Search for resistance to *Sclerotinia sclerotiorum* in exotic and indigenous *Brassica* germplasm

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ABSTRACT

The fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary is distributed worldwide and causes rot in *Brassica* and 400 more plant species. Application of fungicides, bioagents and crop rotation are currently the major methods of controlling this disease. However, fungicides are expensive, environmentally unsafe, and not always effective. Locating effective sources of host resistance offers the best long-term prospect for improved management of this disease. For these reasons, indigenous and exotic genotypes of *Brassica napus* and *B. juncea* were screened in the *Sclerotinia*-infested field for resistance to *S. sclerotiorum* at DRMR, Bharatpur using stem inoculation and spray-inoculation techniques. Results indicated significant differences among susceptible and tolerant genotypes. Among the *B. juncea* genotypes, EC 597328 showed high tolerance, with a mean stem lesion length of <0.05 cm compared to 26.75 cm in the susceptible check cv. Rohini.

Key words: Disease resistance, *Sclerotinia sclerotiorum, Brassica*

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary is a ubiquitous necrotrophic fungal pathogen capable of infecting about 408 plant species among 75 families (Boland and Hall, 1994). It causes rot disease and is considered to be one of the most damaging pathogen. *Sclerotinia* rot causes an estimated annual loss of US $ 200 million in the US alone (Bolton et al*,* 2006). In India, earlier it was considered to be minor problem. But now it has become a serious problem in some parts of the country like Punjab, Haryana, Rajasthan and Bihar. This disease gained importance particularly in areas where farmers practised monocropping of Indian mustard, which led to complete crop failure with more than 80 per cent disease incidence recorded in some parts of Punjab and Haryana. *Sclerotinia* has the potential to cause up to 39.4% reduction in yield (Chattopadhyay et al*.,* 2003)

Sclerotinia sclerotiorum overwinters as mycelia within plants or as sclerotia. The sclerotia germinate and form apothecia, which produce asci. Ascospores discharged from the apothecia in soil at the base of the plants constitute an important primary source of infection. Two main pathogenicity factors, the secretion of oxalic acid and hydrolytic enzymes, work in concert to bring about the maceration of plant tissues and subsequent necrosis (Collmer and Keen, 1986). Oxalic acid acidifies and sequesters calcium in the middle lamellae creating an environment ideal for activity of cellulolytic and pectinolytic enzymes such as endopolygalacturonase, exopolygalacturonase and pectin methylesterase.

The aim of the present study is to determine the differential responses of *Brassica* germplasm from India, China and Australia to identify the source of host resistance and also to evaluate the expression and relationship of resistance to *Sclerotinia.*

MATERIALS AND METHODS

Germplasm

Fifty-six *Brassica* genotypes were obtained from India, China and Australia for screening against *Sclerotinia sclerotiorum* during 2007-08, 2008-09 crop seasons through the Australian

Centre for International Agricultural Research (ACIAR)-funded programme. Genotypes were sown on 25 Oct 2007, 30 Oct. 2008 in paired rows of 3m with 30 cm x 10 cm spacing using cv. Rohini of Indian mustard as susceptible check.

Sclerotinia sclerotiorum inoculum

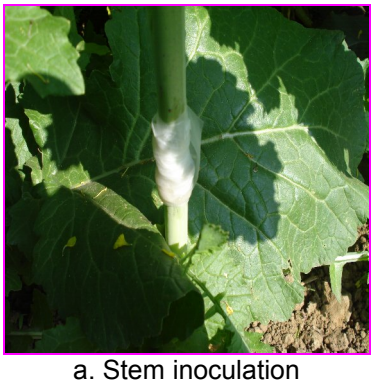
Bharatpur isolate of *Sclerotinia sclerotiorum* BHP was originally collected from infected *B. juncea* from the experimental farm of the Directorate of Rapeseed Mustard Research, Bharatpur, India. A single sclerotium of Bharatpur isolate of *Sclerotinia sclerotiorum* was surface sterilised in 1% (v/v) sodium hypochlorite and 70% ethanol for 4 min followed by three washes in sterile distilled water for 1 min as described by Clarkson et al. (2003). The sclerotium was cut in half and placed on potato dextrose agar (PDA) under sterilized conditions. *S. sclerotiorum* was sub-cultured and maintained at 25°C on PDA under 12 h alternate fluorescent light in culture room.

Inoculation

Pathogen inoculum was mass multiplied in laboratory on autoclaved *Sesbania* leaves in glass jars and artificially mixed with FYM in soil prior to sowing. The test lines were sprayed by automizer at 45 days after sowing with mycelial suspension of the pathogen after growing them in the laboratory on potato dextrose broth. Further, the plants were inoculated at 60 days after sowing on the stem with the pathogen growing on agar blocks and tied to the stem with parafilm (Li et al., 2007).

Disease scoring

Disease incidence was assessed by recording the average size of the lesion on stem and diameter of the stem. Sclerotinia rot intensity of 10 randomly selected plants was recorded by using 0-4 scales. The scale is 0= healthy or no visible symptoms; 1= 0.1-2 cm lesion length on stem; 2= 2.1-4 cm lesion length on stem; 3= 4.1-6 cm lesion length on stem, and 4= > 6 cm lesion length on stem or complete dry plant.



a. Stem inoculation

b. Stem girdling

c. Sclerotia development

d. Necrotic spot

Fig 1. Sclerotinia inoculation, girdling, sclerotia development and the appearance of necrotic stem spots.

RESULTS

Since the pathogen has systematic and aerial infection at both stages by myceliogenic and carpogenic germination of sclerotia, the symptoms of disease development appeared after 10-15 days of inoculation. None of the *Brassica* germplasm lines/accessions exhibited complete resistance to *Sclerotinia* rot. The genotypes of *B. juncea* namely, EC 597328 (Montara), EC 597329 (Berry) and EC 597331 (Ringot I) of Chinese origin were tolerant whereas, none of the Indian lines were tolerant (table 1). In *B. napus*, EC 597258 (BLN 3343) of Australian origin was observed tolerant. The level of tolerance also varied among the genotypes.

The stem diameter of the plants were from 0.53 to 3.70 cm, with the range of < 0.60 cm one, 0.61-1.09 cm eleven, 1.10-150 cm twenty three, 1.51-2.00 cm thirteen, 2.01-2.50 cm five and > 2.51 cm were two. Hence, the majority falling in the range of 1.10-1.50 cm.

Table 1. Response of *Brassica* genotypes for *Sclerotinia sclerotiorum* resistance (DRMR, Bharatpur isolate) (continued next page)

Genotype	Source	Origin	Avg. Size of lesion (cm)	Avg. Diameter of stem (cm)
EC-597254 (CB Tanami)	*B. napus*	Australia	4.78	1.00
EC-597256 (BLN3245)	*B. napus*	Australia	2.40	1.58
EC-597258 (BLN3343)	*B. napus*	Australia	0.28	1.15
EC-597259 (BLN3344)	*B. napus*	Australia	3.68	1.58
EC-597260 (BLN3345)	*B. napus*	Australia	1.25	0.53
EC-597265 (BLN3189)	*B. napus*	Australia	2.25	2.05
EC-597266 (BLN3348)	*B. napus*	Australia	0.50	1.70
EC-597269 (BLN3352)	*B. napus*	Australia	1.00	1.35
EC-597273 (BLN3579)	*B. napus*	Australia	1.98	1.10
EC-597274 (BLN3630)	*B. napus*	Australia	12.90	1.90
EC-597275 (RT006)	*B. napus*	Australia	12.25	1.30
EC-597276 (RT057)	*B. napus*	Australia	3.80	1.70
EC-597277 (RT076)	*B. napus*	Australia	1.13	1.35
EC-597279 (RT117)	*B. napus*	Australia	4.83	2.40
EC-597283 (RT125)	*B. napus*	Australia	1.15	1.00
EC-597294 (ZY002)	*B. napus*	Australia	1.55	1.35
EC-597311 (JM06003)	*B. juncea*	Australia	0.75	0.95
EC-597312 (JM06004)	*B. juncea*	Australia	2.15	1.75
EC-597313 (JM06006)	*B. juncea*	Australia	4.83	1.73
EC-597314 (JM06009)	*B. juncea*	Australia	1.45	1.34
EC-597315 (JM06010)	*B. juncea*	Australia	22.10	1.15
EC-597316 (JM06011)	*B. juncea*	Australia	1.25	1.21
EC-597317 (JM06012)	*B. juncea*	Australia	0.92	1.04
EC-597318 (JM06013)	*B. juncea*	Australia	1.85	1.35
EC-597319 (JM06014)	*B. juncea*	Australia	19.45	1.20

Table 1 (cont.). Response of *Brassica* genotypes for *Sclerotinia sclerotiorum* resistance (DRMR, Bharatpur isolate)

Genotype	*Source*	Origin	Avg. Size of lesion (cm)	Avg. Diameter of stem (cm)
EC-597320 (JM06015)	*B. juncea*	Australia	0.70	1.40
EC-597321 (JM06018)	*B. juncea*	Australia	1.55	1.25
EC-597322 (JM06019)	*B. juncea*	Australia	5.45	1.20
EC-597323 (JM06020)	*B. juncea*	Australia	1.80	1.25
EC-597324 (JM06021)	*B. juncea*	Australia	2.10	1.30
EC-597325 (JM06026)	*B. juncea*	Australia	3.50	1.40
EC-597328 (Montara)	*B. juncea*	Chinese	0.05	2.95
EC-597329 (Berry)	*B. juncea*	Chinese	0.40	2.05
EC-597295 (ZY003)	*B. napus*	Chinese	2.63	1.05
EC-597305 (ZY013)	*B. napus*	Chinese	2.63	1.25
EC-597306 (ZY014)	*B. napus*	Chinese	1.15	1.33
EC-597307 (ZY015)	*B. napus*	Chinese	7.45	1.35
EC-597331 (Ringot I)	*B. juncea*	Chinese	0.45	1.90
EC-597339 (Yilihuang)	*B. juncea*	Chinese	1.50	1.25
EC-597341 (Jinshahuang)	*B. juncea*	Chinese	6.90	0.70
EC-597342 (Manasihuang)	*B. juncea*	Chinese	1.85	2.05
EC-597344 (Brassica juncea 2)	*B. juncea*	Chinese	1.35	1.65
EC-597345 (Brassica juncea 3)	*B. juncea*	Chinese	0.75	1.20
EC-597327-R1-P4-S	*B. juncea*	Chinese	1.40	1.63
EC-597327-R1-P5-S	*B. juncea*	Chinese	0.57	1.58
EC-597327-R2-P1-S	*B. juncea*	Chinese	1.20	1.51
EC-597327-R2-P2-S	*B. juncea*	Chinese	1.50	1.67
EC-597329-R2-P3-S	*B. juncea*	Chinese	1.05	2.09
EC-597329-R2-P4-S	*B. juncea*	Chinese	0.40	1.62
Rohini	*B. juncea*	Indian	26.75	3.70
GSC-5	*B. juncea*	Indian	2.75	0.85
TERI(00) R 9903	*B. juncea*	Indian	3.82	0.75
JM-1	*B. juncea*	Indian	1.41	0.75
MAYA	*B. juncea*	Indian	4.70	0.65
RGN13	*B. juncea*	Indian	2.70	0.75
Bio-902	*B. juncea*	Indian	0.80	1.10
CD ($P < 0.05$)			2.610	0.643

DISCUSSION

To date, complete resistance to the pathogen has not been identified, although partial resistance was reported in *B. napus* cv. Zhongyou 827 (Buchwaldt et al., 2003). The heritability of *Sclerotinia* resistance is high in *B. napus* controlled by nuclear genes and unlinked to low erucic acid trait. Nine genotypes viz. Cutton, ZYR 6, PSM 169, PDM 169 Wester, PYM 7, Parkland, Tobin and Candle showed resistance to Sclerotinia rot in India (Shivpuri et al. 1997). *Brassica napus* and *B. juncea* cv. Rugosa have been reported to possess resistance against

Sclerotinia rot in the field as well as in green house conditions (Singh et al., 1994). Four genotypes viz. PCR 10, RW 8410, RW 9401 and RGH 8006 had resistance against *S. sclerotium* as compared to susceptible check (Pathak et al, 2002). It has been observed that in resistant cvs of *B. juncea*, there is more accumulation of phenolics at the infection site in the infected stems and relatively low level of enzyme activity as compared to that of susceptible cvs. Based upon the available level of tolerance, it is advocated that the identified genotypes could be utilized to further enhance the level of tolerance for incorporating resistance against *Sclerotinia* rot.

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