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New host resistances to *Pseudocercosporella capsellae* and implications for white leaf spot management in *Brassicaceae* crops

Niroshini Gunasinghe [a], Ming Pei You [a], Xi Xiang Li [c], Surinder S. Banga [d], Shashi K. Banga [d], Martin J. Barbetti [a, b, *]

[a] School of Plant Biology, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia
[b] The UWA Institute of Agriculture, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia
[c] Department of Germplasm Resources, Institute of Vegetable and Flowers, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China
[d] Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India

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ABSTRACT

Effective host resistance is the most cost-effective long term prospect for successful management of white leaf spot disease (*Pseudocercosporella capsellae*) in *Brassicaceae*. In two separate field trials, 168 genotypes were screened. In the first trial, lines of *Brassica oleracea* var. *capitata* (59), *Brassica napus* (34), *Brassica juncea* (6) and *B. juncea* containing wild weedy *Brassicaceae* introgression(s) (14) were arranged; and in the second, Australian historic and current *B. napus* (45) and *B. juncea* (10) varieties were screened. There was wide variation in expression of resistance, from complete resistance to highly susceptible as assessed by two disease parameters, viz. (i), Area Under Disease Progress Curve (AUDPC) for percent leaves diseased (values 0–221.2) and (ii) Percent Leaf Collapse Index (%LCI) values for leaf collapse due to disease (0–38.7). *Brassica oleracea* var. *capitata* was overall the most resistant species, while *B. juncea* the most susceptible with the majority having AUDPC values >75 and *B. napus* was intermediate. Five *B. oleracea* var. *capitata* genotypes were completely resistant, with 0 AUDPC and %LCI values. Pioneer® 45Y22 (RR) 'Mystic' and 'Wahoo' were also highly resistant, with the least %LCI (<3.7) and AUDPC (<20) of the Australian *B. napus* varieties. In contrast, 'Thunder TT' (AUDPC -133.6; %CLI − 15.6) and 'Carbine' (AUDPC − 73.8; %CLI − 12.5) were the most susceptible lines in first and second trials, respectively. The particularly high susceptibility of newly released *B. juncea* varieties such as 'Xceed OasisCL' highlights the risk of significant losses in such susceptible varieties when deployed in areas with high degree of pressure for white leaf spot disease. There was no association between AUDPC or % CLI with year of Australian varietal release, indicating that Australian breeding programs not made improvement for resistance to white leaf spot over the past two or more decades. Resistant varieties identified in this experiment can now not only be utilized in breeding programs to significantly improve overall crop resistance and management of white leaf spot disease, but also directly deployed to lower the severe inoculum load challenging current varieties.

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1. Introduction

White leaf spot (*Pseudocercosporella capsellae*) occurs across many *Brassicaceae* including oilseed, vegetable, condiment and fodder *Brassica* species, and results in significant yield losses worldwide (Barbetti and Khangura, 2000; Crossan, 1954; Deighton, 1973; Koike et al., 2007; Penaud, 1987; Petrie and Vanterpool, 1978). For example, in France, Penaud (1987) and Amelung and Daebeler (1988) reported white leaf spot as a major threat to oil seed rape; while in Western Oregon in the USA it seriously impacts commercial seed fields of forage *Brassica* and "field" turnip.

In Australia, yield losses of 15–20% are not uncommon on the more susceptible oilseed *Brassica* varieties (Barbetti and Khangura, 2000; Khangura et al., 2014). There, it is common on both oilseed rape (*Brassica napus*) and on Indian mustard (*Brassica juncea*) (Eshraghi et al., 2007). It also occurs in *Brassicaceae* on *Brassica rapa* and *B. juncea* vegetable types, some *Brassica oleracea* types, such as *B. oleracea* var. *botrytis* (Lancaster, 2006) and *B. oleracea* var. *italica*

* Corresponding author. School of Plant Biology, Faculty of Science, The University of Western Australia, 35 Stirling Highway, Crawley, WA, 6009, Australia.
E-mail address: martin.barbetti@uwa.edu.au (M.J. Barbetti).

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(Lancaster, 2006), and on *Brassica campestris* var. *chinensis*, *B. campestris* var. *rapa*, and *B. napus* var. *naprobrassica* (Shivas, 1989).

Over the past two decades, the significance of white leaf spot has increased in Australia, particularly since the deployment of the 'Surpass type' major gene resistance against blackleg disease (*Leptosphaeria maculans*) as these varieties were inherently susceptible to white leaf spot disease (M.J. Barbetti, unpubl.). The prevalence of white leaf spot significance has also increased in the UK, and global change in climate has been suggested as a cause due to warmer/wetter winters that favour disease spread and development (Inman et al., 1997).

Cultural and fungicidal controls remain the focus for management, but they generally provide inadequate control and are considered cost-prohibitive for managing disease in broad-acre *Brassica* production (Inman, 1992). The general increasing severity of this disease has focused research into finding more effective and reliable control measures to cost-effectively manage this disease. However, breeding for resistance against white leaf spot has not been given priority in breeding programs due to other pathogens being considered of greater economic significance (Inman, 1992). This is despite recent field screening studies in Australia by Eshraghi et al. (2007) and Gunasinghe et al. (2013) having demonstrated opportunities to locate resistance for commercially important oilseed, vegetable, and weedy *Brassica* species. Hence, field studies were undertaken to screen 168 *Brassica* genotypes for their relative resistances to *P. capsellae*. In one trial, *B. oleracea* var. *capitata*, *B. napus, B. juncea* and also *B. juncea* genotypes containing introgressions from wild weedy *Brassicaceae* from Australia, India or China were screened. A second trial included historic and current Australian *B. napus* and *B. juncea* commercial varieties.

2. Materials & methods

2.1. Field site details

Two separate field screening trials were undertaken during the Australian 2013 winter/spring cropping season to determine relative host resistances present across different *Brassica* species accessed from Australia, India and China. Trials were conducted in a nylon mesh covered area (to exclude insect pests) at the University of Western Australia Field Station in Shenton Park, Western Australia. Test genotypes were a arranged in complete randomized block design with four replications. Fifteen seeds per genotype were sown in single 1 m rows with 0.22 m spacing between rows. Certain genotypes were repeated across both trials to allow comparisons between the two trials.

2.2. Individual field trials

Field trial 1 was sown on 23 May 2013 with 113 genotypes from Australia, China and India including *B. oleracea* var. *capitata* (59), *B. napus* (34) and *B. juncea* (20) with *B. juncea* containing wild weedy *Brassicaceae* introgressions (14). Field trial 2 was sown on 7 June 2013 and with historic and current Australian *B. napus* (45) and *B. juncea* (10) varieties.

2.3. P. capsellae isolates used

Four single spore isolates of *P. capsellae* were used: UWA Wln-15, UWA Wlra-7, UWA Wlr-8 and UWA Wlj-5 that had been isolated from different diseased host species in Western Australia. UWA Wln-15 was derived from infected *B. napus* leaves collected from Calingiri, Western Australia (WA) in 2007; isolate UWA Wlra-7 was from *Raphanus raphanistrum* (wild radish) leaves collected in 2005 from West Calingiri, WA; isolates UWA Wlr-8 and UWA Wlj-5 were collected in 2005 from white leaf spot lesions of *B. rapa* at Perth, Western Australia, and *B. juncea* at Shenton Park, Western Australia, respectively. These different isolates were used as they are considered to represent variation found within *P. capsellae* populations in Australia (Gunasinghe et al., 2016) and this approach moderates possible pathotype-specific or race-specific responses to the pathogen on test genotypes. Subsequently, isolates of *P. capsellae* were maintained as lyophilised ampoules until these trials were initiated. Each isolate was revived by sub-culturing onto freshly made Malt Extract Agar medium (MEA: malt extract 20.0 g L^{-1}, glucose 20.0 g L^{-1}, agar 15.0 g L^{-1} and peptone 1.0 g L^{-1}).

2.4. Inoculum preparation and inoculation of plants

A mixture of mycelial fragments (4×10^6 mL^{-1}) across the four isolates (used in equal proportion) was utilized as the inoculum (Eshraghi et al., 2007; Gunasinghe et al., 2013). A mycelial suspension was chosen over a conidial suspension to initiate the disease epidemic as the pathogen, at best, sporulates poorly on agar media (Crossan, 1954; Miller and McWhorter, 1948).

Isolates sub-cultured on to MEA were incubated at 20 °C for at least two weeks and mycelium scraped from the leading edge of each isolate were aseptically transferred into Erlenmeyer flasks (250 mL) containing 150 mL of Malt Extract Broth (MEB: malt extract 20.0 g L^{-1}, glucose 20.0 g L^{-1}, and peptone 1.0 g L^{-1}). The liquid cultures were incubated on a rotary platform shaker (Innova™ 2100, New Brunswick Scientific) at 150 rpm at 25 °C. After 14 days, cultures of all four isolates with abundant mycelial growth were mixed in equal volumes and blended for 5 min (Kambrook®, Mega Blender) to make the inoculum mixture of *P. capsellae* mycelial fragments. The final concentration of mycelial fragments was adjusted to 4×10^6 mL^{-1} fragments using a haemocytometer. Plants at the 4–6 leaf stage (approximately 7–8 weeks of age) were spray inoculated with the mycelial fragments mixture (4×10^6 mL^{-1}) using a hand-held and hand-operated aerosol sprayer. Thereafter, another two sequential inoculations were made at two-week intervals. Inoculum was applied in the late afternoon followed by the conducive, high humidity environment overnight which would maximise infection.

2.5. Disease assessments

Plants were assessed weekly for white leaf spot disease from approximately 30 days after the third inoculation for four weeks. Disease incidence and the amount of leaf collapse due to white leaf spot were assessed on each genotype in both field trials as follows.

First, the percentage leaves diseased was assessed for each of 10 plants per each row for each genotype using a 0–10 disease incidence scale for white leaf spot disease (Barbetti, 1987; Eshraghi et al., 2007), where 0 = nil disease, 1 = 1–10%, 2 = 11–20%, 3 = 21–30%, 4 = 31–40%, 5 = 41–50%, 6 = 51–60%, 7 = 61–70%, 8 = 71–80%, 9 = 81–90%, 10 = 91–100% of leaves diseased. Area under disease progress curve (AUDPC) values for each genotype were subsequently calculated from these independent disease incidence scores using the formula $Y = \sum [(X_i + X_{i+1})/2] (t_{i+1} - t_i)$, where Y is the AUDPC, X_i is the white leaf spot disease incidence of the ith evaluation, X_{i+1} is the white leaf spot score of the i+1st evaluation and $(t_{i+1} - t_i)$ is the number of days between two evaluations (Campbell and Madden, 1990). AUDPC was chosen because resistance against *P. capsellae* is likely quantitative such that AUDPC provides the most appropriate estimate for where the accumulated disease incidence and/or severity is assessed multiple times (Vale et al., 2001). Secondly, the extent of leaf collapse caused by white leaf spot disease was assessed on a 0–10 scale where 0 = nil collapse and 10 = >90% of leaves collapsed from *P. capsellae*

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