Eur J Plant Pathol (2010) 126:305–315
DOI 10.1007/s10658-009-9547-7

Pathogenicity of morphologically different isolates of *Sclerotinia sclerotiorum* with *Brassica napus* and *B. juncea* genotypes

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Received: 11 December 2008 / Accepted: 5 October 2009 / Published online: 25 October 2009

Abstract Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* is a serious threat to oilseed production in Australia. Eight isolates of *S. sclerotiorum* were collected from Mount Barker and Walkway regions of Western Australia in 2004. Comparisons of colony characteristics on potato dextrose agar (PDA) as well as pathogenicity studies of these isolates were conducted on selected genotypes of *Brassica napus* and *B. juncea*. Three darkly-pigmented isolates (WW-1, WW-2 and WW-4) were identified and this is the first report of the occurrence of such isolates in Australia. There was, however, no correlation between pigmentation or colony diameter on PDA with the pathogenicity of different isolates of this pathogen as measured by diameter of cotyledon lesion on the host genotypes. Significant differences were observed between different isolates ($P \leq 0.001$) in two separate experiments in relation to pathogenicity. Differences were also observed between the different *Brassica* genotypes ($P \leq 0.001$) in their responses to different isolates of *S. sclerotiorum* and there was also a significant host × pathogen interaction ($P \leq 0.001$) in both experiments. Responses between the two experiments were significantly correlated in relation to diameter of cotyledon lesions caused by selected isolates ($r=0.79$; $P<0.001$, $n=48$). Responses of some genotypes (e.g., cv. Charlton) were relatively consistent irrespective of the isolates of the pathogen tested, whereas highly variable responses were observed in some other genotypes (e.g., Zhongyou-ang No. 4, Purler) against the same isolates. Results indicate that, ideally, more than one *S. sclerotiorum* isolate should be included in any screening programme to identify host resistance. Unique genotypes which show relatively consistent resistant reactions (e.g., cv. Charlton) across different isolates are the best for commercial exploitation of this resistance in oilseed *Brassica* breeding programmes.

Keywords Canola · Mustard · Oilseed rape · Pathogenicity · Sclerotinia stem rot

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Introduction

Sclerotinia stem rot (SSR) caused by the ascomycete *Sclerotinia sclerotiorum*, is a serious threat to oilseed rape production with substantial yield losses from this disease recorded world-wide including Australia,

Europe and North America (McCartney et al. 1999; Hind et al. 2003; Sprague and Stewart-Wade 2002; Koch et al. 2007; Malvarez et al. 2007). While, *S. sclerotiorum* is considered to exhibit little host specificity (Purdy 1979), it is important to understand the diversity of this pathogen for the development of effective screening strategies to identify and deploy host resistance. The diversity and pathogenicity studies of this pathogen have been investigated for different crops in Canada and the USA (Auclair et al. 2004; Pratt and Rowe 1991; Hambleton et al. 2002; Maltby and Mihail 1997; Kull et al. 2003). Some past studies have investigated the genetic diversity of *S. sclerotiorum* but genetic diversity was not related to the pathogenicity of the pathogen in these studies (Kohn et al. 1991; Kohli et al. 1992; Cubeta et al. 1997; Sun et al. 2005; Malvarez et al. 2007). Further, only limited studies have been conducted so far, to understand the diversity and pathogenicity of *S. sclerotiorum* on Brassicas or other hosts in Australia. These include the work of Sexton et al. (2006) who demonstrated genotypic diversity among *S. sclerotiorum* isolates collected from oilseed rape crops from south-east Australia, utilising microsatellite markers, and Ekins et al. (2007), who compared aggressiveness of *S. sclerotiorum* isolates collected also from south-east Australia on sunflower.

Differences in the morphology of *S. sclerotiorum* isolates have previously been observed by Li et al. (2003) and Garrabrandt et al. (1983) where isolates producing tan sclerotia were identified. Very few reports exist to date describing darkly-pigmented isolates of *S. sclerotiorum*, such as those from Canada and the south-western region of the USA (Lazarovits et al. 2000; Sanogo and Puppala 2007). Primarily, the dark colour of the colonies results from the production of melanin, the main role of which in this pathogen is to protect the sclerotia from adverse biological and environmental conditions (Butler and Day 1998; Lazarovits et al. 2000). An association of melanin with pathogenicity has also been reported in other pathogens. For example, heavily-melanised variants of the ascomycete fungus *Gaeumannomyces graminis* var. *tritici* are non-pathogenic (Goins et al. 2002), while *Magnaporthe grisea* and *Colletotrichum lagenarium* require melanin for pathogenicity (Kubo et al. 2000). However, there are no reports of a relationship between pigmentation and pathogenicity in *S. sclerotiorum.* Furthermore, colony characteristics including radial growth rates of isolates of *S. sclerotiorum* in vitro have been related to pathogenicity of this pathogen under controlled environmental conditions. (e.g., Ziman et al. 1998; Durman et al. 2003). However, no such studies have been undertaken with Australian isolates of *S. sclerotiorum*.

For this study, isolates of *S. sclerotiorum* were obtained from the Mount Barker and Walkaway regions of Western Australia in 2004 where significant losses from the disease have been reported on *B. napus*. The aims of our study were firstly, to relate colony characteristics to the pathogenicity for eight isolates of *S. sclerotiorum* from the two different regions, and secondly, to evaluate the differences in their pathogenicity to selected genotypes of *B. napus* and *B. juncea*, under controlled environmental conditions.

Materials and methods

Sclerotinia sclerotiorum isolates

Eight isolates of *S. sclerotiorum* were used to study isolate × cultivar interactions. Four isolates (viz MBRS-1, MBRS-2, MBRS-3 and MBRS-5) collected from the Mount Barker and four isolates (WW-1, WW-2, WW-3 and WW-4) from the Walkaway regions of Western Australia in 2004 were used in this study. The initial cultures were then sub-cultured on to water agar and stored at 4°C. All isolates were subsequently sub-cultured to PDA as this medium allows the best expression of any pigmentation occurring in *S. sclerotiorum* colonies (Lazarovits et al. 2000; Sanogo and Puppala 2007).

Molecular identification of different isolates

Single nucleotide polymorphism (SNP) based diagnostics were conducted to identify the eight strains of *Sclerotinia* isolated in the present study. SNP data from seven loci as described by Carbone (2000) were analysed to develop species-specific oligonucleotides for identification among *S. sclerotiorum*, *S. minor*, and *S. trifoliorum.* Oligonucleotides using Primer 3 (Rozen and Skaletsky 2000) were designed to hybridise to SNPs in regions that had high relative sequence similarity to ensure hybridisation across the board. In addition to the eight isolates used in this study, two confirmed isolates of *S. sclerotiorum* (1980, the

genome sequence reference isolate http://www.broad.mit.edu/annotation/genome/sclerotinia_sclerotiorum/Home.html and LMK211), one of *S. trifoliorum* (LMK36) and one of *S. minor* (FA2-1) were also included as positive controls, with an isolate of *Botrytis cinerea* (LMK 18) included as a negative control (L. M. Kohn and M. Andrew, unpublished).

Comparison of colony characteristics

Mycelial plugs of each isolate were taken from the growing margins of colonies grown on PDA for 3 days and inoculated on to fresh PDA. All cultures were incubated at 20°C and colony diameter measured after 24 and 48 days of incubation. Eight replications with two plates per replication were used for each isolate.

Pathogenicity of different isolates

Fungal isolates All eight isolates (viz. MBRS-1, MBRS-2, MBRS-3, MBRS-5, WW-1, WW-2, WW-3, and WW-4) were used singly to determine the reaction of various genotypes to each isolate in experiment 1. In experiment 2, four isolates viz. MBRS-2,-5 (both highly pathogenic), WW-3 (moderately pathogenic) and WW-4 (least pathogenic) were used to provide a similar range of pathogenicity for both experiments in order that host responses and host-pathogen interactions identified in experiment 1 could be compared.

Genotypes tested Sixteen genotypes (twelve *B. napus* and four *B. juncea*) from Australia and China as listed in Table 4 were selected for evaluation on the basis of differences in their resistance reaction as identified earlier by Li et al. (2006) and Garg et al. (2008). Seed was obtained from Australia and China through an Australian Centre for International Agricultural Research (ACIAR) collaborative programme. The experiment was repeated (experiment 2) with the same sixteen cultivars as described above.

Test conditions and inoculum production All procedures, test conditions and inoculum production were as described previously by Garg et al. (2008). Briefly, the host genotypes screened were grown in 13.7×6.6×4.9 cm trays, each having eight cells and containing a soil-less compost mixture. Groups of four trays were placed in 10 l plastic storage boxes (34×13×23 cm). Three seeds of each genotype were sown in each cell and thinned to a single seedling per cell after emergence. A complete randomised block design was utilised with four replications and two plants per replication of each genotype × isolate combination. All experiments were conducted under controlled environment growth room conditions of 18±1°C day and 14±1°C night, with light intensity of 150 μE m^{-2} s^{-1}. Seedlings were grown until cotyledons were fully expanded, equivalent to growth stage 1.00 on the scale given by Sylvester-Bradley and Makepeace (1984).

Seven agar plug discs (each 5 mm^2 diam) were cut from the actively growing margin of 3 day-old colonies of *S. sclerotiorum* on PDA at 20°C and transferred to 250 ml flasks containing 75 ml of sterilised liquid medium (potato dextrose broth 24 g, peptone 10 g, H_2O 1 l). Flasks were rotated on an Innova® 2300 platform shaker at 120 rpm min^{-1}. After 3 days, colonies of *S. sclerotiorum* were harvested and washed twice with sterilised deionised water. The fungal mats obtained were transferred to 125 ml of the same liquid medium and mycelia macerated in a Breville® food grinder for 3 min. The macerated mycelial suspension was then filtered through four layers of cheesecloth and the concentration adjusted with the same liquid medium to 1×10^4 fragments ml^{-1} using a haemocytometer (SUPERIOR®, Berlin, Germany).

Inoculations Inoculations were carried out when cotyledons were 10 days-old. A total of four droplets of mycelial suspension each of 10 μl were deposited on each plant using a micropipette, with a single drop on each cotyledon lobe. While inoculating, the mycelial suspension was shaken regularly to maintain a homogeneous mycelial suspension. A 2.5 cm-deep layer of water was added at the bottom of the plastic containers to maintain high humidity. In addition, a very fine mist of water was sprayed both over cotyledons and on the inside of the container lids. Together, these procedures allowed maintenance of a relative humidity level of *ca.* 100% within the plastic containers. Following inoculation, the boxes were placed for 2 days under the benches in the controlled environment room and maintained at a low light intensity of approximately 13 μE m^{-2} s^{-1} for 4 days up to time of disease assessment. Previous studies (Garg et al. 2008) had shown this technique to be a

reliable means of determining the level of resistance in Brassica genotypes.

Disease assessment At 4 days post-inoculation (dpi), box covers were removed and lesions assessed on the basis of lesion diameter (mm) as described by Garg et al. (2008).

Data analyses The lesion rating data from initial experiment and the repeat experiment were separately analysed by analysis of variance (ANOVA) using Genstat (9th edition, Lawes Agricultural Trust). Fisher's least significant differences $P<0.05$ were used to calculate the differences between the genotypes and isolates. The relationships between the two experiments were assessed by computing Pearson correlation coefficients using the data analysis function in Microsoft Excel.

Results

Molecular identification of different isolates

Three loci (CAL-calmodulin, RAS-ras protein, IGS-intergenic spacer region) were amplified and sequenced in the SNP-based diagnostic. Two sites were chosen to be diagnostic for each species, and the sites chosen for *S. sclerotiorum* were CAL19 and CAL448. Tables 1 and 2 show the sequencing results for these sites, including representative isolates of each species and all eight test isolates. The isolates of *Sclerotinia* from Western Australia we tested had identical sequences at the SNP site to the representative isolates from *S. sclerotiorum* as listed in Tables 1 and 2. All the isolates used in this study shared SNP sequences with previously identified *S. sclerotiorum* isolates. Additionally, there was no hybridisation of the isolates used in this study at SNP sites that were diagnostic for *S. trifoliorum* and *S. minor* and no hybridisation to the probes for the negative control.

Colony characteristics of isolates

Pigmentation Of the eight isolates, three (viz. WW-1, WW-2, and WW-4) were found to be darkly-pigmented. However, intensity of melanisation varied among the pigmented isolates. Pigmentation was most pronounced in WW-1 as compared to WW-2 and WW-4 following 1 month of incubation at 20°C.

Colony diameter There were significant differences between different isolates in relation to the colony diameter measured after 24 and 48 h of incubation (Table 3). However, there was no significant correlation between pathogenicity and the colony diameter of different isolates (data not shown).

Responses of various genotypes to different isolates of *S. sclerotiorum*

Experiment 1 Small necrotic and water-soaked lesions were observed after 24 h post-inoculation,

Table 1 Sequences of Australian isolates (WW-1,2,3,4; MBRS-1,2,3,5) and representative isolates of *S. sclerotiorum, S. trifoliorum*, *S. minor* species at site CAL19, where T is diagnostic for *S. sclerotiorum*

Isolate	Sequence at CAL19
S. sclerotiorum (1980)	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
S. sclerotiorum (LMK211)	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
WW-1	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
WW-2	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
WW-3	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
WW-4	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
MBRS-1	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
MBRS-2	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
MBRS-3	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
MBRS-5	TCTTTGTAAGTTCATCTC **T** CTAACTTTTACAATCTCAG
S. trifoliorum (LMK36)	TCTTTGTGAGTTCATCTC **C** CTAACTTTTACAATCTCAG
S. minor (FA2-1)	TCTTTGTAAGTTCATCTC **C** CTGACTTTTATAATCTCAG

Table 2 Sequences of Australian isolates and representative isolates of *S. sclerotiorum, S. trifoliorum*, *S. minor* at site CAL448, where A is diagnostic for *S. sclerotiorum*

Isolate	Sequence at CAL448/500
S. sclerotiorum (1980)	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
S. sclerotiorum (LMK211)	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
WW-1	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
WW-2	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
WW-3	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
WW-4	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
MBRS-1	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
MBRS-2	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
MBRS-3	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
MBRS-5	CCATTGATTTCCCAGGTACGGC **A** AAGCATAATATAGT
S. trifoliorum (LMK36)	CCATTGATTTCCCAGGTACGGC **G** AAGCATAATATAGT
S. minor (FA2-1)	CCATTGATTTCCCAGGTACGGC **T** AAGCATGACATAGT

their size depending upon the isolate used. After 24 h, an increase in lesion size was observed across the different genotypes when inoculated with MBRS-1, MBRS-2, MBRS-3, MBRS-5, WW-1 and WW-3. In contrast, no such progression in lesion size after 24 h was observed in the genotypes when inoculated with isolates WW-2 or WW-4, with necrotic lesions only developing directly underneath where the drops of the inoculum had been applied (data not shown).

Significant differences were observed between the genotypes ($P<0.001$, Table 4) in relation to the severity of lesions on cotyledons across the isolates tested. Cultivar Charlton was identified as the most resistant cultivar with mean lesion diameter of 1.72 mm, whereas cv. Rivette was found to be the most susceptible genotype with a mean lesion diameter of 6.82 mm at 4 dpi.

Table 3 Colony diameter (cm) of different *S. sclerotiorum* isolates growing on PDA after 48 h incubation

Isolate	Colony diam
MBRS-1	7.96
MBRS-2	6.65
MBRS-3	6.67
MBRS-5	8.06
WW-1	7.64
WW-2	5.97
WW-3	6.14
WW-4	7.08

$P<0.001$; l.s.d ($P\leq0.05$)=0.72

Experiment 2 There were significant differences between genotypes ($P<0.001$, Table 5) in relation to cotyledon lesion diameter across the isolates tested. Cultivar Charlton was again found to be the most resistant cultivar with a mean lesion length of 1.24 mm and while *B. napus* lines AV-Sapphire and Ding 474 were the most susceptible genotypes with a mean lesion length of 6.2 mm.

Pathogenicity of different isolates of *S. sclerotiorum*

Significant differences were observed between eight isolates ($P<0.001$) in relation to their pathogenicity towards the sixteen *Brassica* genotypes used in Experiment 1 and Experiment 2. Overall, MBRS-5 was the most pathogenic isolate with a mean lesion length of 8.6 and 5.9 mm in Experiments 1 and 2 mm, respectively, at 4 dpi. Similarly, WW-4 was the least pathogenic isolate with a mean lesion length of 0.8 (Experiment 1) and 1.7 (Experiment 2) mm at 4 dpi.

Host × pathogen interaction

A significant ($P<0.001$) host × pathogen interaction was observed in both experiments. However, some of the genotypes (e.g., cv. Charlton) performed consistently better in relation to cotyledon lesion diameter against most of the isolates used in this study (viz. top-ranked resistant genotype for five isolates, rank 5 or 6 for two other isolates, rank 16 for the isolate which was the least pathogenic and where the differences in the lesion diameters between the more

Table 4 Experiment 1: Reaction (lesion diam, mm) and rank order (numbers in parenthesis) of twelve *Brassica napus* and four *B. juncea* genotypes from Australia and China to different isolates of *S. sclerotiorum* (viz. MBRS-1, 2, 3 and 5; WW-1, 2, 3, and 4) 4 dpi on the cotyledons

Genotype	Origin	Type	MBRS-1	MBRS-2	MBRS-3	MBRS-5	WW-1	WW-2	WW-3	WW-4	Means
AG-Spectrum	Australia	*B. napus*	6.2(9)	9.7(12)	7.1(12)	9.8(12)	5.5(8)	1.5(7)	3(7)	0.4(2)	5.3
AV-Sapphire	Australia	*B. napus*	10.1(15)	9.8(14)	7.1(12)	7.1(3)	7(10)	1.6(8)	3.6(13)	1(11)	5.8
Charlton	Australia	*B. napus*	1(1)	2.5(1)	1.7(1)	0.8(1)	1.6(1)	3.1(16)	2.5(5)	0.7(6)	1.7
Ding 474	China	*B. napus*	7.7(14)	8.5(10)	5.3(3)	9.3(8)	6.3(9)	1(3)	3.1(8)	0.3(1)	5.1
Fan168	China	*B. napus*	5.8(7)	5.9(5)	7.8(14)	9.4(9)	5.2(6)	1.2(5)	3.2(10)	0.7(7)	4.9
JM16	Australia	*B. juncea*	5.1(6)	6.7(7)	5.4(4)	10.7(13)	7.7(11)	1.3(6)	3.2(9)	0.8(8)	5.0
JN010	Australia	*B. juncea*	2.6(3)	6.1(6)	6.2(7)	9.7(11)	3(3)	0.9(1)	1(1)	0.4(3)	3.7
JN028	Australia	*B. juncea*	6.9(10)	5.8(4)	5.7(5)	7.3(4)	2.3(2)	0.9(2)	1.4(2)	0.5(4)	3.8
JR042	Australia	*B. juncea*	5.9(8)	5.7(3)	6.5(10)	9(7)	5.4(7)	1.1(4)	1.6(3)	0.6(5)	4.4
Mystic	Australia	*B. napus*	1.5(2)	4.3(2)	4.3(2)	3.9(2)	3.7(4)	1.9(10)	2(4)	1(10)	2.8
P617	China	*B. napus*	7.1(11)	9.7(13)	6.4(9)	9.4(10)	8.2(14)	2.1(11)	2.6(6)	1.3(16)	5.7
Purler	Australia	*B. napus*	4.1(4)	8.2(9)	6(6)	10.6(14)	8.3(15)	2.5(13)	4.1(14)	1.3(15)	5.6
Rivette	Australia	*B. napus*	10.1(16)	7.7(8)	8.9(15)	12(16)	8(13)	2.9(15)	4.5(16)	1(9)	6.8
RQ001-02M2	Australia	*B. napus*	7.5(12)	10.9(15)	6.2(8)	10.9(15)	11.2(16)	2.2(12)	4.2(15)	1.1(12)	6.7
RR013	Australia	*B. napus*	7.5(13)	12(16)	7(11)	8.7(6)	7.9(12)	2.7(14)	3.3(12)	1.1(14)	6.2
Zhongyou-ang No. 4	China	*B. napus*	4.9(5)	8.7(11)	9(16)	8.7(5)	5.2(5)	1.8(9)	3.3(11)	1.1(13)	5.3
Means			5.9	7.6	6.3	8.6	6	1.8	2.9	0.8	

Significance of genotypes $P<0.001$; l.s.d ($P\leq0.05$)=0.60

Significance of isolates $P<0.001$; l.s.d ($P\leq0.05$)=0.42

Significance of genotypes × isolates $P<0.001$; l.s.d ($P\leq0.05$)=1.70

Table 5 Experiment 2: Reaction (lesion diam, mm) and rank order (numbers in parenthesis) of twelve *Brassica napus* and four *B. juncea* genotypes from Australia and China to different isolates of *S. sclerotiorum* (viz. MBRS-2, and 5; WW-3, and 4) 4 dpi on the cotyledons

Genotype	MBRS-2	MBRS-5	WW-3	WW-4	Means
AG-Spectrum	3.6(5)	7.7(12)	6(9)	1.3(6)	4.7
AV-Sapphire	5.4(13)	7.8(13)	9.33(16)	2.1(12)	6.2
Charlton	1.0(1)	2.0(2)	1.36(1)	0.5(1)	1.2
Ding 474	7.2(16)	8.1(14)	7.34(13)	2.1(11)	6.2
Fan168	5.4(12)	7.6(11)	6.66(10)	2.3(15)	5.5
JM16	5(9)	6.4(8)	5.21(6)	1.3(4)	4.5
JN010	2.7(2)	6.9(9)	5.13(5)	1.2(3)	4.0
JN028	5.2(10)	8.5(16)	3.55(3)	1.4(7)	4.7
JR042	3.3(4)	5.9(7)	5.22(7)	1.3(5)	3.9
Mystic	3.3(3)	1.2(1)	2.4(2)	1.2(2)	2.0
P617	5.0(8)	4.2(5)	8.61(15)	2.8(16)	5.2
Purler	6.6(14)	5.1(6)	4.97(4)	2.0(10)	4.7
Rivette	6.6(15)	7.3(10)	6.79(12)	2.2(14)	5.7
RQ001-02M2	5.2(11)	3.5(3)	5.59(8)	1.7(8)	4.0
RR013	4.2(6)	8.4(15)	6.73(11)	2(9)	5.3
Zhongyou-ang No. 4	4.9(7)	4.1(4)	8.13(14)	2.2(13)	4.8
Means	4.7	5.9	5.8	1.7	

Significance of genotypes $P<0.001$; l.s.d ($P\leq0.05$)=0.95

Significance of isolates $P<0.001$; l.s.d ($P\leq0.05$)=0.48

Significance of genotypes × isolates $P<0.001$; l.s.d ($P\leq0.05$)=1.91

resistant genotypes were very small; Table 4). Some of the genotypes (e.g., cv. AG-Spectrum, Purler, Zhongyou-ang No. 4) were found to be strongly and differentially responsive to isolates of *S. sclerotiorum* tested, suggesting that there can be a strong interaction between genotypes and the isolates of the pathogen used in this study.

Correlation of responses of genotypes between experiments

Overall, there was a significant positive correlation (r=0.78; P<0.001, n=16, Fig. 1) between Experiments 1 and 2 for mean values for genotypes in relation to cotyledon lesion diameter.

There was significant positive correlation between Experiments 1 and 2 for individual values for cotyledon lesion diameter of different genotypes across three isolates of *S. sclerotiorum* (MBRS-2, MBRS-5 and WW-4) (r=0.79; P<0.001, n=48, Fig. 2). However, when *S. sclerotiorum* isolate WW-3 was included in analysis the r value decreased from 0.79 to 0.56, but was still significant (r=0.56; P<0.001, n=64, Fig. 3). There was a significant positive correlation in relation to diameter of the lesions on cotyledons for various genotypes inoculated with MBRS-1 in this study when compared with responses for these same genotypes reported previously (Garg et al. 2008; r=0.84, P=0.002, n=10, Fig. 4).

Discussion

In comparing the colony characteristics of the eight isolates of *S. sclerotiorum* isolated, we obtained three darkly-pigmented colonies (viz. WW-1, WW-2, and

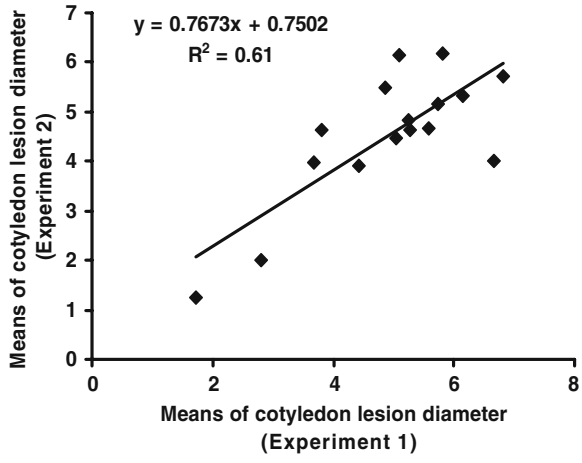

Fig. 1 Correlation between experiments 1 and 2 for overall mean values of diameter of cotyledon lesions in each experiment across each of the twelve *B. napus* and four *B. juncea* genotypes 4 dpi with *S. sclerotiorum*

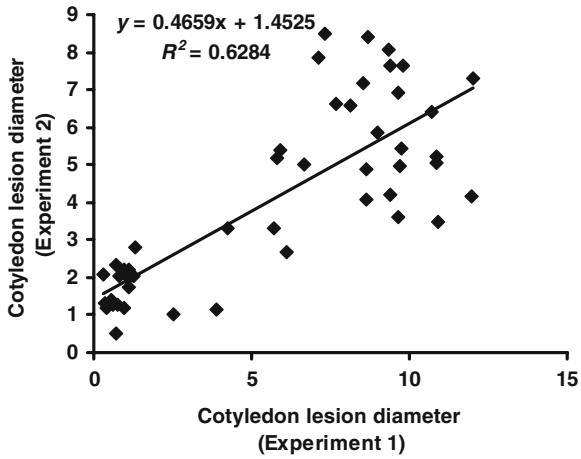

Fig. 2 Correlation between experiments 1 and 2 for individual values for cotyledon lesion diameter 4 dpi with three isolates of *S. sclerotiorum* (viz. MBRS-2, MBRS-5 and WW-4) onto twelve *B. napus* and four *B. juncea* genotypes

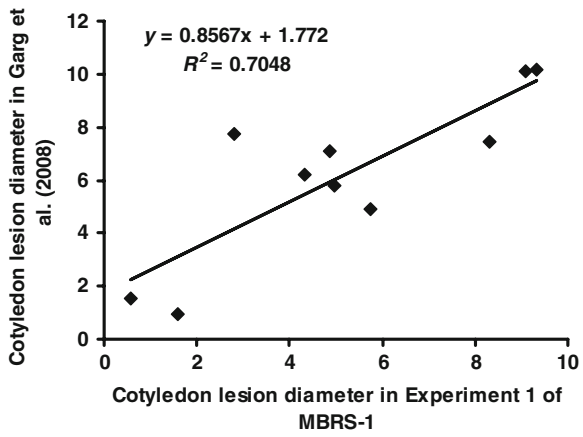

Fig. 4 Correlation of data for diameter of cotyledon lesions on ten *B. napus* genotypes 4 dpi with *S. sclerotiorum* in experiment 1 with data taken from Garg et al. (2008) when inoculated with MBRS-1

WW-4). We, however, found no correlation between pathogenicity and either pigmentation or colony diameter (data not shown). This is the first report of the occurrence of darkly pigmented mycelial isolates of *S. sclerotiorum* on Brassicas in Australia. Three of our isolates (viz. WW-1, WW-2, and WW-4) with varying intensities of pigmentation were identified from the Walkaway region of Western Australia. Of the three pigmented isolates we found, WW-1 was relatively more pathogenic than isolates WW-2 and WW-4. A darkly-pigmented isolate was also identified as pathogenic by Sanogo and Puppala (2007). However, our results demonstrate that pigmentation and colony diameter do not necessarily relate to pathogenicity of *S. sclerotiorum*. This is, however, in contrast to previous reports, for example, Zhou and Boland (1999) examined factors affecting virulence and found that while a hypo-virulent isolate showed reduced mycelial growth, pathogenicity was mainly affected by reduced or delayed production of oxalic acid.

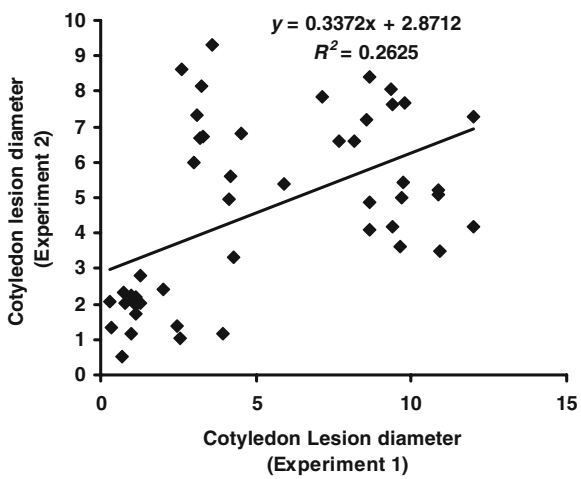

Fig. 3 Correlation between experiments 1 and 2 for individual values of cotyledon lesion diameter 4 dpi with four isolates of *S. sclerotiorum* (viz. MBRS-2, MBRS-5, WW-3, WW-4) onto twelve *B. napus* and four *B. juncea* genotypes

There were significant differences observed among different *Brassica* genotypes when challenged by different strains of *S. sclerotiorum* and also among different isolates in relation to their pathogenicity as measured by cotyledon lesion diameter on different *Brassica* genotypes. There was also a significant host × pathogen interaction in both experiments. It is noteworthy that some genotypes showed consistent host responses irrespective of the isolates of *S. sclerotiorum* used in this study, whereas others showed a variable pattern of responses depending upon the isolate used. For instance, in terms of lesion length, cv. Charlton consistently showed a high level of resistance to most of the isolates. It is interesting that where variable ranking for cv. Charlton was observed, it was against the *S. sclerotiorum* isolates that were least pathogenic (viz. WW-2, WW-3 and WW-4). Similarly, cv. Rivette and RQ001–02M2 were also consistent in terms of their responses, showing susceptible reactions against most of the isolates used (Table 4). In contrast, the ranking of certain other genotypes (e.g., Zhongyou-ang No. 4, AV-Sapphire) was highly variable in response to

inoculation with different isolates of *S. sclerotiorum*. These particular genotypes showed responses ranging from resistant to highly susceptible, depending upon the isolate. Genotypes that show the most consistent and promising responses across a range of *S. sclerotiorum* isolates should be used as standards in disease screening programmes and in commercial breeding programmes, as these are the most likely genotypes to perform consistently across different national and international geographic locations. Such differences in responses of genotypes depending upon the isolate, also suggest that resistance in Brassicas against *S. sclerotiorum* is most likely to be polygenic.

There are very few published reports of significant genotype × *S. sclerotiorum* interactions of the type we observed in our study, probably because in most of previous studies either more isolates were included but with only a very small number of genotypes or *vice-versa* (e.g., Riddle et al. 1991; Ekins et al. 2007; Morrall et al. 1972; Auclair et al. 2004; Pratt and Rowe 1995). It is interesting that no significant interaction was observed between *Glycine max* and *S. sclerotiorum* isolates (Auclair et al. 2004) in Canada where five host cultivars and four pathogen isolates were involved or with five isolates and seven alfalfa cultivars (Pratt and Rowe 1995) in the USA. The host genotypes we used in our study were representative of a wide genetic diversity of *Brassica* germplasm available from Australia and China, which could have enhanced the significant host-pathogen interactions observed.

Significant differences were observed among the isolates of *S. sclerotiorum* in relation to their pathogenicity. At 4 dpi, some isolates were less pathogenic (WW-2, WW-4) irrespective of the host genotypes tested, whereas others (MBRS-5, MBRS-2) were highly pathogenic to almost all of the *Brassica* genotypes evaluated in this study. This may be related to some form of physiological specialisation in *S. sclerotiorum* in Western Australia. This further illustrates the advantage of screening *Brassica* genotypes with a range of *S. sclerotiorum* isolates so that the reaction of different host genotypes can be precisely identified. Where such a spectrum of isolates is not readily available, it is best to use a highly pathogenic isolate such as MBRS-1, as used by Li et al. (2006, 2007).

We used only eight isolates in this study. However, similar differences among *S. sclerotiorum* isolates have been reported by other workers even where a larger number of isolates were investigated (e.g., Riddle et al. 1991; Ekins et al. 2007; Morrall et al. 1972). While variations in pathogenicity have been reported among isolates, the differences did not justify grouping of *S. sclerotiorum* isolates on the basis of their pathogenicity. This is further supported by the observations of Melzer and Boland (1996) working on lettuce and Morrall et al. (1972) on 23 different hosts, who defined a 'continuum' (in contrast to distinct categories) in the pathogenicity of this pathogen. Even where significant differences in pathogenicity among isolates of this pathogen occur, their responses were found to be overlapping (e.g., Riddle et al. 1991; Ekins et al. 2007).

We found that some isolates (e.g., MBRS-2, MBRS-5 and WW-4) behaved consistently across the two experiments as compared to others (e.g., WW-3). Differences in pathogenicity of this pathogen observed between repeated experiments have also been reported by other workers e.g., Pratt and Rowe (1995) on alfalfa; Errampalli and Kohn (1995) on canola; Brenneman et al. (1988) on peanuts, when only a few isolates were tested, or when large number of isolates were involved (e.g., Ekins et al. 2007 on sunflower; Riddle et al. 1991 on dandelion).

In conclusion, this study is the first to identify darkly-pigmented isolates of *S. scleortiorum* in Australia, although the pathogenicity of the pathogen was not influenced by pigmentation or colony diameter of the isolates. Significant differences in pathogenicity were observed between different isolates across different genotypes, suggesting a form of physiological specialisation occurs in this pathogen in Western Australia. For this reason, it is best to include more than one *S. sclerotiorum* isolate in any germplasm screening programme. Furthermore, genotypes such as cv. Charlton which showed consistent resistant reactions to different isolates in our study, and also showed useful resistance under field conditions (Li et al. 2006), are those most likely best suited for commercial exploitation of this resistance in oilseed rape breeding programmes. This is especially so in relation to developing cultivars for deployment in areas where physiological specialisation occurs.

Acknowledgements The first author gratefully acknowledges the financial assistance from the Australian Centre for International Agricultural Research by way of a John Allwright PhD Fellowship. We thank Dr Caixia Li for provision of the eight isolates of *S. sclerotiorum* used in this study and for advice on statistical analyses.

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