Sclerotinia rot tolerance in oilseed *Brassica*

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Abstract

Sclerotinia rot of *Brassica* species incited by *Sclerotinia sclerotiorum* (Lib) De Bary is a devastating disease and proving threat to the cultivation of *Brassica* species in different oilseeds Brassica growing countries of the globe. The present study was carried out at CCS Haryana Agricultural University, Hisar (India) which is situated at 29° 10'N and 75° 46E'. The efforts were made to identify sources lines tolerant to *Sclerotinia* rot. Ninety one (43 *B. juncea*, 48 *B. napus*) assembled germplasm accessions of Australia, China and India origin were screened by soil and stem inoculation techniques at seedling and 80% blooming stage (90 DAS old plants, siliquae development), respectively, under screen house conditions, to identify sources of resistance/tolerance at seedling and siliquae development stage, 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 5-6 days of inoculation and seedlings collapsed within 15 days after inoculation. None of the assembled oilseed Brassica germplasm lines/accessions exhibited complete resistance to *Sclerotinia* rot. The genotypes of *B. juncea* namely, JO 009, JN 031 & JN 033 of Australian origin were observed tolerant whereas, none of the Indian and Chinese lines were observed tolerant at seedling stage. In *B. napus,* AG Outback, Rainbow, RQ 011 and RQ 011-02M2 of Australian origin, Neelam and GSL 1 of Indian origin and YU 178 of Chinese origin were observed tolerant at seedling stage. The variation in degree of incidence may be attributed due to the pathotype and plant age chosen for this study. The genotypes observed tolerant at seedling stage doesn't hold true at siliquae formation stage and *vice-versa*. However, the genotype RQ 011 was observed tolerant at both seedlings as well as at siliquae formation stage. The level of tolerance also varied among the genotypes. Based upon the available level of tolerance, it is advocated that the identified genotypes could be utilized to further enhance the level of resistance/tolerance rather than as donor parents for incorporating resistance against *Sclerotinia* rot.

Key words: *Sclerotinia*, Indian mustard (*Brassica juncea* Czern & Coss.), Swede Rape (*Brassica napus*), Screening, Oilseed Brassica.

Introduction

Sclerotinia sclerotorium (Lib) De Bary has been reported to infect more than 400 plant species including cultivated crops and Oilseed Brassica (Boland, 1994). This ascomycete fungus have systemic and aerial infection by myceliogenic and carpogenic germination of sclerotia surviving in soil even under adverse environmental condition. Being ubiquitous necrotroph pathogen, it is proving bottleneck and thwarting to cultivation of Oilseed Brassica grown in different countries. It causes *Sclerotinia* rot of Oilseed Brassica spp. The primary method of managing it with non host crops and fungicide application may not achieve an economic benefit. If genetic resistance to *Sclerotinia* rot is available reliance on fungicide would lessen and production of oilseed Brassica crops would become more profitable. Hence evaluation of Brassica spp for resistance is important. An efficient, reliable and inexpensive screening method would allow large scale evaluation. Methods that have been used to identify the resistant source include soil and stem inoculation at seedling and siliquae initiation stage (mid January) under screen house condition.

Materials and methods

A diverse range of ninety one accessions of Brassica spp of Australian, Chinese and Indian origin were used in this study having different growth habit and seed qualities. Seed were planted in 12 cm diameter pots and allow to grow in natural environment (2-25°C) even during screening period. Inoculum was raised by using boiled and sterilized wheat grain. For obtaining pure mycelia suspension, potato dextrose booth was used. Fifteen days old inoculum having mycelia and sclerotia

were inoculated by placing near collar region of 45 days old seedling of each plant of every genotype in all the five replications. Disease incidence/intensity was calculated on the basis of seedling collapsed. Genotype contracted less than 25 percent disease was categorized as tolerant, whereas genotype having 25-50% disease incidence as susceptible and >50% as highly susceptible.

Stem inoculation method was adopted as described by Zhao *et al* (2004) with some modification. The fifth leaf of 90 days old plants of each replication of every cultivar was severed near petiole junction using razor blade and inoculated by placing mycelia and sclerotia raised on boiled and sterilized wheat grain covered with cellotape. In second method by syringe inoculation of mycelia suspension near petiole junction and in third method ascospore suspension ($1X10^3$ ascospore/ml) was prepared from apothecia raised on sandy loam soil by induction of carpogenic germination of sclerotia (saturated moisture for 15 days) of sandy loam kept at $12+2^oC$ along with tube light continuously and inoculated by syringe..

Disease incidence was calculated on the basis of average of all three methods of inoculation. Disease incidence is measured till stem break and irreversible wilting occurred. Days to wilting were calculated from symptom appearance to irreversible wilting and stem break (Zhao *et al* 2004).

Results

In soil inoculations method seedling collapsed within 15 days of inoculation. Water soaked lesion appeared within 4-6 days after inoculation near collar region and collapsed within 15 days. None of the genotype was observed free from incidence of *Sclerotinia* rot. In stem inoculation symptom appeared within 4 -7 days depending upon method of inoculation and genotype. Water soaked lesion symptom first appeared in syringe inoculation of mycelia suspension followed by mycelia and sclerotia and ascosporic suspension inoculation. After symptom appearance, rate of disease progress also varied among genotype.

Among *Brassica juncea* genotype JO 009 (18-20%), JN 031(23.8%) and JN 033 (25.00%) of Australian origin was observed as tolerant where as none of the Indian and Chinese lines of *Brassica juncea* expressed tolerance. In *Brassica napus* AG outback (15.8%), Rainbow (20.00%), RQ 011(18.2%) and RQ 001-02 M2 (22.7%) of Australian origin, Neelam (25.00%) and GSL-1 (22.7%) of Indian origin and YU-178 (25.00%) of Chinese origin were observed as tolerant. The variation in degree of incidence, level of tolerance may be attributed because of the used pathotype and plant age. Seedling stage inoculation was carried out at this stage since systemic infection takes place by prevailing environmental condition for myceliogenic germination of sclerotia and ultimately resulting into *Sclerotinia* stem rot of *Brassica juncea* in/under Indian field condition. If the plant/genotype get escaped by systematic infection even than it can get infected at lateral stage i.e. blooming/siliquae initiation stage in mid January by ascospore produced as a result of induction of carpogonic germination of sclerotia. Using stem inoculation near petiole junction revealed that the genotypes RQ 011(25.00), RR 001(25.00), M 616 (25.00) of *B. juncea* of Australian origin expressed tolerant as the stem crecked within 15 days and categorized as tolerant since they can also be protected by adopting curative chemical means to check the progress of disease. None of Indian and China origin observed as tolerant.

Discussion

Screening through stem inoculation of mycelial suspension seems to be a promising method for screening of germplasm under screen house natural environmental condition for large collections. Differences in level of susceptibility do help in identification of genotype with tolerance to *Sclerotinia*. Similarly Zhao *et al* (2004), Bradley *et al* (2004) Singh and Tripathi (1994), Pathak *et al* (2002) and Steward Wade (2003) used petiole inoculation, detached leaf array, oxalic acid and soil inoculation for screening of different germplasm of Brassica species. The differences observed in the level of susceptibility under lab or green house screening method could accurately be confirmed in the field. Therefore, it is advocated to conform the level of tolerance under sick fields.

Conclusions

None of the assembled oilseed Brassica germplasm lines/accessions exhibited complete resistance to *Sclerotinia* rot. The genotypes of *B. juncea* namely, JO 009, JN 031 & JN 033 of Australian origin were observed tolerant whereas, none of the Indian and Chinese lines were observed tolerant at seedling stage. In *B. napus,* AG Outback, Rainbow, RQ 011 and RQ 011-02M2 of Australian origin, Neelam and GSL 1 of Indian origin and YU 178 of Chinese origin were observed tolerant at seedling stage. The variation in degree of incidence may be attributed due to the pathotype and plant age chosen for this study. The level of tolerance also varied among the genotypes. Interestingly, the genotype RQ 011 was observed tolerant at both seedlings as well as at siliquae formation stage. Based upon the available level of tolerance, it is advocated that the identified genotypes could be utilized to further enhance the level of resistance/tolerance rather than as donor parents for incorporating resistance against *Sclerotinia* rot.

References

Bradley C.A., Hension, R.A., Porter, P.M, Le Gare, D.G., Rio L.E del and Khot S.D. (2006).: Response of canola cultivars to *Sclerotinia sclerotorium* in controlled and field environments. Plant disease:90:215-219

Pathak, A.K.; Godika, Shalesh, Jain J.P and Murija Suresh (2002). Screening of *Brassica* genotypes agent stem rot disease of mustard caused by *Sclerotinia sclerotiorum*. (Lib) de Bary, J. Mycol Pt. Patho 32:111-112.

Singh Rajender and Tripathi, N.N. (1994): Asssured method of inoculation and screening of Brassica spp. Against *Sclerotinia sclerotiorum* (Lib) De Bary. Crop Research 8(3):570-574.

Stewart Wade, S.M (2003): Assessment of resistance of canola quality Brassica lines to *Sclerotinium* stem rot. In 8th International congress of Plant Pathology

held at Christ church in Mar 2003. PP 296 (abstract).
Zhao, A.J. Petlier, Meng, J., Osborn T.C. and Gran C.R. (2004): Evaluation of *Sclerotinia* stem rot in Oilseed *Brassica napus* using a petiole inoculation technique under greenhouse condition. Plant disease: 88:1033-39

Table-1 Screening of Brassica species germplasm accessions/varieties of Indian, Chinese and Australian origin against *Sclerotinia* rot (*Sclerotinia sclerotiorum*)

Sr. No.	Accession Number	Name of Accession	Soil Inoculation	Stem Inoculation		Sr. No.	Accession Number	Soil Inoculation	Stem Inoculation	
			Per cent disease Incidence	Per cent disease Incidence	Days to stem break			Per cent disease Incidence	Per cent disease Incidence	Days to stem break
Australian *B. napus* accessions						**Indian *B. napus* accessions**				
1	EC 552585	Lanthen	40.0	43.3	13.2	26	Neelam	25.0	65.3	11.0
2	EC 552586	AG outback	15.8	35.0	14.0	27	GSL-1	22.7	90.0	8.5
3	EC 552587	Trigold	36.8	40.0	13.5	28	GSL-2	50.0	85.0	9.0
4	EC 552588	Monty	57.1	51.0	12.4	**Chinese *B. napus* accessions**				
5	EC 552589	Rainbow	20.0	40.0	13.5	29	EC 557008	76.2	65.3	
6	EC 552590	Rivette	38.1	45.0	13.0	30	EC 557009	55.0	58.6	11.0
7	EC 552591	RQ 011	18.2	25.0	11.3	31	EC 557010	81.8	40.0	10.7
8	EC 552592	Tranby	42.9	37.3	13.8	32	EC 557011	61.9	68.6	13.5
9	EC 552593	RR 002	50.0	33.3	14.2	33	EC 557012	68.2	66.6	10.7
10	EC 552594	AV Sapphire	33.3	N.G.	N.G.	34	EC 557013	95.0	53.3	10.9
11	EC 552595	BST 702M2	63.2	29.3	14.6	35	EC 557014	57.1	77.3	12.2
12	EC 552596	RQ 001-02M2	22.7	40.0	13.5	36	EC 557015	61.9	55.0	9.8
13	EC 552597	RR 013	47.4	29.3	14.6	37	EC 557016	86.4	59.6	8.0
14	EC 552598	RR 009	95.8	38.6	13.7	38	EC 557017	50.0	77.3	11.6
15	EC 552599	Surpass 400	40.0	41.3	13.4	39	EC 557018	66.6	50.0	9.8
16	EC 552600	RR 005	80.0	51.3	12.4	40	EC 557019	63.6	55.0	12.5
17	EC 552601	Scar	68.4	30.0	14.5	41	EC 557020	52.3	50.0	12.0
18	EC 552602	Mystic	59.1	31.3	14.4	42	EC 557021	52.3	61.3	12.5
19	EC 552603	RR 001	54.5	25.0	15.0	43	EC 557022	25.0	80.0	11.4
20	EC 552604	Charlton	81.8	32.6	14.3	44	EC 557023	54.5	83.3	9.5
21	EC 552605	Skipton	57.9	40.0	13.5	45	EC 557024	80.9	50.0	9.3
22	EC 552606	Trilogy	80.0	30.0	9.4	46	EC 557025	52.3	N.G.	12.5
23	EC 552607	AG Spectrum	47.4	85.0	9.0	47	EC 557026	95.0	73.3	N.G.
24	EC 552608	TQ 0055-02W2	73.7	85.0	9.0	48	EC 557027	95.4	45.0	10.2
25	EC 552609	Purler	75.0	43.3	13.3					
Australian accessions *B. juncea*										
1	EC 552573	JN 004	47.6	43	13.3	13	Varuna	80.9	43.3	13.2
2	EC 552574	JN 010	80.9	43	13.2	14	Seeta	95.2	43.3	13.2
3	EC 552575	JN 028	52.4	49	12.7	15	Sanjukta-Asesh	76.2	40.0	13.5
4	EC 552576	JN 031	23.8	45	13.0	16	RH-30	96.2	35.0	14.0
5	EC 552577	JN 032	28.6	96	8.0	17	RL 1359	80.0	50.0	12.6
6	EC 552578	JN 033	25	55	12.0	18	Prakash	63.2	27.3	14.8
7	EC 552579	JM 016	48.3	25	15.0	19	RH 781	47.4	33.3	14.2
8	EC 552580	JM 018	61.9	35	14.0	20	PBR 97	76.2	35.0	14.0
9	EC 552581	JO 006	38.1	70	10.6	21	RH 819	52.4	28.6	14.7
10	EC 552582	JO 009	18.2	85	9.0	22	Durgamani	61.9	33.3	14.2
11	EC 552583	JR 042	45.5	56	12.3	23	Sej-2	95.8	35.0	14.0
12	EC 552584	JR 049	76.2	55	12.0	24	RH 8113	50.0	50.0	12.5
Chinese accessions *B. juncea*						25	Kranti	42.9	35.0	14.0
1	EC 564640	CBJ 001	61.9	49	12.7	26	PCR 7 (Rajat)	33.3	50.0	12.0
2	EC 564641	CBJ 002	35	65	11.0	27	Vardan	83.3	35.0	14.0
3	EC 564642	CBJ 003	80.9	70	10.6	28	RH 8812	80.9	30.0	14.5
4	EC 564643	CBJ 004	95	50	12.5	29	GM 1	80.0	25.0	15.0
5	EC 564644	TABP	66.7	55	12.0	30	Vaibhav	71.4	43.3	13.2
6	EC 564645	MPJR	96.2	35	14.0	31	PBR 91	57.1	26.6	14.9
7	EC 564646	XINYOU-4	N.G.			32	Rohini	38.1	32.6	14.3
8	EC 564647	XINYOU-5	N.G.			33	RLM 619	33.3	28.3	14.7
9	EC 564648	XINYOU-8	95.4	30	14.5					
10	EC 564649	XINYOU-9	52.4	65	11.0					

N.G Denotes not germinate

Sr. No.		Name of Germplasm line	%disease Incidence	Disease reaction	Disease Incidence	Days to stem break	Name of Germplasm line	%disease Incidence	Disease reaction	Disease Incidence	Days to stem break
1	EC 552585	Lanthen	40.0	S	43.3	13.2					
2	EC 552586	AG outback	15.8	T	35.0	14.0					
3	EC 552587	Trigold	36.8	S	40.0	13.5					
4	EC 552588	Monty	57.1	S	51.0	12.4					
5	EC 552589	Rainbow	20.0	T	40.0	13.5					
6	EC 552590	Rivette	38.1	S	45.0	13.0					
7	EC 552591	RQ 011	18.2	T	25.0	11.3					
8	EC 552592	Tranby	42.9	S	37.3	13.8					
9	EC 552593	RR 002	50.0	S	33.3	14.2					
10	EC 552594	AV Sapphire	33.3	S	N.G.	N.G.					
11	EC 552595	BST 702M2	63.2	HS	29.3	14.6					
12	EC 552596	RQ 001-02M2	22.7	T	40.0	13.5					
13	EC 552597	RR 013	47.4	S	29.3	14.6					
14	EC 552598	RR 009	95.8	HS	38.6	13.7					
15	EC 552599	Surpass 400	40.0	S	41.3	13.4					
16	EC 552600	RR 005	80.0	HS	51.3	12.4					
17	EC 552601	Scar	68.4	HS	30.0	14.5					
18	EC 552602	Mystic	59.1	HS	31.3	14.4					
19	EC 552603	RR 001	54.5	HS	25.0	15.0					
20	EC 552604	Charlton	81.8	HS	32.6	14.3					
21	EC 552605	Skipton	57.9	HS	40.0	13.5					
22	EC 552606	Trilogy	80.0	HS	30.0	9.4					
23	EC 552607	AG Spectrum	47.4	S	85.0	9.0					
24	EC 552608	TQ 0055-02W2	73.7	HS	85.0	9.0					
25	EC 552609	Purler	75.0	HS	43.3	13.3					
Indian origin (*B. napus)*											
1	Neelam		25.0	T	65.3	11.0					
2	GSL-1		22.7	T	90.0	8.5					
3	GSL-2		50.0	HS	85.0	9.0					