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Short communication

New host resistances in *Brassica napus* and *Brassica juncea* from Australia, China and India: Key to managing Sclerotinia stem rot (*Sclerotinia sclerotiorum*) without fungicides

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ABSTRACT

Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, is a serious problem in oilseed rape and mustard worldwide. Locating effective sources of host resistance to this disease offers the best long term prospects for its improved management. For this reason, 19 *Brassica napus* genotypes from Australia (6), China (7) and India (6) and 34 *Brassica juncea* genotypes from Australia (6), China (3) and India (25), were screened for resistance to SSR under field conditions using a stem inoculation test. There were significant differences ($P < 0.001$) among the *B. napus* and *B. juncea* test genotypes in relation to the stem lesion length. The most resistant *B. napus* genotypes were Oscar from Australia, Zhongyou-za No. 8, Fan 168 and Ding 110 from China, all with stem lesion lengths ≤4.1 cm. The most susceptible *B. napus* were GSL2 from India, and 03-p74-11 from China, with stem lesion lengths ≥12 cm. The most resistant *B. juncea* genotypes were Aravali and Bio-902 from India with stem lesion lengths ≤5.7 cm; while the most susceptible were JM018 from Australia, Xinyou 8 and Xinyou 9 from China, Sanjucta Ascsh and Basanti from India, all with stem lesion lengths >10 cm. In particular, this high level resistance in *B. napus* Oscar from Australia, Zhongyou-za No. 8, Fan 168 and Ding 110 from China provides sources of resistance for oilseed *Brassica* breeding programs in Australia; particularly resistance to pathotype 76, the dominant prevailing *S. sclerotiorum* pathotype. It is noteworthy that progenies from *B. napus* crosses of Oscar with Ding 110 express isolate-independent host resistance, making Oscar and Ding 110, and potentially their progenies, an ideal target to exploit in developing new commercial rapeseed cultivars that not only have more effective resistance to SSR, but resistance that is effective across multiple pathotypes of this pathogen. These findings ensure successful management of SSR based on host resistance rather than fungicides is now possible providing breeders take the opportunities now presented.

Sclerotinia stem rot (SSR) of oilseed rape and mustard (*Brassica napus* and *Brassica juncea*, respectively) poses a major threat to oilseed rape production in Australia (Li et al. 2006, 2007, 2009; Barbetti et al. 2014), China (Zhou et al. 1994; Zhao et al. 2004, 2006) and, in India, particularly to mustard (Singh et al. 2008, 2010; Goyal et al. 2011). Currently, cultural and chemical control measures provide only partial control and can be cost prohibitive, particularly for broad-acre oilseed rape crops in Australia and elsewhere (Barbetti et al. 2011). Hence, locating effective resistance to SSR remains a high priority for cruciferous crops in Australia, China and India as this is viewed as the most economic and sustainable method for disease control (Barbetti et al. 2011). Given the quantitative nature of the resistance to SR identified so far (Zhou et al. 1994; Zhao and Meng, 2003; Zhao et al. 2006), there is a need to continue to quantify the full range of host resistances

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available across both *B. napus* and *B. juncea* to ensure that selections with high resistance to SSR are identified for inclusion in breeding programs. Identifying improved sources of host resistance to SSR is an important prerequisite for its effective management across the three countries.

Partial (polygenic) resistance has been reported in some Chinese genotypes of *B. napus* (Zhou et al. 1994; Li et al. 1999; Zhao et al. 2004) and in some *B. napus* and *B. juncea* genotypes in Australia (e.g., Li et al. 2006, 2007, 2009). Particularly high level resistance has been reported in some introgression lines derived from hybridization between wild crucifers and *B. napus* and *B. juncea* in India (Garg et al., 2010b), but these resistances are not yet available outside of India. There remains significant potential to identify additional sources of resistance to SSR from wider screening of germplasm. As part of an international *Brassica* collaboration initiative between Australia, China and India, we evaluated *B. napus* and *B. juncea* exchanged germplasm from China, India and Australia under Western Australian field conditions for resistance to SSR. We highlight how these new host resistances can ensure successful management of SSR in their own right and replace current reliance upon fungicides.

The germplasm for this study was provided by breeders in China, India and Australia through a joint project funded by the Australian Centre for International Agricultural Research (ACIAR) and the Grains Research and Development Corporation (GRDC). The basis for the test genotypes/cultivars in this study was inclusion of an agreed shared set of test genotypes to be tested across India, Australia and China for a range of disease and other characteristics. These were 19 *B. napus* genotypes, from Australia (6), China (7) and India (6), and 34 *B. juncea* genotypes, from Australia (6), China (3) and India (25) (Table 1). The genotypes were tested in a nylon mesh screen house at the University of Western Australia Shenton Park Field Station, Perth, Western Australia. All genotypes were sown on 6 May 2009 in single rows of 1 m length and with 0.6 m between rows. Twenty seeds per genotype were sown and plants were thinned to 12–13 plants per row two weeks after germination. Rows of each genotype were arranged in a randomized complete block design with four replications.

A single isolate of *Sclerotinia sclerotiorum* (MBRS1) was used in this study. This isolate was obtained from a sclerotium collected from infected oilseed rape at Mount Barker Research Station in Western Australia in 2004 and stored as air-dried sclerotia maintained at 4 °C. MBRS 1 is now known as a highly aggressive isolate that belongs to the prevailing pathotype (pathotype 76) occurring in Western Australia (Ge et al. 2012). It is also an isolate that has been used for resistance screening of *B. juncea* and *B. napus* genotypes in the field (Li et al. 2006, 2007). Ten plants in each test genotype were randomly selected and inoculated at the flowering stage when 50% of the plants of each genotype in the rows had at least one opened flower. Overhead sprinklers were used at inoculation to maintain consistency of high humidity conditions. Stem inoculation was undertaken using the method of Buchwaldt et al. (2005), but as modified by Li et al. (2006). A single agar plug disc (5 mm diameter) was used as inoculum for each plant. The agar disc was cut from the actively growing margin of a 3-day-old colony on a glucose-rich medium (peptone 10 g, glucose 20 g, Agar 23 g, KH_2PO_4 0.5 g, H_2O 1 L, adjusted to pH 4.0 with HCl before autoclaving) and wrapped onto the first internode above the middle node of each stem using Parafilm®. Plants were also irrigated by overhead sprinklers when natural rainfall was insufficient.

Stem lesion length was measured with a ruler at 3 weeks after inoculation, as any impact from different times of flowering in the populations due to differing maturity is rendered insignificant by delaying the disease assessment to a single assessment at this timing after inoculation (Li et al. 2007). Further, this particular inoculation and assessment method used significantly reduces variability in the responses commonly observed in screening for resistance against SSR (Li et al. 2007). Control plants were inoculated with uncolonized agar plugs to ensure that all infections observed were due to inoculation with strain MBRS1. In addition, stem diameters of all plants per row were also measured using a linear ruler and flowering dates (days from sowing) of each genotype were recorded. A single factor analysis of variance was conducted using Genstat (14th edition, Lawes Agricultural Trust). Fisher's least significant difference (LSD) at 95% significant level was used to test the differences among genotypes. Genotypes were ranked according to their means in relation to stem lesion length.

Table 1
Field screening of 20 *Brassica napus* genotypes [from Australia (7), China (8) and India (5)] and 33 *B. juncea* genotypes [from Australia (6), China (2) and India (25)], under field conditions at Shenton Park, Western Australia, following stem inoculation. The stem lesion length (cm) was measured 3 weeks after inoculation.

Genotypes	Origin	Species	Lesion length
Oscar	Australia	*B. napus*	2.74
Zhongyou-za No.8	China	*B. napus*	3.02
Fan 168	China	*B. napus*	3.14
Ding 110	China	*B. napus*	4.09
06-6-3792	China	*B. napus*	4.09
RT108	Australia	*B. napus*	4.31
Zhongshu-ang N0.4	China	*B. napus*	4.42
Ag-Spectrum	Australia	*B. napus*	4.84
Rivette	Australia	*B. napus*	4.96
Zhongyou 821	China	*B. napus*	5.26
Trilogy	Australia	*B. napus*	5.59
GSC 5	India	*B. napus*	6.16
Lantern	Australia	*B. napus*	6.46
Teri (00)R9903	India	*B. napus*	7.23
RR002	Australia	*B. napus*	7.92
GSL1	India	*B. napus*	8.10
Neelam	India	*B. napus*	9.70
GSL2	India	*B. napus*	10.52
03-p74-11	China	*B. napus*	12.38
Aravali,	India	*B. juncea*	5.40
Bio-902	India	*B. juncea*	5.66
JM06018	Australia	*B. juncea*	6.20
Urvashi	India	*B. juncea*	6.54
CS-54	India	*B. juncea*	6.66
Kranti	India	*B. juncea*	6.76
JN028	Australia	*B. juncea*	6.77
JM-2	India	*B. juncea*	7.02
Maya	India	*B. juncea*	7.12
RH8812	India	*B. juncea*	7.50
Laxmi	India	*B. juncea*	7.76
RGN-13	India	*B. juncea*	7.81
CS-52	India	*B. juncea*	7.86
GM-2	India	*B. juncea*	7.94
Geeta	India	*B. juncea*	7.99
Jagannath	India	*B. juncea*	8.03
JO006	Australia	*B. juncea*	8.10
GM-3	India	*B. juncea*	8.11
JM-1	India	*B. juncea*	8.29
JM-3	India	*B. juncea*	8.31
Vardan	India	*B. juncea*	8.43
JR042	Australia	*B. juncea*	8.44
Ashirwad	India	*B. juncea*	8.51
Narendra Swarna Rai-8	India	*B. juncea*	8.60
Pusa Mahak	India	*B. juncea*	8.70
CBJ-003	China	*B. juncea*	8.71
Narendra Ageti Rai–4	India	*B. juncea*	8.72
Swarna Jyoti	India	*B. juncea*	9.20
Vasundhra	India	*B. juncea*	9.26
JM018	Australia	*B. juncea*	10.80
Sanjucta Ascsh	India	*B. juncea*	10.87
Xinyou 9	China	*B. juncea*	11.35
Xinyou 8	China	*B. juncea*	12.62
Basanti	India	*B. juncea*	12.75
$P <$			0.001
l.s.d. ($P \leq 0.05$) =			4.28