrick. Inoculation, data collection, and AUDPC calculation were carried out following the same procedure as previous experiments.

Determination of aggressiveness levels of nine *S. sclerotiorum* isolates on accessions Karab_084 and Deste_063. In a subsequent investigation, the aggressiveness of nine isolates characterized above

was reassessed on the two wild *Cicer* accessions, Karab_084 and Deste_063, identified to have the greatest resistance. Inoculation, data collection, and AUDPC calculation were carried out following the same procedure as previous experiments.

Experimental design and statistical analysis. *Experimental design.* To assess the susceptibility of chickpea accessions, the aggressiveness of *S. sclerotiorum* isolates and their interactions, randomized complete block designs (RCBDs) were applied for the first three hoophouse experiments, and a split-plot design was applied for the fourth glasshouse experiment. Negative controls with PDA-only agar plugs were included for all experiments. All experimental designs were generated using the experimental design tool DiGger in R (Coombes 2018).

The first experiment for assessing *S. sclerotiorum* pathogenicity was designed to be an RCBD with cultivar and isolate as treatments in 12 replicates to compare three chickpea cultivars (Besev_079, PBA HatTrick, S2Drd_065) and nine isolates (CU4.2, CU6.1, CU8.20, CU8.24, CU10.12, CU10.17, CU11.4, CU11.7, CU11.19).

The second experiment for screening wild *Cicer* accessions for *S. sclerotiorum* resistance was designed to be an RCBD with cultivar and isolate as treatments in four replicates to compare 86 wild *Cicer*

Table 1. Australian *Sclerotinia sclerotiorum* isolates used for pathogenicity tests

Isolate	MCG ^a	IGS ^b	Genome sequenced	Original host	Collection location	Year collected
CU4.2	I	7	Yes	<i>Brassica napus</i>	Mount Barker, WA	2014
CU6.1	II	7	Yes	<i>Brassica napus</i>	Mount Barker, WA	2014
CU8.20	III	5	Yes	<i>Brassica napus</i>	South Stirling, WA	2014
CU8.24	IV	3	Yes	<i>Brassica napus</i>	South Stirling, WA	2014
CU10.12	VI	3	Yes	<i>Brassica napus</i>	Geraldton, WA	2014
CU10.17	VII	5	Yes	<i>Brassica napus</i>	Geraldton, WA	2014
CU11.4	IX	7	No	<i>Lupinus albus</i>	Eneabba, WA	2014
CU11.7	X	5	Yes	<i>Lupinus albus</i>	Eneabba, WA	2013
CU11.19	XI	5	Yes	<i>Lupinus albus</i>	Geraldton, WA	2014

^a MCG = mycelial compatibility group from Denton-Giles et al. (2018).

^b IGS = intergenic spacer region haplotype from Clarkson et al. (2017).

Table 2. Wild chickpea accessions used in this study, classified according to provinces, collection site, species, genetic group, names, and code number based on von Wettberg et al. (2018) for material collected in 2013/2014 from southeastern Turkey

Province	Collection site	Species ^a	N ^b	Prefix ^c	Suffix ^d and accession code no.
Adiyaman	Oyali	<i>C. ret</i>	4	Oyali	081, 084, 101, 105
Mardin	Baristepe 1	<i>C. ret</i>	3	Bari1	064, 068, 092
	Baristepe 2	<i>C. ret</i>	5	Bari2	062, 064, 067, 072, 074
	Baristepe 3	<i>C. ret</i>	4	Bari3	072, 091, 100, 106D
	Besevler	<i>C. ret</i>	8	Besev	061, 062, 065, 066, 074, 075, 079, 083
	Dereici	<i>C. ret</i>	8	Deric	062, 065, 066, 069, 070, 072, 074, 075,
	Kayatepe	<i>C. ret</i>	7	Kayat	061, 063, 064, 066, 070, 077, 080
	Sarikaya	<i>C. ret</i>	9	Sarik	061, 064, 065, 066, 067, 073, 074, 077, 080
	Savur	<i>C. ret</i>	1	Savur	063
Diyarbakir	Cermik	<i>C. ech</i>	3	Cermi	072, 073, 075
	Kesentas	<i>C. ret</i>	3	Kesen	062, 065, 075
	Egil	<i>C. ret</i>	3	Egill	065, 066, 073
	Kalkan	<i>C. ret</i>	4	Kalka	061, 064, 066, 074
	Gunasan	<i>C. ech</i>	2	Guna	062, 100
Urfa	Destek	<i>C. ech</i>	3	Deste	063, 071, 075
	Karabahce	<i>C. ech</i>	4	Karab	067, 084, 085C, 092
	Ortanca	<i>C. ech</i>	2	Ortan	061, 066
	Siv-Diyar	<i>C. ech</i>	5	S2Drd	062, 065, 100, 101, 102
Sirnak	CudiA	<i>C. ret</i>	4	CudiA	122, 124, 127, 152
	CudiB	<i>C. ret</i>	4	CudiB	009, 016, 018, 022C
	Sirnak	<i>C. ech</i>	1	Sirna	60

^a *C. ret* = *Cicer reticulatum*; *C. ech* = *Cicer echinospermum*.

^b N = number of accessions.

^c Prefix = code used before the suffix number as the name of each accession.

^d Suffix indicates the individual accession number from a specific location (prefix name).

accessions (for details see Supplementary Table S2) along with domestic desi cultivars (PBA HatTrick and Kyabra) and two isolates (CU10.12, CU8.20).

The third experiment for validating the partial stem resistance of wild *Cicer* accessions was designed to be an RCBD with cultivar as treatments in 12 replicates to compare the susceptibility of the 11 wild *Cicer* accessions (Deste_063, CudiA_122, Oyali_105, Sarik_065, Oyali_084, Egill_065, Karab_084, Besev_074, Besev_079, Deric_062, S2Drd_065) along with domestic desi cultivar PBA HatTrick using the highly aggressive isolate CU8.20.

The fourth experiment for determining aggressiveness levels of nine *S. sclerotiorum* isolates on accessions Karab_084 and Deste_063 was designed to be a split-plot design with cultivar as main-plot and isolate as subplot in four replicates to compare two wild *Cicer* accessions (Karab_084, Deste_0630) and nine isolates (CU4.2, CU6.1, CU8.20, CU8.24, CU10.12, CU10.17, CU11.4, CU11.7, CU11.19).

All experiments were conducted between January 2018 and June 2019 in the Curtin University field trial area of the Bentley campus. Both hoophouse and glasshouse experiments were conducted under natural light and an average temperature of $\sim 24^{\circ}\text{C}$.

Statistical analysis. Linear mixed models (LMMs) were fitted using ASReml-R (Butler et al. 2018) to examine the spatial variations including local autocorrelations, global trends, and extraneous variations and produce predicted values. As the main objective of the experiments is to determine the difference between specific pairs of treatments, the cultivar, isolate, and the interaction term between cultivar and isolate were fitted as fixed effects (Smith et al. 2005). The blocking structures of the experiments were fitted as random effects. Spatial trends and residual variances with autoregressive correlations at first-order for rows, and columns were examined and fitted when the global trends and autocorrelations are significant. Likelihood ratio tests were used for random effects, and conditional Wald tests (Kenward and Roger 1997) were used for fixed effects. Residual diagnostics were performed to examine the validity of the model assumption of normality and homogeneity of variance. For each of the fitted models, the empirical best unbiased linear estimates (eBLUEs) were produced. The R package asremlPlus (Brien 2021) was used to compute the least significant difference (LSD with $\alpha = 0.05$) values.

The *Cicer* screening data (Exp 2) were also analyzed by factorial nested analysis of variance (ANOVA) in Genstat (V20) to partition variance within and between species, collection sites, and accessions and to identify interaction with isolate type. Orthogonal contrasts were used to define differences between wild and domestic *Cicer* and between the two wild species (*C. echinospermum* and *C. reticulatum*). Block effects were fitted to take advantage of the RCBD, residual plots prepared to identify outliers and to confirm that the ANOVA expectations of random and independent error distribution was met.

Results

A robust *S. sclerotiorum* phenotyping assay shows significant differences in aggressiveness across nine genetically distinct *S. sclerotiorum* isolates on three different *Cicer* species. A robust phenotyping assay was developed, which measures the lesion length over time following inoculation of a chickpea stem with an agar plug containing *S. sclerotiorum*. This allowed us to calculate the AUDPC to investigate the response of *Cicer* germplasm to *S. sclerotiorum* isolates, which showed differences in the aggressiveness of *S. sclerotiorum* isolates on chickpea.

Inoculation of the three representatives of *C. reticulatum* (Besev_079), *C. echinospermum* (S2Drd_065), and *C. arietinum* (PBA HatTrick) with nine *S. sclerotiorum* isolates resulted in a consistent infection in all three genotypes (Fig. 1). The interaction between genotype and isolate showed significant differences ($P < 0.05$, to $\text{LSD}_{0.05} = 803.6$) with Besev_079 having higher resistance (lower AUDPC scores) to all the isolates except CU11.19 and CU10.12 compared with PBA HatTrick and S2Drd_065 (Fig. 1A; Supplementary Table S1). There were significant differences ($P < 0.05$, $\text{LSD}_{0.05} = 464$) between the aggressiveness of the nine *S. sclerotiorum* isolates (Fig. 1B). Overall, isolates CU8.20, CU10.17, CU4.2, CU8.24, CU11.7, and CU6.1 caused significantly ($P \leq 0.05$, $\text{LSD}_{0.05} = 464$) higher AUDPC values (1,382 to 2,875) than isolates CU10.12, CU11.4 and CU11.19 (723 to 1,327) (Fig. 1B;

Supplementary Table S1). Therefore, isolates CU10.12, CU11.4, and CU11.19 were placed into the moderately aggressive isolate group and isolates CU8.24, CU4.2, CU10.17, CU 6.1, CU 11.7, and CU8.20 in the highly aggressive isolate group. The genotypes exhibited differences ($P < 0.05$, $\text{LSD}_{0.05} = 267.9$) with Besev_079 showing significantly higher resistance than PBA HatTrick and S2Drd_065 (Fig. 1C). From this initial test, we selected a highly aggressive isolate (CU8.20) and a moderately aggressive isolate (CU10.12) for subsequent screening for *S. sclerotiorum* resistance in a collection of wild *Cicer* germplasm.

Wild *Cicer* accessions exhibit a wide range of responses to *S. sclerotiorum* inoculation. To investigate resistance levels of 86 wild *Cicer* accessions, and two Australian cultivars to *S. sclerotiorum*, the AUDPC value was determined after inoculation with the highly aggressive (CU8.20) or the moderately aggressive (CU10.12) isolate. We used a factorial nested ANOVA approach to partition variance within and between species, collection sites, and accessions and to identify interaction with isolate type, and orthogonal contrasts to define differences between wild and domestic *Cicer* and between the two wild species (Supplementary Table S2).

Orthogonal contrasts revealed large differences between wild and domestic responses and isolate type ($P < 0.006$) (Supplementary Table S2). *C. echinospermum* and *C. reticulatum* were both more resistant to CU10.12 than domestic chickpea ($P < 0.001$), whereas there were no wild by domestic differences for the more aggressive CU8.20 ($P_{\text{diff}} = 0.735$). Within these species, there was a broad range of responses to both isolates which are examined individually (Fig. 2; Supplementary Table S3) and in terms of interaction (see Fig. 3). AUDPC scores of individual accessions ranged from 67.5 to 897.5 for highly aggressive CU8.20 (Fig. 2A) and 35.0 to 615.0 for moderately aggressive isolate CU10.12 (Fig. 2B). The cultivar PBA HatTrick had lower AUDPC scores to both isolates compared with cultivar Kyabra, with the difference to isolate CU10.12 being significant (Fig. 2). Several wild *Cicer* accessions displayed low mean AUDPC scores to both isolates (*C. echinospermum* accession Cerri_073 and *C. reticulatum* accessions Sarik_073, CudiB_018 and Egill_073). In contrast, accessions Deric_062, Besev_074, Deste_063, and S2Drd_065 showed high mean AUDPC scores and were classed as susceptible to both isolates. The conditional Wald statistic test of the fixed effects from the fitted model provided strong evidence to suggest that the main effects of accession and isolate are statistically significant ($P < 0.001$), and the interactive effect between genotype and isolate was statistically significant ($P = 0.0099$).

To visualize this interaction between accessions and isolates directly, we plotted mean AUDPC scores of the aggressive (CU8.20) against the moderately aggressive (CU10.12) and included a 1:1 line for reference (Fig. 3). (Accessions within 1 LSD of the 1:1 line showed no significant isolate interaction; those significantly larger or smaller favored one isolate over the other.) Thus, in domestic chickpea, Kyabra shows no interaction, being consistently sensitive to both isolates while PBA HatTrick was resistant to CU10.12 and moderately resistant to CU8.20. By plotting species separately and identifying collection site membership, it becomes possible to visualize the variance partitioning identified by the nested ANOVA model (Supplementary Table S2).

The greater aggression of CU8.20 over CU10.12 is indicated by higher AUDPC scores (i.e., significantly above the 1:1 line) in most accessions of both wild species (Fig. 3). Most *C. echinospermum* accessions showed resistance to the moderately aggressive isolate and a wide range of responses to the highly aggressive isolate (Fig. 3A). Exceptions include Ortanca accessions, which are moderately resistant to both isolates, and Destek 071, which was resistant to the aggressive CU 8.20 but sensitive to the less aggressive CU10.12. *C. echinospermum* collection sites were mostly similarly resistant to CU10.12 (except for Ortanca and Destek, mentioned above) but did not discriminate well for responses to the more aggressive CU8.20. Cermik, Karabahce, and particularly Destek responses to CU8.20 ranged from susceptible to resistant (Fig. 3A), highlighting the significant variance within collection sites. Only the Gunasan, Ortanca, and to a lesser extent Siv-Diyar accessions responded in a uniform way.

C. reticulatum showed a wider range of responses to both isolates (Fig. 3B). Accessions within one LSD of 1:1 line were similarly

Fig. 1. Aggressiveness of nine *Sclerotinia sclerotiorum* isolates on *Cicer reticulatum* (Besev_079), *Cicer echinospermum* (S2Drd_065), and *Cicer arietinum* (PBA HatTrick) based on a hoophouse experiment with 12 replicates per *Cicer* species for each isolate: **A**, mean area under the disease progress curve (AUDPC) for each isolate by genotype interaction; **B**, mean AUDPC for each isolate on all three *Cicer* species; and **C**, mean AUDPC for three chickpea genotypes across all *S. sclerotiorum* isolates. The vertical bars represent the least significant differences (LSD with $\alpha = 0.05$). The control indicates the lesion length of mock-inoculated plants with a PDA-agar plug without *S. sclerotiorum* mycelium. Different letters above bars represent significant differences between treatments at significance level of 0.05.

responsive to both isolates, ranging from consistently resistant (Sarik_073, Deric_075, CudiB_018) to consistently moderately susceptible (Bari3_106D, CudiA_127, Bari2_062). Cudi B was the only collection site returning consistently tolerant accessions to both isolates (but was represented by only two accessions in this study). Most *C. reticulatum* accessions had strong isolate response interactions: typically resistant to CU10.12 but highly or moderately susceptible to CU8.20 (Fig. 3B). However, this behavior was not well defined by collection site: most *C. reticulatum* collection sites show a wide range of accession responses to CU8.20 (e.g., Kayatepe, Oyali). Only the Kesentas accessions showed consistent isolate responses: resistant to CU10.12, moderately resistant to CU8.20. Other collection sites showed the opposite response: stable for CU8.20, but variable for CU10.12. Thus, Baristepe 1, Cudi A, and Egil accessions were all moderately susceptible to the highly aggressive CU8.20 but varied in response to CU10.12 (Fig. 3B). Finally, some collection sites such as Baristepe 2, Sarikaya, and Kalkan returned widely different accession responses to both isolates.

Two *Cicer echinospermum* accessions exhibit robust resistance to *S. sclerotiorum*. To further evaluate the wild material, 11 *Cicer* accessions with diverse responses to the two isolates in the previous experiment were selected, including the moderately resistant *C. arietinum* cultivar PBA HatTrick as a control. ANOVA for AUDPC of a subset of the wild *Cicer* accessions showed Karab_084 and Deste_063 resistance responses to *S. sclerotiorum* to be significantly different at 12 dpi ($P < 0.05$) from nine other accessions (Fig. 4; Supplementary Table S4), while the mean AUDPC scores were significantly lower for Karab_084 and Deste_063 compared with Egill_065 and Deric_062 (LSD_{0.05} = 226.6) (Fig. 4).

Based on AUDPC, *C. echinospermum* accession Karab_084 exhibited strong resistance to *S. sclerotiorum* as compared with accessions Besev_074, Oyali_084, Besev_079, S2Drd_065, Egill_065, and Deric_062 (Fig. 4). The two accessions Karab_084 and Deste_063 were screened for resistance to the previously characterized nine *S. sclerotiorum* isolates (Fig. 5). The isolate CU8.20 and CU10.12 were found to be significantly ($P < 0.05$) different in aggressiveness to both Karab_084 (LSD_{0.05} = 537.6) (Fig. 5A) and Deste_063 (LSD_{0.05} = 797.9) (Fig. 5B), confirming our previous findings.

Discussion

This is the first study utilizing the collection of wild *Cicer* species (von Wettberg et al. 2018) for the evaluation of Sclerotinia stem rot resistance. In the collection, we identified accessions with higher levels of partial resistance to *S. sclerotiorum*, including *C. reticulatum* and *C. echinospermum* when compared with the cultivated *C. arietinum* varieties PBA HatTrick and Kyabra. Two findings in our present study have important implications for future investigation of SSR on chickpea. First, a robust stem inoculation assay showed the diverse pathogenicity levels of Western Australian *S. sclerotiorum* isolates on chickpea. Second, wild *Cicer* accessions with improved resistance compared with moderately resistant domesticated cultivars were identified.

The status of resistance to *S. sclerotiorum* has been reported after screening various legume host crops. A study by Vuong et al. (2004) reported significant differences in *S. sclerotiorum* resistance in soybean (*G. max*), and dry bean (*Phaseolus vulgaris*) cultivars, which were screened following a cut stem inoculation method. To improve *S.*

Fig. 2. The area under the disease progress curve (AUDPC) for 86 wild *Cicer* accessions and two domestic cultivars inoculated with *Sclerotinia sclerotiorum* isolate based on a hoophouse experiment. **A**, CU8.20 representing a highly aggressive isolate (LSD_{0.05} = 383.2). **B**, CU10.12 representing a moderately aggressive isolate (LSD_{0.05} = 307.3). The mean AUDPC scores are derived from four replicates per accession per isolate, where accessions shaded black represent *Cicer reticulatum*, gray represent *Cicer echinospermum*, and white represent *Cicer arietinum* species. The accessions highlighted with a hash (#) for both CU8.20 and CU10.12 indicate the genotypes selected for rescreening. The vertical bar represents the least significant difference (LSD with $\alpha = 0.05$) value across all accessions.

Fig. 3. Interaction between accessions and isolates represented by plotting AUDPC accession mean values of CU8.20 (highly aggressive) against CU10.12 (moderately aggressive) *Sclerotinia sclerotiorum* isolates. **A**, *Cicer echinospermum* (▲) and **B**, *Cicer reticulatum* (●) are plotted separately and collection sites identified by unique markers to assist in visualizing the variance partitioning among isolate interactions within/between species, collection sites within species, and accessions indicated by nested ANOVA. *Cicer arietinum* (*) is included on both **A** and **B** as domestic chickpea controls. Vertical and horizontal error bars represent least significant difference (LSD with $\alpha = 0.05$) values ($P < 0.05$) for CU8.20 and CU10.12, respectively. A 1:1 line (—) is included for reference to help identify significant interactions among accessions. (Accessions within 1 LSD of the 1:1 line show no significant isolate interaction; those significantly larger or smaller favor one isolate over the other.)

sclerotiorum management in pea (*Pisum sativum*), screening of a core collection for resistance to *S. sclerotiorum* under different controlled conditions identified two partially resistant accessions that could be used in pea breeding programs (Porter 2012). These reports indicate that partial resistance is an important attribute in breeding for legumes with SSR resistance and can be a vital resource in optimizing breeding strategies to increase production.

Researchers have developed several methods of screening for SSR resistance on different hosts (Denton-Giles et al. 2018; Hoffman et al. 2002; Porter 2012). *S. sclerotiorum* inoculation methods can use either whole plant or different parts of the plant and inoculation with the fungus; however, the stem inoculation method has been more reliable (Bennett et al. 2016; Denton-Giles et al. 2018; Li et al. 2009). *S. sclerotiorum* naturally infects its host and limits the flow of water and nutrients through the xylem (Seifbarghi et al. 2017). Therefore, the stem inoculation method used in this study simulated the infection strategy naturally employed by *S. sclerotiorum* in the field (Denton-Giles et al. 2018; Garg et al. 2010; Uloth et al. 2013). In the current study, inoculation was conducted on the main stem just before chickpea flowering (Fuhlbohmer et al. 2003). For these reasons, introgression of the identified resistance into chickpea cultivars would likely reduce natural *S. sclerotiorum* infection in the field.

The aggressiveness of a pathogen is its relative ability to attack, colonize, and cause damage, and partial resistance of a host to a pathogen can result in a significant reduction of disease rather than a total absence of the disease (Deacon 1997; Denton-Giles et al. 2018). Previous research has reported partial resistance and characterization of *S. sclerotiorum* aggressiveness levels in different hosts including canola (*B. napus*) (Denton-Giles et al. 2018), sunflower (*Helianthus annuus* L.) (Seiler et al. 2017; Vuong et al. 2004), soybean (*G. max*) (Hoffman et al. 2002), pea (*P. sativum* L.) (Porter 2012), and dry beans

(*P. vulgaris*) (Miorini et al. 2019). A study by Miorini et al. (2019) showed that *S. sclerotiorum* pathotypes could influence the outcome of host resistance assays. Therefore, knowledge of the aggressiveness of *S. sclerotiorum* isolates on chickpea is an important resource for any future chickpea–*S. sclerotiorum* infection study. The distinction in the aggressiveness of *S. sclerotiorum* isolates in different plant species was previously shown to be because of physiological specialization of the pathogen (Garg et al. 2010). For instance, two separate studies on *B. napus* (Denton-Giles et al. 2018; Taylor et al. 2015) and one study on dry beans (Viteri et al. 2015) found *S. sclerotiorum* isolates to have different levels of pathogenicity on these host crops.

The nine Australian isolates used in this study showed different levels of aggressiveness in chickpea similar to previous isolate aggressiveness classifications found by Denton-Giles et al. (2018). However, *S. sclerotiorum* isolate CU11.19 was found to be moderately aggressive in our study and highly aggressive in *B. napus* Denton-Giles et al. (2018). This suggests some degree of isolate-host specialization, which is reported as a possible *S. sclerotiorum* characteristic (Liang and Rollins 2018); however, further work is needed with a greater range of chickpea genotypes to test this hypothesis. Aggressiveness variations of *S. sclerotiorum* when infecting different hosts have been reported in other regions of the world. For instance, isolates collected from different plant species in the United Kingdom exhibited a different range of aggressiveness when inoculated onto *B. napus* (Taylor et al. 2015). In the current study, differential levels of pathogenicity of nine Australian isolates on chickpea was demonstrated, and the results enabled selection of two isolates, moderately and highly aggressive, for screening a collection of wild *Cicer* germplasm.

In a range of crops, adoption of partial resistance to *S. sclerotiorum* is an alternative to improve susceptible cultivars especially in crop species with low genetic diversity and high disease pressure (Denton-Giles

Fig. 4. Area under disease progress curve (AUDPC) for 11 selected wild *Cicer* accessions and one *Cicer arietinum* cultivar (PBA HatTrick) inoculated with the highly aggressive *Sclerotinia sclerotiorum* isolate CU8.20 based on a hoophouse experiment. The mean AUDPC values are derived from 12 replicates per accession/cultivar. The vertical bar represents the least significant difference (LSD with $\alpha = 0.05$) value across all accessions. Different letters above bars of each genotype represent significant differences at significance level of 0.05.

et al. 2018; Hoffman et al. 2002; Porter 2012). Wild *Cicer* spp. offer desirable resistance to multiple chickpea diseases compared with *C. arietinum* (Croser et al. 2003). Improved resistance to Ascochyta blight and root-lesion nematodes has been found in *C. echinospermum* (Collard et al. 2003a, b; Newman et al. 2021; Reen et al. 2019). Despite not finding total resistance to *S. sclerotiorum*, our findings demonstrate that wild *Cicer* accessions also possess promising sources of *S. sclerotiorum* resistance, with *C. echinospermum* accession Karab_084 showing a greater level of resistance among the 88 genotypes assessed. Plant breeders look for resistance to specific diseases by screening available germplasm for resistance, but more often than not, complete resistance is not identified (Li et al. 2015). In the current study, a quantitative continuum of responses to SSR was observed among wild *Cicer* accessions. However, it must be recognized that this study represents only the beginning of the characterization of wild *Cicer* as a source of resistance. While the numbers of accessions screened in this study are large in a historical context, far exceeding the world's germplasm resource prior to this collection (Berger et al. 2003), there is much more material that remains to be evaluated in both *C. reticulatum* and *C. echinospermum*. Our wild chickpea germplasm resistance assessment strategy has been employed in previous studies aiming to identify improved resistance to chickpea diseases from wild *Cicer* accessions (Croser et al. 2003; Knights et al. 2008; Newman et al. 2021; Reen et al. 2019). To the authors' knowledge, this is the first report of resistance to *S. sclerotiorum* in wild *Cicer* accessions.

The wild *Cicer* accessions used in the current study were collected from 21 sites with a narrow geographical area and a wide range of physical and environmental properties such as location, altitude, slope, climate, and soil type (von Wettberg et al. 2018). Our data demonstrates

that it is very important to consider collection site when searching for SSR resistance because there has clearly been selection for different disease responses across sites. However, these interactions are complex and resist classification along climatic or even geographic lines. For instance, accessions from the three Baristepe collection sites showed very different responses to *S. sclerotiorum* despite being collected in an apparently homogenous environment within 5 km of each other. *S. sclerotiorum* is a pathogen with a wide range of hosts (Boland and Hall 1994); hence, the disease will vary around the collection sites depending on the presence/absence of other hosts. While collection sites can have big effects, the present study indicates they are very hard to understand and probably reflect complex interactions between geography, climate, and biological history. This suggests that future sampling strategies should try to balance accessions and collection sites to maximize diversity in respect to SSR resistance. Therefore, future collection of wild *Cicer* accessions may need to take collection data into consideration before embarking on screening studies to avoid unbalanced comparisons where collection sites are both underrepresented or overrepresented.

A range of tests for SSR resistance have been recommended for screening germplasm for diseases resistance (Taylor et al. 2015). The results in our study highlight the consistency of found resistance in wild *Cicer* accession, Karab_084, after rescreening and analyzing the rate of stem lesion development (calculated as AUDPC). Furthermore, the levels of aggressiveness of nine isolates initially used to develop the assay were also consistent when reevaluating the two most resistant accessions (Karab_084 and Deste_063) with these isolates. This assessment revealed that there was a lack of genotype by isolate interaction. The analysis of resistance based on *S. sclerotiorum* responses in multiple experiments increased the confidence of identifying the most resistant genotype. Thus, it can be concluded that an agar plug stem inoculation method employed herein can be used to robustly screen chickpea germplasm for partial stem resistance to *S. sclerotiorum* infection and that this aligns with studies in canola and other legumes (Denton-Giles et al. 2018; Webster et al. 2020).

Screening for SSR resistance in the field has been reported to be challenging because of it being highly dependent upon favorable weather conditions (Denton-Giles et al. 2018). We, therefore, recommend further studies on developing reproducible assays for field *S. sclerotiorum* resistance testing on chickpea. These assays will facilitate validation of the identified wild *Cicer* partial stem resistance for durability in the field. It is vital to identify resistance to local aggressive *S. sclerotiorum* isolates; however, it will also be interesting to test the resistance of the identified wild *Cicer* accessions using other national and international isolates. The development of recombinant inbred lines by crossing the partially resistant wild accession with a susceptible domestic cultivar will also enable genetic dissection of the underlying loci conferring resistance to *S. sclerotiorum* in wild *Cicer* species.

In conclusion, we have developed a robust phenotyping assay to screen domestic and wild *Cicer* species for their response to *S. sclerotiorum* infection. Using this approach, wild *Cicer* accessions have been identified that show partial levels of resistance to *S. sclerotiorum*. Introgression of the resistance into modern chickpea cultivars has the potential to improve resistance to this economically damaging disease. Furthermore, isolates with distinct levels of aggressiveness on *Cicer* species were identified, and these results provide further insight into the *S. sclerotiorum*–chickpea interaction and sources of partial resistance to improve future chickpea varieties.

Literature Cited

- Abbo, S., Berger, J., and Turner, N. C. 2003. Viewpoint: evolution of cultivated chickpea: four bottlenecks limit diversity and constrain adaptation. *Funct. Plant Biol.* 30:1081.
- Akem, C., and Kabbabeh, S. 1999. Screening for resistance to sclerotinia stem rot in chickpea: a simple technique. *Pak. J. Biol. Sci.* 2:277-279.
- Ashtari Mahini, R., Kumar, A., Elias, E. M., Fiedler, J. D., Porter, L. D., and McPhee, K. E. 2020. Analysis and identification of QTL for resistance to *Sclerotinia sclerotiorum* in pea (*Pisum sativum* L.). *Front. Genet.* 11:587968.
- Bardin, S. D., and Huang, H. C. 2001. Research on biology and control of Sclerotinia diseases in Canada. *Can. J. Plant Pathol.* 23:88-98.
- Barrantes, W., López-Casado, G., García-Martínez, S., Alonso, A., Rubio, F., Ruiz, J. J., et al. 2016. Exploring new alleles involved in tomato fruit quality in an introgression line library of *Solanum pimpinellifolium*. *Front. Plant Sci.* 7:1172.

Fig. 5. The mean area under the disease progression curve (AUDPC) scores for wild *Cicer* accessions **A**, Karab_084 and **B**, Deste_063 following inoculation with nine *Sclerotinia sclerotiorum* isolates in a glasshouse experiment. The mean AUDPC values are derived from 12 replicates per accession for each isolate. The vertical bar represents the least significant difference (LSD with $\alpha = 0.05$) value across all isolates. Different letters above bars of each isolate represent significant differences at significance level of 0.05.

- Bennett, R., Payton, M. e., and Chamberlin, K. 2016. Three inoculation methods for evaluating sclerotinia blight resistance in peanut. *Peanut Sci.* 43:52-58.
- Berger, J., Abbo, S., and Turner, N. C. 2003. Ecogeography of annual wild *Cicer* species. *Crop Sci.* 43:1076-1090.
- Boland, G. J., and Hall, R. 1994. Index of plant hosts of *Sclerotinia sclerotiorum*. *Can. J. Plant Pathol.* 16:93-108.
- Boudhrioua, C., Bastien, M., Torkamaneh, D., and Belzile, F. 2020. Genome-wide association mapping of *Sclerotinia sclerotiorum* resistance in soybean using whole-genome resequencing data. *BMC Plant Biol.* 20:195.
- Brien, C. 2021. Employing asremlPlus, in conjunction with asreml, to calculate and use information criteria. <http://cran.fhcrc.org/web/packages/asremlPlus/vignettes/Wheat.infoCriteria.pdf>
- Brooks, K. D., Bennett, S. J., Hodgson, L. M., and Ashworth, M. B. 2018. Narrow windrow burning canola (*Brassica napus* L.) residue for *Sclerotinia sclerotiorum* (Lib.) de Bary sclerotia destruction. *Pest Manag. Sci.* 74:2594-2600.
- Butler, D. G., Cullis, B. R., Gilmour, A. R., Gogel, B. J., and Thompson, R. 2018. A SREML-R Reference Manual Version 4. VSN International Ltd, Hemel Hempstead, UK.
- Chen, W., Schatz, B., Henson, B., McPhee, K. E., and Muehlbauer, F. J. 2006. First report of Sclerotinia stem rot of chickpea caused by *Sclerotinia sclerotiorum* in North Dakota and Washington. *Plant Dis.* 90:114.
- Chen, Y., and Wang, D. 2005. Two convenient methods to evaluate soybean for resistance to *Sclerotinia sclerotiorum*. *Plant Dis.* 89:1268-1272.
- Clarkson, J. P., Warmington, R. J., Walley, P. G., Denton-Giles, M., Barbeti, M. J., Brodal, G., et al. 2017. Population structure of *Sclerotinia subarctica* and *Sclerotinia sclerotiorum* in England, Scotland and Norway. *Front. Microbiol.* 8:490.
- Collard, B. C. Y., Ades, P. K., Pang, E. C. K., Brouwer, J. B., and Taylor, P. W. J. 2001. Prospecting for sources of resistance to ascochyta blight in wild *Cicer* species. *Australas. Plant Pathol.* 30:271-276.
- Collard, B. C. Y., Pang, E. C. K., Ades, P. K., and Taylor, P. W. J. 2003a. Preliminary investigation of QTLs associated with seedling resistance to ascochyta blight from *Cicer echinospermum*, a wild relative of chickpea. *Theor. Appl. Genet.* 107:719-729.
- Collard, B. C. Y., Pang, E. C. K., and Taylor, P. W. J. 2003b. Selection of wild *Cicer* accessions for the generation of mapping populations segregating for resistance to ascochyta blight. *Euphytica* 130:1-9.
- Coomes, N. 2018. DiGGER: DiGGER design generator under correlation and blocking. R package version 1.0.3. <http://nswdipibiom.org/austatgen/software>
- Coyne, C. J., Kumar, S., von Wettberg, E. J. B., Marques, E., Berger, J. D., Redden, R. J., Ellis, T. H. N., Brus, J., Zablazka, L., and Smýkal, P. 2020. Potential and limits of exploitation of crop wild relatives for pea, lentil, and chickpea improvement. *Legume Sci.* 2:e36.
- Croser, J., Ahmad, F., Clarke, H., and Siddique, K. 2003. Utilisation of wild *Cicer* in chickpea improvement - progress, constraints, and prospects. *Aust. J. Agric. Res.* 54:429-444.
- Deacon, J. W. 1997. Glossary of plant-pathological terms. *New Phytol.* 137: 371-372.
- Denton-Giles, M., Derbyshire, M. C., Khentry, Y., Buchwaldt, L., and Kamphuis, L. G. 2018. Partial stem resistance in *Brassica napus* to highly aggressive and genetically diverse *Sclerotinia sclerotiorum* isolates from Australia. *Can. J. Plant Pathol.* 40:551-561.
- Fuhlbohmer, M. J., Tatnell, J. R., and Ryley, M. J. 2003. First report of stem rot and wilt of chickpea caused by *Sclerotinia minor* in Queensland, Australia. *Australas. Plant Pathol.* 32:323-324.
- Garg, H., Li, H., Sivasithamparam, K., Kuo, J., and Barbeti, M. J. 2010. The infection processes of *Sclerotinia sclerotiorum* in cotyledon tissue of a resistant and a susceptible genotype of *Brassica napus*. *Ann. Bot. (Lond.)* 106: 897-908.
- Haware, M. P. 1990. Fusarium wilt and other important diseases of chickpea in the Mediterranean area. *Fusarium wilt and other important diseases of chickpea in the Mediterranean area. Options Mediterr. Ser. Semin.*, 61-64.
- Hoffman, D. D., Diers, B. W., Hartman, G. L., Nickell, C. D., Nelson, R. L., Pedersen, W. L., et al. 2002. Selected soybean plant introductions with partial resistance to *Sclerotinia sclerotiorum*. *Plant Dis.* 86:971-980.
- Jeger, M. J., and Viljanen-Rollinson, S. L. H. 2001. The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. *Theor. Appl. Genet.* 102:32-40.
- Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., and Chibbar, R. N. 2012. Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): a review. *Br. J. Nutr.* 108 (S1):S11-S26.
- Kenward, M. G., and Roger, J. H. 1997. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics* 53:983-997.
- Kim, H. S., Sneller, C. H., and Diers, B. W. 1999. Evaluation of soybean cultivars for resistance to Sclerotinia stem rot in field environments. *Crop Sci.* 39: 64-68.
- Knights, E. J., Southwell, R. J., Schwingamer, M. W., and Harden, S. 2008. Resistance to *Phytophthora medicaginis* Hansen and Maxwell in wild *Cicer* species and its use in breeding root rot resistant chickpea (*Cicer arietinum* L.). *Aust. J. Agric. Res.* 59:383-387.
- Koseoglu, K., Adak, A., Sari, D., Sari, H., Oncu Ceylan, F., and Tokar, C. 2017. Transgressive segregations for yield criteria in reciprocal interspecific crosses between *Cicer arietinum* L. and *C. reticulatum* Ladiz. *Euphytica* 213:116.
- Kukreja, S., Salaria, N., Thakur, K., and Goutam, U. 2018. Fungal disease management in chickpea: current status and future prospects. Pages 293-309 in: *Fungi and Their Role in Sustainable Development: Current Perspectives*. P. Gehlot and J. Singh, eds. Springer, Singapore.
- Kull, L. S., Vuong, T. D., Powers, K. S., Eskridge, K. M., Steadman, J. R., and Hartman, G. L. 2003. Evaluation of resistance screening methods for Sclerotinia stem rot of soybean and dry bean. *Plant Dis.* 87:1471-1476.
- Leite, M. E., Dias, J. A., de Souza, D. A., Alves, F. C., Pinheiro, L. R., dos Santos, J. B., et al. 2016. Increasing the resistance of common bean to white mold through recurrent selection. *Sci. Agric.* 73:71-78.
- Li, C. X., Liu, S. Y., Sivasithamparam, K., and Barbeti, M. J. 2009. New sources of resistance to Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* in Chinese and Australian *Brassica napus* and *B. juncea* germplasm screened under Western Australian conditions. *Australas. Plant Pathol.* 38:149-152.
- Li, H., Rodda, M., Gnanasambandam, A., Aftab, M., Redden, R., Hobson, K., et al. 2015. Breeding for biotic stress resistance in chickpea: progress and prospects. *Euphytica* 204:257-288.
- Liang, X., and Rollins, J. A. 2018. Mechanisms of broad host range necrotrophic pathogenesis in *Sclerotinia sclerotiorum*. *Phytopathology* 108:1128-1140.
- Liang, Y., Cason, J. M., Baring, M. R., and Septiningsih, E. M. 2020. Identification of QTLs associated with Sclerotinia blight resistance in peanut (*Arachis hypogaea* L.). *Genet. Resour. Crop Evol.* 68:629-637.
- Michael, P. J., Lui, K. Y., Thomson, L. L., Stefanova, K., and Bennett, S. J. 2020. Carpopogenic germinability of diverse *Sclerotinia sclerotiorum* populations within the southwestern Australian grain belt. *Plant Dis.* 104:2891-2897.
- Miorini, T. J. J., Kamvar, Z. N., Higgins, R. S., Raetano, C. G., Steadman, J. R., and Everhart, S. E. 2019. Differential aggressiveness of *Sclerotinia sclerotiorum* isolates from North and South America and partial host resistance in Brazilian soybean and dry bean cultivars. *Trop. Plant Pathol.* 44:73-81.
- Miranda, J. H. 2019. Single plant selection for improving root rot disease (*Phytophthora medicaginis*) resistance in Chickpeas (*Cicer arietinum* L.). *Euphytica* 215:91.
- Muñoz, N., Liu, A., Kan, L., Li, M.-W., and Lam, H.-M. 2017. Potential uses of wild germplasms of grain legumes for crop improvement. *Int. J. Mol. Sci.* 18:328.
- Newman, T. E., Jacques, S., Grime, C., Kamphuis, F. L., Lee, R. C., Berger, J., et al. 2021. Identification of novel sources of resistance to ascochyta blight in a collection of wild *Cicer* accessions. *Phytopathology* 111:369-379.
- Ociepa, T. 2019. The oat gene pools – review about the use of wild species in improving cultivated oat. *J. Cent. Eur. Agric.* 20:251-261.
- Perchepe, L., Balagué, C., Riou, C., Claudel-Renard, C., Rivière, N., Grezes-Besset, B., et al. 2010. Nitric oxide participates in the complex interplay of defense-related signaling pathways controlling disease resistance to *Sclerotinia sclerotiorum* in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact.* 23:846-860.
- Porter, L. D. 2012. Pea germplasm with partial resistance to *Sclerotinia sclerotiorum* extends the time required by the pathogen to infect host tissue. *Crop Sci.* 52:1044-1050.
- Porto, A. C. M., Cardon, C. H., Vasconcellos, R. C. C., Novaes, E., Leite, M. E., Chalfun-Junior, A., et al. 2019. Expression of candidate genes related to white mold resistance in common beans. *Trop. Plant Pathol.* 44:483-493.
- Pulse Australia. 2020. APB Chickpea IDM strategies. <http://www.pulseaus.com.au/growing-pulses/bmp/chickpea/idm-strategies>
- Qi, L., Long, Y., Talukder, Z. I., Seiler, G. J., Block, C. C., and Gulya, T. J. 2016. Genotyping-by-sequencing uncovers the introgression alien segments associated with Sclerotinia basal stalk rot resistance from wild species. I. *Helianthus argophyllus* and *H. petiolaris*. *Front. Genet.* 7:219.
- Reen, R. A., Mumford, M. H., and Thompson, J. P. 2019. Novel sources of resistance to root-lesion nematode (*Pratylenchus thornei*) in a new collection of wild *Cicer* species (*C. reticulatum* and *C. echinospermum*) to improve resistance in cultivated chickpea (*C. arietinum*). *Phytopathology* 109:1270-1279.
- Seifbarghi, S., Borhan, M. H., Wei, Y., Coutu, C., Robinson, S. J., and Hegedus, D. D. 2017. Changes in the *Sclerotinia sclerotiorum* transcriptome during infection of *Brassica napus*. *BMC Genomics* 18:266.
- Seiler, G. J., Misar, C. G., Gulya, T. J., Underwood, W. R., Flett, B. C., Gilley, M. A., et al. 2017. Identification of novel sources of resistance to Sclerotinia basal stalk rot in South African sunflower germplasm. Online publication. *Plant Health Prog.* 18. doi: 10.1094/PHP-01-17-0007-RS.
- Shakiba, E., and Eizenga, G. C. 2014. Unraveling the secrets of rice wild species. Pages 1-58 in: *Rice - Germplasm, Genetics and Improvement*. J. Bao, ed. IntechOpen, London.
- Siddique, K. H. M., Erskine, W., Hobson, K., Knights, E. J., Leonforte, A., Khan, T. N., et al. 2013. Cool-season grain legume improvement in Australia—use of genetic resources. *Crop Pasture Sci.* 64:347-360.
- Singh, K. B., and Ocampo, B. 1997. Exploitation of wild *Cicer* species for yield improvement in chickpea. *Theor. Appl. Genet.* 95:418-423.
- Smith, A. B., Cullis, B. R., and Thompson, R. 2005. The analysis of crop cultivar breeding and evaluation trials: an overview of current mixed model approaches. *J. Agric. Sci.* 143:449-462.
- Taylor, A., Coventry, E., Jones, J. E., and Clarkson, J. P. 2015. Resistance to a highly aggressive isolate of *Sclerotinia sclerotiorum* in a *Brassica napus* diversity set. *Plant Pathol.* 64:932-940.

- Thudi, M., Chitikineni, A., Liu, X., He, W., Roorkiwal, M., Yang, W., et al. 2016. Recent breeding programs enhanced genetic diversity in both desi and kabuli varieties of chickpea (*Cicer arietinum* L.). *Sci. Rep.* 6:38636.
- Uloth, M. B., You, M. P., Finnegan, P. M., Banga, S. S., Banga, S. K., Sandhu, P. S., et al. 2013. New sources of resistance to *Sclerotinia sclerotiorum* for crucifer crops. *Field Crops Res.* 154:40-52.
- Viteri, D. M., Otto, K., Terán, H., Schwartz, H. F., and Singh, S. P. 2015. Use of four *Sclerotinia sclerotiorum* isolates of different aggressiveness, three inoculations per plant, and delayed multiple evaluations to select common beans with high levels of white mold resistance. *Euphytica* 204: 457-472.
- von Wettberg, E. J. B., Chang, P. L., Başdemir, F., Carrasquilla-Garcia, N., Korbu, L. B., Moenga, S. M., et al. 2018. Ecology and genomics of an important crop wild relative as a prelude to agricultural innovation. *Nat. Commun.* 9:649.
- Vuong, T. D., Hoffman, D. D., Diers, B. W., Miller, J. F., Steadman, J. R., and Hartman, G. L. 2004. Evaluation of soybean, dry bean, and sunflower for resistance to *Sclerotinia sclerotiorum*. *Crop Sci.* 44:777-783.
- Webster, R. W., Roth, M., Reed, H., Mueller, B., Groves, C., McCaghey, M., et al. 2020. Identification of soybean (*Glycine max*) Check lines for evaluating genetic resistance to *Sclerotinia* stem rot. Online publication. *Plant Dis.* <https://apsjournals.apsnet.org/doi/10.1094/PDIS-10-20-2193-RE>
- Xing, Q., Dekker, S., Kyriakopoulou, K., Boom, R. M., Smid, E. J., and Schutyser, M. A. I. 2020. Enhanced nutritional value of chickpea protein concentrate by dry separation and solid state fermentation. *Innov. Food Sci. Emerg. Technol.* 59:102269.