

Field evaluation of Indian and exotic oilseed *Brassica napus* and *B. juncea* germplasm against *Sclerotinia* stem rot

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ABSTRACT

Sclerotinia stem rot, incited by *Sclerotinia sclerotiorum* (Lib) De Bary, continues to be a serious disease across oilseed *Brassica* growing areas in India, China and Australia. As it is not possible to manage this disease using chemical or cultural means, sources of host resistance need to be identified against this pathogen. Germplasm, comprising genotypes of *Brassica napus* (L.) and *Brassica juncea* (L.Czern & Coss.) from Australia, China and India were screened under field conditions in north India. Using a stem inoculation test, resistance sources were identified among genotypes from Australia including the cultivars 'Ag Spectrum' and 'Ag Outback', accessions 'RR002' and 'JM 018' and from China including accessions 'RH 13', 'Ringot', 'Brassica juncea 1' and 'Brassica juncea 2'. Using a soil inoculation test, sources of resistance in *B. juncea* genotypes from India were also identified including 'Sanjuncta asesh', 'PCR 7', 'RH 819' and 'Vardan'. These sources of resistance will be useful towards developing new cultivars of *B. napus* and *B. juncea* with improved host resistance to *S. sclerotiorum*.

Key words: *Sclerotinia sclerotiorum*, *Brassica napus*, *Brassica juncea*, Sclerotinia stem rot

Sclerotinia stem rot, a major disease of oilseed *Brassica* has been recorded with incidences up to 80% in the Punjab and Haryana (Kang and Chahal 2000). In Haryana, 5–20% plants affected by Sclerotinia stem rot are common, depending upon crop growth stage and region (Singh *et al.* 2008). Estimated yield losses from Sclerotinia stem rot vary throughout the world, such as 13% in North Dakota and Minnesota (Lamey and Bradley 2002); from approximately 20% (Fitt *et al.* 1992) up to 50% (Pope *et al.* 1989) in the UK; from 0.4–1.5 tonnes/ha in Australia (Kirkegaard *et al.* 2006) and 10–70% in China (Deng and Tang 2006).

Sclerotinia of *S. sclerotiorum* can germinate either myceliogenically or carpogenically, giving rise to stem base infection and aerial infections, respectively. Being a ubiquitous necrotrophic pathogen with many different hosts Sclerotinia stem rot is difficult to manage. The primary method of management relies upon use of non-host crops, fungicide application and manipulation of management

practices, but all have proved unreliable and frequently of little, if any economic benefit. Genetic resistance to Sclerotinia stem rot is needed if usage of fungicides is to lessen and productions of oilseed *Brassica* crops are to become more profitable. For this reason, using stem and soil inoculation techniques, we evaluated oilseed *Brassica* germplasm from *Brassica napus* and *B. juncea* from India, China and Australia for resistance to *S. sclerotiorum*.

MATERIALS AND METHODS

Seed of accessions of *B. napus* and *B. juncea* was obtained from Australia, China and India through an Australia Centre of International Agricultural Research (ACIAR) collaborative programme between these 3 countries. The *B. napus* and *B. juncea* genotypes tested are as listed in Tables 1, 2.

Experiment I

Ninetyeight genotypes (29 *B. napus* and 69 *B. juncea*) were tested in the field at the Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. The germplasm was hand sown on 23 October 2007 and 27 October 2008 in plot size of 5 m×2 m. Row-to-row and plant-to-plant spacing of 45 cm×15 cm was maintained throughout the experiment. All recommended agronomic practices were followed, and these included 100 kg N/ha, 30 kg P₂O₅/ha fertilizer application at sowing, 2 irrigation at 45 and 90 days

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Table 1 Evaluation of Oilseed Brassica germplasm of Indian and Exotic origin under field condition at CCS HAU-Hisar by mycelial agar plug inoculation technique and their clustering

Cluster	Genotype	Mean plant age at time of inoculation and range (days)	Mean stem diameter and range(mm)	Mean stem lesion length and range (mm)	Percent dead/wilted plants and range	D ² value
I	JN004, JO008, JN031, JN033, JM06002, JM06012, JM06013, JM06021, JM06026, JM06006, JM06014, JM06015, JM06011, JM016, JM06010, JO009, JR042, JN010, JN032, JM06020 (A, Bj)*, Vasundera, RGN13, Bio902, Aravali, Jagannath, Swaran jyoti, Kranti, GM2, Geeta, JM 1, Pusa mahak, (I, Bj)	81 (76–84)	11 (8–16)	92 (70–118)	38.16 (26.66–53.33)	1.17
11	JR049, JM06001, JN028 (A, Bj), CS 54, Ashirwad, GM 3, Basanti, CS 52 (I, Bj)	79(78–81)	9(8–18)	73(48–85)	25.33(20–30)	1.51
111	Laxmi, Narendra ageti, JM 2, JM 3, Maya, Urvashi (I, Bj), JM06004, JM06003 (A, Bj)	78 (77–81)	10 (8–12)	118 (108–150)	60.00 (53.33–73.33)	1.08
IV	Trigold, RR013, Trilogy, RR009 (A, Bn), Datonghuangiie, RL, Loiret, Montana, AmoraIII, Jinshahuang, Tunliuhuangiie, TQ0055–02W2, Yilihuang, Qianxianjiecai (C, Bj), Teri 9903, GSL 1 (I, Bn)	103 (99–105)	16 (11–20)	104 (78–140)	43.56 (30–56.66)	1.25
V	Surpass 400, Scar, Mystic (A, Bn), JM06018, JM06019 (A, Bj)	88 (81–102)	20 (18–24)	122 (95–150)	64.46 (56.66–70)	1.95
V1	Rivette, RR005, Lantern, RQ 001–02M2, Purler, Skipton, RR001(A, Bn), Hatianyocai (C, Bj)	102 (101–105)	22 (19–25)	91 (74–105)	41.11 (26.66–46.66)	1.02
V11	Monty, RR002, Ag Outback, Ag Spectrum (A, Bn), Manasihuang, Brassica juncea 2, Brassica juncea 1, Brassica juncea 3, RH 13, Ringot (C, Bj), JM018 (A, Bj)	102 (85–106)	15 (11–21)	20 (2–30)	3.00 (0–10.00)	1.14
V111	Rainbow, Tranby, RQ011, Av Sapphire, Charlton, BST–702N2 (A, Bn)Ekla, RK 2, Haoyou II, Berry (C, Bj), GSC 5, HNS0501 (I, Bj)	101 (96–102)	16 (14–23)	70 (42–110)	24.5 (10–36.66)	1.10

Fig in parenthesis (*) indicate A=Australia, C=China, I=India, Bj=Brassica juncea, Bn=Brassica napus

after sowing in sandy loam soil having pH 8.0 and electrical conductivity 3.5 mMhos/cm. A complete randomised block design with 3 replicates was used.

The single isolate of *S. sclerotiorum* ('CCS HAU-Hisar') used was isolated from sclerotia collected from diseased *B. juncea* plants in previous crop season. Collected sclerotia were dried in sun for a week at 35–40°C and stored in petriplates at room temperature (25°C) for further future use. Sclerotia were purified with 0.1% mercuric chloride solution in distilled water under aseptic condition in laminar flow and raised on potato dextrose agar medium in 90 mm sterilized petriplate (200 g peeled potato, 20 g dextrose and 20 g Agar-agar in 1litre distilled water and autoclaved at 121.7 °C for 15 min). Thirty plants within each replication were arbitrarily picked and inoculated at the flowering stage (when 50% of plants in the row had at least one opened flower; at GS 61 BBCH Scale of Lancashire *et al.* (1991) or GS 41–42 Scale of Harper and BerkenKamp (1975). Stem

inoculation was carried out according to the method used by Buchwaldt *et al.* (2005) in both crop seasons. A single 5 mm diameter agar plug disc cut from the *S. sclerotiorum* colony edge of a 3–4-days old-culture growing on a glucose rich medium (Glucose 20 g, NaOH 1 g, NH₄ NO₃ 2 g, MgSO₄ 7 H₂O 0.1 g, Malic acid 3 g, KH₂ PO₄ 1 g and Agar-agar 30 g in 1 litre of distilled H₂O) was used to inoculate each plant. The agar plug was placed, mycelium side up, on a small piece of parafilm (about 5 cm long) and the plug (with the mycelium in contact with stem) was then secured on the first internode above middle internode of each stem by twisting the ends of the parafilm strip around the stem. Care was taken not to crush the agar plug. A wet cotton swab was also wrapped around the stem just above the top of parafilm strip to maintain high humidity during the infection period.

Stem diameter was measured by Vernier callipers on all inoculated stems after inoculation as reported by Li *et al.* (2006). Stem lesion length (mm) was measured daily with

Table 2 Evaluation of oilseed *Brassica* germplasm of Indian and Australian origin under field condition at DWR (ICAR), Regional Station, Dalang Maidan Lahul and Spiti (Himachal Pradesh) India by soil inoculation technique and their clustering

Cluster	Genotype	Per cent disease incidence and range	Stem diameter of infected plant and range (cm)	D ² Value
I	JN042, JM018 (A, Bj) RR002 (A, Bn), Vaibhav, RH 819, Sej2, Vardan, PCR 7, Sanjuncta asesh (I.Bj)*	2.50 (0.5–5.0)	0.75 (0.63–0.82)	1.02
II	JN031, JN010 (A, Bj) Purler, RR005, Skipton, TQ055–02W, Trigold, Charlton, Surpass 400, RR001(A, Bn)	6.10 (5.6–7.0)	0.91 (0.80–0.95)	0.48
III	JO009, JR049, JR042, JO006 (A, Bj), Rainbow, Ag Outback, Monty (A, Bn), RH 781(I.Bj)	3.50 (2.30–5.00)	1.0 (0.95–1.05)	0.55
IV	Lantern, RR013, BST 702N2, RQ001–2M2, Ag Spectrum (A, Bn), GSL 2(I, Bn), Neelam (I, Bj)	5.70 (4.00–7.50)	1.20 (1.10–1.30)	0.94
V	GSL 1, HNS0501, HNS0502, HNS0401, HNS09605 (I, Bn)	8.40 (7.50–10.0)	1.30 (1.20–1.50)	0.91
VI	JN028, JN033, JN032 (A, Bj), RLM 619, PBR 91, PBR97, RL1359, Kranti, Pusa Bold, Seetha, GM 1, Durgamani(I.Bj)	7.10 (6.0–10.0)	0.66 (0.61–0.82)	0.75
VII	Varuna (I, Bj), RR009, Rivette, Mystic (A, Bn)	10.50 (9.50–12.50)	1.10 (1.05–1.20)	0.79
VIII	Trilogy, RQ011, Oscar, Tranby, Av Sapphire (A, Bn) RH 30, RH8113, RH8812, Rohini, (I, Bj)	10.10 (8.30–12.50)	0.89 (0.78–0.95)	0.81

Fig in parentheses (*) indicate A, Australia; C, China; I, India; Bj, *Brassica juncea*; Bn, *Brassica napus*

ruler up to 9 weeks after inoculation (wai) but data presented are of 3 weeks after inoculation were categorized resistant and susceptible as this has been shown to be the time most ideal to demonstrate differences in host responses to the pathogen (Li *et al.* 2007). Average/mean of both crop seasons data is being presented. During test period, there was no natural rainfall, temperature ranged from –3.2 to 23°C and relative humidity ranged from 29 to 100%. The dates of inoculation and of flowering for each genotype were recorded.

Experiment 2

Experiment was conducted at the Directorate of Wheat Research (ICAR) Regional Station at Dalang Maidan Lahul Spiti, in Himachal Pradesh (India). Seed of 32 lines of *B. napus* and 32 lines of *B. juncea* were hand sown in plot size 5 m × 2 m on 25 May 2007. The row-to-row and plant-to-plant spacings were maintained as in Experiment 1. Each furrow/row was inoculated with 20 sclerotia randomly of a single isolate of *S. sclerotiorum* ('DWR, R.S. Lahul Spiti') at the same date as sowing. Sclerotia were collected from diseased *B. juncea* plants in previous crop season. As in experiment 1, collected sclerotia were dried in sun for a week at 35–40°C and stored in Petriplates at room temperature for further future use. Sclerotia were purified as in experiment 1. Purified sclerotia were used for soil inoculation. Test genotypes were arranged in a randomized complete block design with 3 replicates. The per cent incidence of SSR was calculated on basis of per cent infected plants mortality at crop maturity of GS 89 BBCH Scale described by Lancashire *et al.* (1991) or GS 54–55 Scale (Harper and BerkenKamp 1975). Stem diameter at the third basal internode was measured by Vernier callipers of infected plants (Li *et al.*

2006). All infections were from myceliogenic sclerotial germination that subsequently resulted in a basal stem rot.

A single factor of variance was conducted using SPSS 7.5. The relationship between stem lesion length and percentage of plant dead from infection was tested using the regression and linear models procedure of SPSS. The relationship between percentage of plant death and stem diameter was assessed by quadratic and linear regression analysis as appropriate using SPSS 7.5 for window software. The pooled data were subjected to D² analysis (Mahalanobis 1930). Genotypes were grouped into different clusters as described by Rao (1952).

RESULTS AND DISCUSSION

Experiment 1

Inoculated stems with the *S. sclerotiorum* mycelial discs showed symptoms of a water soaked bleached lesion after 4–5 days after inoculation. White cottony mycelial growth was observed on stem surface in some plants. Sclerotia also formed both inside and outside of infected stems by the time of stem lesion length progressed after 3 weeks of inoculation in some genotype. There were significant differences among genotypes in relation to stem lesion length (3 cm) by 3 wai. Based on stem lesion length and percentage of plant dead/wilted (<10%) observed by Li *et al.* (2006), resistant genotypes of *B. juncea* included 'RH13', 'Ringot', 'Manasihuang', 'Brassica juncea 1', 'Brassica juncea 2', 'Brassica juncea 3' from China and 'JM 018' from Australia. For *B. napus* germplasm tested, 'Ag Outback', 'Monty', 'RR002', 'Ag Spectrum' from Australia showed useful resistance of Cluster VII (Table1). There were no significant differences among tested genotypes in respect of percentages of plant death. There was no significant relationship between

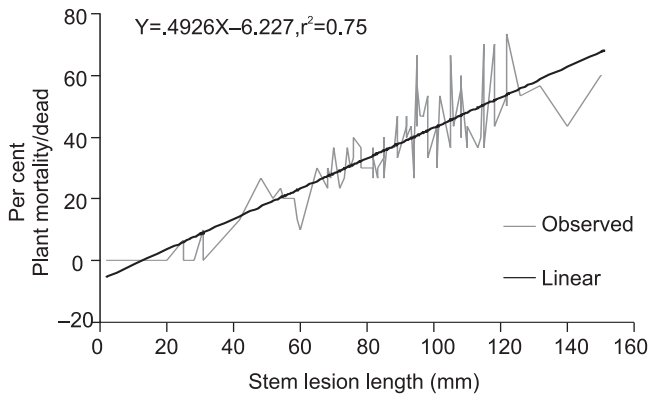


Fig 1 The relationship between stem lesion length at 3 weeks after inoculation with *Sclerotinia sclerotiorum* mycelia agar plug disc and percentage plant mortality/dead at 9 weeks after inoculation ($r=0.86$, $p<0.005$, $n=98$) at CCSHAU-Hisar

stem lesion length and stem diameter after 3 weeks of inoculation ($r^2=0.028$) and mortality and stem diameter after 9 weeks of inoculation by mycelial agar plug disc ($r^2=0.027$). Stem lesion length was significantly and positively correlated with percentage of plant death at maturity for both mycelial plug inoculations ($r^2=0.75$, $P=0.05$, $n=98$) (Fig 1).

Experiment 2

Genotypes 'JM018', 'JO009', 'JR042' from Australia, and 'Sanjuncta asesh', 'PCR 7', 'RH819' and 'Vardan' from India expressed useful resistance with < 3% plant death following soil inoculation of Cluster I and Cluster III (Table 2). There was no significant relationship between stem diameter and plant death ($r^2=0.019$).

Genotypes from India, China and Australia tested expressed varying levels of resistance to SSR (Table 1). Following stem inoculation by the mycelia disc technique, stem lesion length and incidence of plant death were important parameters to measure comparative resistance in genotypes by also considering/measuring the stem diameter as used by Buchwaldt *et al.* (2005), Li *et al.* (2004) and Li *et al.* (2006). Present observations also confirm that the stem inoculation technique is useful for identification of field resistance in *B. napus* and *B. juncea* germplasm as found earlier by Li *et al.* (2004, 2006 and 2007). *B. juncea* genotypes were overall more susceptible than *B. napus* in the genotypes tested by either inoculation technique. The strong relationship between stem lesion length and percentage of plant death was noteworthy, as plant mortality is directly related to crop yield losses. This highlights the need to record plant mortality in addition to stem lesion length in all screening tests for *Sclerotinia* stem rot resistance and our findings corroborate those earlier of both Li *et al.* (2007) and of Wegulo *et al.* (1998). Our study also showed that there was no significant quadratic relationship between stem lesion length and stem diameter after 3 wai and plant death/mortality to stem diameter after 9 wai, but a significant positive linear

relationship between plant death and stem lesion length by following mycelial agar plug disc inoculation technique in first experiment. On the basis of the screening technique of Li *et al.* (2006), Li *et al.* (2007) and Buchwaldt *et al.* (2005) having < 3 cm stem lesion length after 3 wai and <10% plant death were categorized as showing useful resistance, the *B. napus* genotypes 'Ag Outback', 'Monty', 'Ag Spectrum', 'RR002' and 'JM018' of *B. juncea* from Australia, 'RH 13', 'Ringot', 'Brassica juncea 1', 'Brassica juncea 2', 'Brassica juncea 3', from China were identified as having useful resistance by the stem inoculation technique. This technique can be effectively utilized for screening and identifying resistance in oilseed *Brassica* against *Sclerotinia* stem rot.

Brassica. juncea genotypes 'PCR7', 'RH819', 'Sanjuncta asesh' and 'Vardan' were identified as having useful resistance under field conditions in north India by soil inoculation. As pathogen survives in form of sclerotia, myceliogenic germination causes systemic infection and result into SSR under natural environmental condition when average temperature is above 15°C and saturated soil moisture prevail during crop growth stage GS 55–75 BBCH Scale; Lancashire *et al.* (1991) or GS 35–55 Scale Harper and BerkenKamp (1975) and personnel communication. The type of inoculation and/or nature of assessment can significantly affect the relative ranking of genotype as has been observed by Li *et al.* (2007). Hence the soil inoculation technique is being adopted. Plants wilted/dead/mortality was considered as percent disease incidence. Although, there was significant variation in results between different locations using the two different methods of inoculation for screening, this observation may be attributed due to strain differences between *S. sclerotiorum* isolates. The variation of genotype performance under different environmental condition as has also been observed by Li *et al.* (2006). Conclusively, *B. napus* genotypes 'Ag Outback', 'Monty', 'Ag Spectrum', 'RR002' and 'JM018' of *B. juncea* from Australia, 'RH 13', 'Ringot', 'Brassica juncea 1', 'Brassica juncea 2', 'Brassica juncea 3', from China were identified as having useful resistance by the stem inoculation technique. In India, in areas with high rainfall or irrigation, *B. napus* is suitable for cultivation while *B. juncea* is more suitable for growing in areas where rainfall is inadequate and irrigation is limited. Both species are significantly adversely affected by *Sclerotinia* stem rot and our findings provide the opportunity for breeders to exploit these resistances to enhance the level of resistance in future cultivars. These identified resistant genotypes can also be directly utilized as commercial varieties in India, if they have suitable agronomic adaptation.

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