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Rakesh Punia
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Ram Avtar
Oilseeds Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India

Manjeet Singh
Oilseeds Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India

Vivek Singh
Oilseeds Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India

Mahavir
Oilseeds Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Rakesh Punia
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Phenotyping of F_2 populations of Indian mustard for Sclerotinia rot caused by *Sclerotinia sclerotiorum* under artificial inoculation conditions

Rakesh Punia, Ram Avtar, Manjeet Singh, Vivek Singh and Mahavir

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Abstract
Sclerotinia rot incited by *Sclerotinia sclerotiorum* is a major threat of rapeseed- mustard worldwide. For over two decades, research on the management of this disease has been directed at various approaches, and yet available methods have not provided effective control. Host resistance offers the environmentally safe and long-term method for effective management of this disease. Therefore, F_2 population of two crosses *i.e.* RH 1138×RH 1222-28 and RH 406× RH 1222-28 were screened for their relative resistance/tolerance to Sclerotinia rot under artificial inoculation and permanent sick plot conditions in field at Research Area of Oilseeds Section, Department of Genetics and Plant Breeding, CCS HAU, Hisar during *rabi* 2018-19. Results indicated that fourteen individuals of F_2 population (RH 406 × RH 1222-28) and only two individuals of F_2 population (RH 1138 × RH 1222-28) exhibited complete resistance to Sclerotinia rot under artificial inoculation conditions.These sources of resistant F_2 plants need to be tested for confirmation for their resistance with other inoculation techniques and after that may be utilized in resistance breeding programme for development of resistant variety of Indian mustard.

Keywords: Brassica, disease resistance, inoculation, *Sclerotinia sclerotiorum*

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) [1]. It is considered to be one of the most damaging pathogen and produce more or less similar symptoms on leaves, stem and siliquae as fluffy white mycelia and sclerotia are produced after mycelial growth when the nutrition is not sufficient or other conditions are favourable for sclerotial development (Rakesh *et al.*, 2016) [5]. Sclerotinia rot causes an estimated annual loss of US $ 200 million in the US alone (Bolton *et al.*, 2006) [2]. 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. Plants infected at or before flower initiation resulted in 100 per cent yield loss, whereas, infection after flowering stage caused more than 50 per cent yield loss (Shukla, 2005) [7]. *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) [3]. 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. Complete resistance to *S. sclerotiorum* is lacking in all cultivated rapeseed-mustard crops, however, partial resistance was identified in some of the *Brassica napus* and to a lesser extent in *B. juncea* genotypes from China, Australia (Li *et al.*, 2008) [4] and India (Singh *et al.*, 2008; Singh *et al.*, 2010) [8, 9]. *B. napus* and *B. juncea* cv. rugosa genotypes have been reported to possess resistance

against Sclerotinia rot in the field as well as in green house conditions (Singh *et al.,* 1994) [10]. Therefore, aim of the present study was to phenotyping of F_2 population of Indian mustard for Sclerotinia rot under artificial inoculation conditions.

Materials and methods

Preparation of pure culture of *Sclerotinia sclerotiorum*

The sclerotia of *S. sclerotiorum* collected from permanent sick plot at Oilseed Research Area, Department of Genetics and Plant Breeding, CCS HAU, Hisar in the month of March, 2018 and brought for further examination (Fig. 1). Sclerotia were air-dried at room temperature for three months and preserved in laboratory conditions. Firstly, these sclerotia were washed under tap water and blot dried. After that these were surface sterilized by dipping in 0.1 percent mercuric chloride solution ($HgCl_2$) for 1 minute followed by rinsing 3-4 times with sterilized water to remove traces of disinfectant. Sclerotia were transferred aseptically into Petri plates containing PDA (Potato Dextrose Agar). The inoculated plates were incubated in BOD incubator at 25 ± 2°C and observed daily for fungal growth. After 48 hrs of incubation, the mycelium emerging from sclerotia was transferred aseptically to PDA slants. The pure culture was maintained on PDA slant and Petri plates at 10±2^{0}C in refrigerator and sub-cultured periodically (Fig. 2).

Fig 1: Sclerotia collected from infected stem of mustard

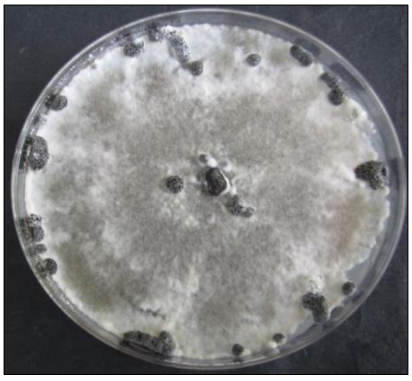

Fig 2: Pure culture of *S. sclerotiorum*

Artificial stem inoculation

The F_2 population of two crosses *viz.,* RH 1138×RH 1222-28 and RH 406× RH 1222-28 were screened for their relative resistance/tolerance against *S. sclerotiorum* under artificial stem inoculation conditions in the field. The F_2 populations of each crosseswere sown during first week of November. Seven days old pure culture of *S.sclerotiorum*was artificially inoculated on stem (third internode) of sixty-five to seventy days old plants. These individuals ofF_2 populations were inoculated by artificial stem inoculation method with slight modification (Zhao *et al.,* 2004) [11]. Seven plants in each row were randomly chosen for stem inoculation. Mycelial discs (5 mm^2) were cut with the help of blade and mycelia disc along with moist cotton swab was then placed on stripe of paraffin wax and was wrapped tightly on main stem after making slight injury at third internode (Fig. 3). Regular watering of inoculated stems and frequent irrigations were given to create high humidity for disease development (Fig. 4). The observations on stem girth and lesion length caused by Sclerotinia rot on all selected individual plants of F_2 population were recorded.

Fig 3: Stem inoculation

Fig 4: Stem rot infection

Disease scoring

Disease rating was recorded according to 0-4 scale [0= no visible lesion on stem (resistant reaction); 1= lesion length on stem 0.1-5.0 cm (moderately resistant); 2= lesion length on stem 5.1-10.0 cm (moderately susceptible); 3= lesion length on stem 10.1-15.0 cm (susceptible); 4= lesion length on stem more than 15.0 cm; highly susceptible).

Results and discussion

The data revealed that fourteen individuals of F_2 population of the cross, RH 406 × RH 1222-28 exhibited complete resistance to Sclerotinia rot under artificial stem inoculation conditions (Table 1). The girth of these individual plants varied from 5.2 to 10.0 cm. The maximum number of plants showed moderately susceptible reaction. Among the F_2 derivatives of RH 1138 × RH 1222-28, two plants showed complete resistance to the disease under artificial inoculation conditions. The stem girth of the individuals of this cross varied from 6.5 to 7.4 cm. Most of the plants of this population also showed moderatly susceptible reaction to the pathogen.

Table 1: Scoring of individuals of F2 populations in field under artificial inoculation conditions

RH 406 × RH 1222-28				RH 1138 × RH 1222-28			
Scale	**Lesion length**	**No. of plants**	**Disease reaction**	**Scale**	**Lesion length**	**No. of plants**	**Disease reaction**
0	0	14	Resistant	0	0	2	Resistant
1	0.1-5.0 cm	35	Moderatelyresistant	1	0.1-5.0 cm	8	Moderately resistant
2	5.1-10.0 cm	101	Moderatelysusceptible	2	5.1-10.0 cm	74	Moderately susceptible
3	10.1-15.0 cm	47	Susceptible	3	10.1-15.0 cm	59	Susceptible
4	>15.0 cm	48	Highly susceptible	4	>15.0 cm	53	Highly susceptible

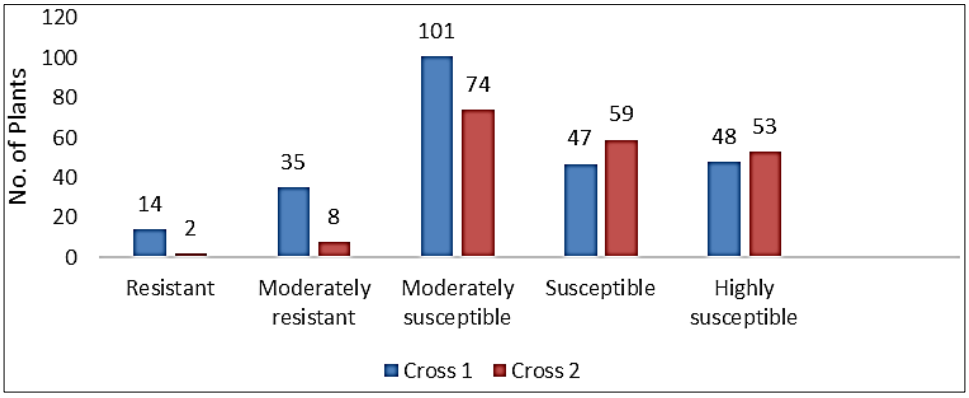

Fig 5: Phenotyping of F_2 population of cross 1(RH 406 × RH 1222-28) and cross 2 (RH 1138 × RH 1222-28)

Among the cross RH 406 × RH 1222-28, thirty-five plants showed lesion length of 0.1-5.0 cm indicating resistance, one hundred one plants showed lesion length of 5.1-10.0 cm, forty-seven plants showed lesion length of 10.1-15.0 cm while forty-eight plants showed highest lesion length of > 15.0 cm indicating highly susceptibility. Similarly, among the cross RH 1138 × RH 1222-28, eight plants showed minimum lesion length of 0.1-5.0 cm indicating resistance, seventy-four plants showed lesion length of 5.1-10.0 cm, fifty-nine plants showed lesion length of 10.1-15.0 cm while fifty-three plants showed maximum lesion length of > 15.0 cm indicating highly susceptibility. Results of present study was in confirmetry with Sharma *et al.,* (2009) [6]. They found that *B. juncea* genotypes EC597328 showed high tolerance, with a mean stem lesion length of <0.05 cm compared to 26.75cm in the susceptible check cv. Rohini.

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