

## Host resistance in oilseed Brassicas against Sclerotinia - renewed hope for managing a recalcitrant pathogen

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### Abstract

Sclerotinia stem rot (SSR) disease of oilseed rape (*Brassica napus*) and mustard (*B. juncea*) is a serious threat to oilseed production in regions of Asia, Europe, North America and Australia. A range of different screening tests were evaluated, including a cotyledon test and different field inoculation types. The cotyledon assay allowed rapid differentiation of reactions of *B. napus* genotypes against SSR and was a relatively reliable indicator of field performance. However, the field stem inoculation method was the most reliable across diverse germplasm sets. Among the *B. napus* genotypes tested in field tests for stem resistance to SSR in Australia, ZY006 showed outstanding resistance, with a mean stem lesion length of <0.45 cm (compared with 15-25 cm for susceptible genotypes). This level of resistance was far superior to that ever identified previously in Australia for *B. napus* or *B. juncea*. *B. napus* genotypes 06-6-3792 and ZY004 from China and genotype RT108 from Australia also showed high levels of resistance, with mean stem lesion lengths <3 cm. The most resistant *B. juncea* genotypes were JM06018 and JM06006 from Australia and *B. juncea* #2 from China, with mean stem lesion lengths of <4.8 cm. Introgression lines developed in India following hybridization of three wild crucifers (viz. *Erucastrum cardaminoides*, *Diplotaxis tenuisiliqua* and *E. abyssinicum*) with *B. napus*, *B. juncea*, *B. rapa* and *B. nigra* showed outstanding levels of resistance to SSR, with ≥98% of progenies of the selected resistant plants exhibiting consistent responses with stem lesion length <1.0 cm across repeat experiments. The outstanding resistance to SSR identified in *B. napus* ZY006 and in the introgression lines provides the first such high level of resistance available for oilseed *Brassica* breeding programs.

**Key words:** *Sclerotinia sclerotiorum*, *Brassica napus*, *Brassica juncea*, sclerotinia stem rot, oilseed rape, mustard

Sclerotinia stem rot (SSR) disease of oilseed rape (*Brassica napus*) and mustard (*B. juncea*) is a serious threat to oilseed rape and mustard production worldwide, including Australia (Hind-Lanoiselet and Lewington 2004), China (Zhou et al. 1994), and India (Singh et al. 2010). Yield loss as high as 24% have been recorded under Australian conditions. Current management options are mainly cultural control and chemical control that at best provide only partial control and can be cost prohibitive. Locating and deploying host resistance remains the only long-term cost-effective and sustainable means of managing this disease, certainly for Australia. As part of a five year international collaborative project between Australia, India and China, studies were undertaken in Western Australia to develop and apply methodologies to identify resistance to SSR in *B. napus* and *B. juncea* germplasm from the three countries.

### Materials and Methods

A range of different screening tests were evaluated, including: a cotyledon test where cotyledons were drop-inoculated using macerated mycelium under controlled environmental conditions; and different field inoculation methods, including application of a spray of a mycelial suspension, natural myceliogenic and/or capogenic germination originating from sclerotia resident in soil; and a stem inoculation test at the flowering stage using a single agar plug disc bearing actively growing mycelium.

**Cotyledon test:** *B. napus* genotypes were grown under controlled environment growth room conditions of 18°C during the day and 14°C at night, with light intensity of 150 µE/m<sup>2</sup>/s. Inoculum production was undertaken using a sterilised liquid medium (potato dextrose broth 24 g, peptone 10 g, H<sub>2</sub>O 1 L). The mycelia were macerated and the concentration was adjusted to 10<sup>4</sup> fragments/mL. When cotyledons were fully expanded, (growth stage 1.00 on the scale given by Sylvester-Bradley and Makepeace, 1984), cotyledons were inoculated with a single 10 µL drop deposited on each cotyledon lobe. Typical hypersensitive and/or necrotic and water soaked lesions were apparent by 1-2 days post-inoculation (dpi). At 4 dpi, lesions assessed on the basis of lesion diameter (mm).

**Field tests 2005-2009:** 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 and the Grains Research and Development Corporation. Ten plants in each test genotype in the field were randomly selected and inoculated at the 50% flowering stage. Stem inoculation was made according to Buchwaldt et al. (2005) where a single agar plug disc (5 mm diameter) 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<sub>2</sub>PO<sub>4</sub> 0.5 g, H<sub>2</sub>O 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<sup>®</sup>. Other inoculation tests were evaluated, including from natural myceliogenic germination and infection of stem base; from natural ascospore infections from germinating ascocarps of the pathogen; and from application of macerated hyphal suspension sprays to flowering plants. Stem lesion length was measured at 1-3 weeks after inoculation and genotypes ranked according to their means in relation to stem lesion length. The most severely affected plants collapsed and died as the result of stem lesion girdling.

**Introgression lines:** Introgression lines were developed for *B. juncea* and *B. napus* by introgression from three wild crucifers viz. *E. cardaminoides* (2n = 18), *D. tenuisiliqua* (2n = 18) and *E. abyssinicum* (2n = 32) by S.S. Banga and co-workers, Punjab Agricultural University, India, by synthesizing intergeneric hybrids, *E. cardaminoides*/*B. rapa* (Chandra et al. 2004), *E. cardaminoides*/*B. nigra* (Chandra et al. 2004), *B. napus*/*E. cardaminoides*, *B. juncea*/*D. tenuisiliqua*, and *B. juncea*/*E. abyssinicum*. Following chromosome doubling, the synthetic amphiploids or the trigonomic hybrids were subsequently used as pollen/seed parents to hybridize with cultivated digenomics, *B. juncea* or *B. napus*. This was followed by three to four generations of selfing using the single pod descent method. Genotypes were selected randomly from the populations (BC1S4/S5) derived from the cross combinations involving wild species and cultivated germplasm. Comparisons were made to lines of *B. napus* and *B. juncea* obtained from Australia, India and China as in the field tests above. Introgression lines that showed stem lesion lengths <1 cm in first test were not only re-inoculated at late flowering stage above the site of the first inoculation to confirm the resistance observed in the first test, but also further evaluated in a subsequent season at the Punjab Agricultural University, India. In each test, 20 seeds per introgression line were hand sown in single rows of 2 m length and with 0.6 m between rows. Resistance responses of all introgression lines were evaluated by using the stem inoculation test as described by Buchwaldt et al. (2005) and as modified by Li et al. (2006). All disease assessments were made at 3 weeks after inoculation. Introgression lines were categorized into five different classes based on their resistant responses, namely highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS) with stem lesion lengths ranging from 0 to <2.5; 2.5 to <5.0; 5.0 to <7.5; 7.5 to 10.0 and >10.0 cm, respectively.

## Results and Discussion

From these studies, a reliable cotyledon test for rapid seedling screening for *B. napus* genotypes and a reliable field stem inoculation test for *B. napus* and *B. juncea* were developed. The cotyledon assay allowed rapid differentiation of reactions of *B. napus* genotypes against SSR and was a relatively reliable indicator of field performance (Garg et al. 2008). However, the stem inoculation method was the most reliable across diverse germplasm sets (Li et al. 2006, 2007, 2009).

In the initial field tests, it was noted that there was a variable impact of the time of inoculation on the disease level depending upon time of assessment post-stem inoculation. However, this impact could be reduced to an insignificant level provided the assessment after stem inoculation was delayed until 3 weeks post-inoculation (Li et al. 2007). These results demonstrated for the first time that the use of appropriate inoculation and assessment methods significantly reduce variability in the responses commonly observed in screening for resistance against SSR. In field tests over 4 seasons, among the *B. napus* genotypes tested in field tests for stem resistance to SSR in Australia, ZY006 showed outstanding resistance, with a mean stem lesion length of <0.45 cm (compared with 15-25 cm for susceptible genotypes) (Li et al. 2009). This level of resistance was far superior to that ever identified previously in Australia for *B. napus* or *B. juncea*. *B. napus* genotypes 06-6-3792 and ZY004

from China and genotype RT108 from Australia also showed high levels of resistance, with mean stem lesion lengths <3 cm (Li et al. 2009). The most resistant *B. juncea* genotypes were JM06018 and JM06006 from Australia and *B. juncea* #2 from China, with mean stem lesion lengths of <4.8 cm (Li et al. 2009).

Introgression lines developed in India following hybridization of three wild crucifers (viz. *Erucastrumcardaminoides*, *Diplotaxistenuisiliqua* and *E. abyssinicum*) with *B. napus*, *B. juncea*, *B. rapa* and *B. nigra* showed outstanding levels of resistance to SSR, with ≥98% of progenies of the selected resistant plants exhibiting consistent responses with stem lesion length <1.0 cm across repeat experiments, suggesting a very high transmission frequency of the gene(s) governing resistance (Garg et al. 2010).

The outstanding resistance to SSR identified in *B. napus* ZY006 and in the introgression lines provides the first such level of resistance available for oilseed *Brassica* breeding programs. Genotypes with such high level resistance ensure excellent future prospects for utilizing host resistance as the basis for SSR management.

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