Epidemiology and recent advances in Sclerotinia rot management in *Brassica juncea L.*

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Sclerotinia sclerotiorum

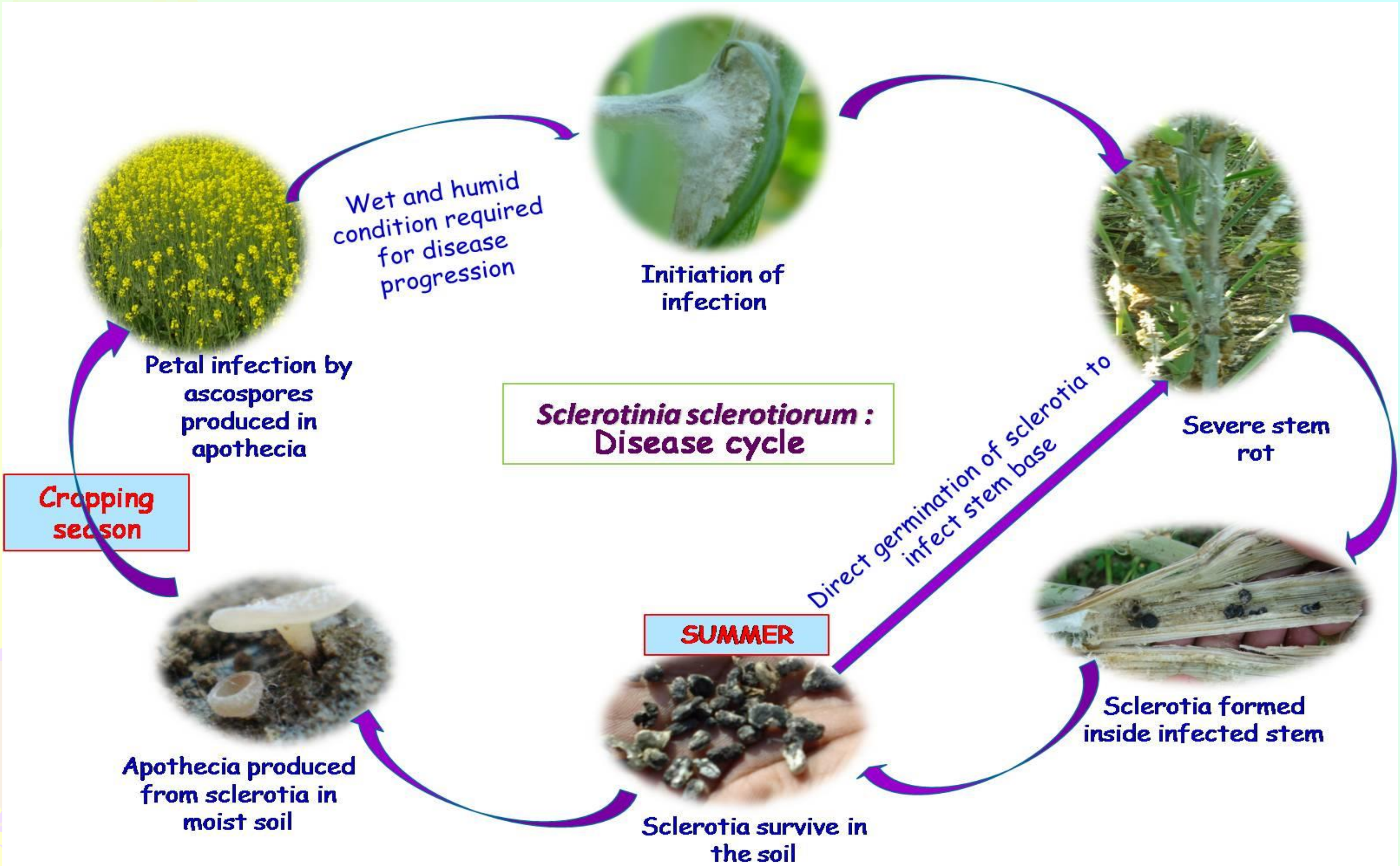
- *Sclerotinia sclerotiorum* (Lib.) de Bary is a ubiquitous necrotrophic fungal pathogen.
- It is capable of infecting >500 plant species among 75 families (Sharma et al., 2015).
- In India, it has become a serious problem in some parts of the country like Punjab, Himachal Pradesh, Haryana, Rajasthan and Bihar.
- This disease gained importance particularly in areas where farmers practiced mono-cropping of Indian mustard, which led to complete crop failure.

- Disease incidence upto 80% has been reported in Punjab and Haryana (Kang and Chahal, 2000; Sharma *et al.*, 2001), and 72% in Uttar Pradesh (Chauhan *et al.*, 1992).
- Kumar and Thakur (2000) from Himachal Pradesh have reported that stem rot appears regularly in mild to severe form in major mustard growing areas and cause considerable loss in yield.
- In Rajasthan, 60% seed yield loss has been reported in severely infected plants (Krishnia *et al.*, 2000; Ghasolia *et al.*, 2004).

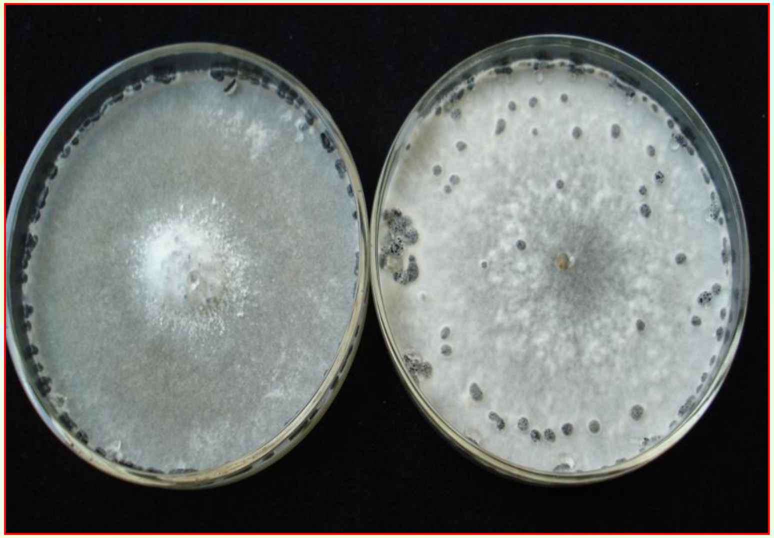
Symptoms of Sclerotinia rot on stem, leaf and pod

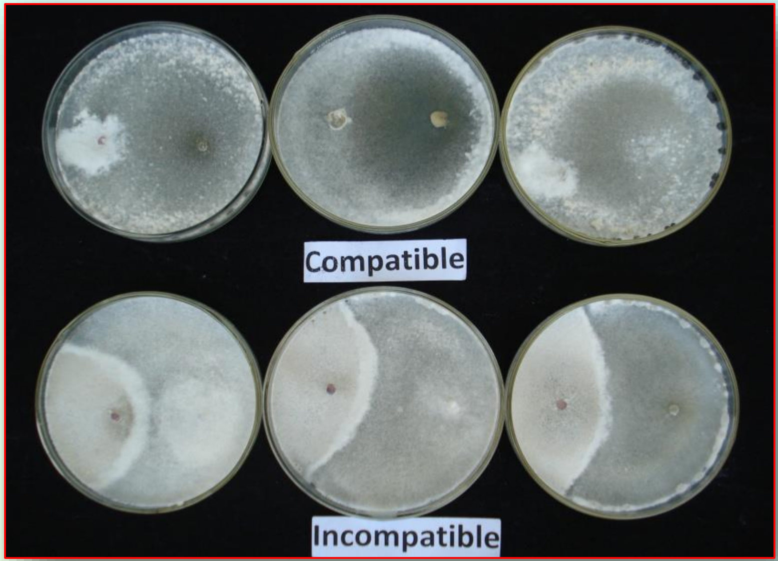
Sclerotia formed on stem, in pith & on root

Sclerotinia sclerotiorum : Disease cycle



Sclerotinia sclerotiorum : Variability





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Patho-biochemical investigations on stem rot (*Sclerotinia sclerotiorum*) of Indian mustard (*Brassica juncea* L.)

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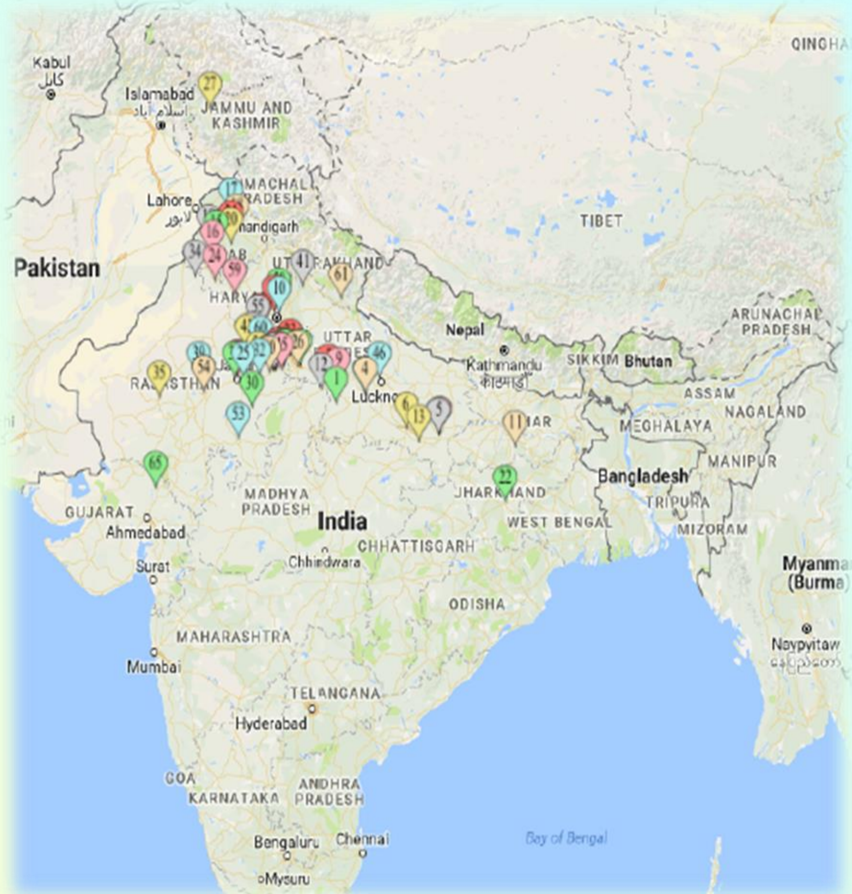
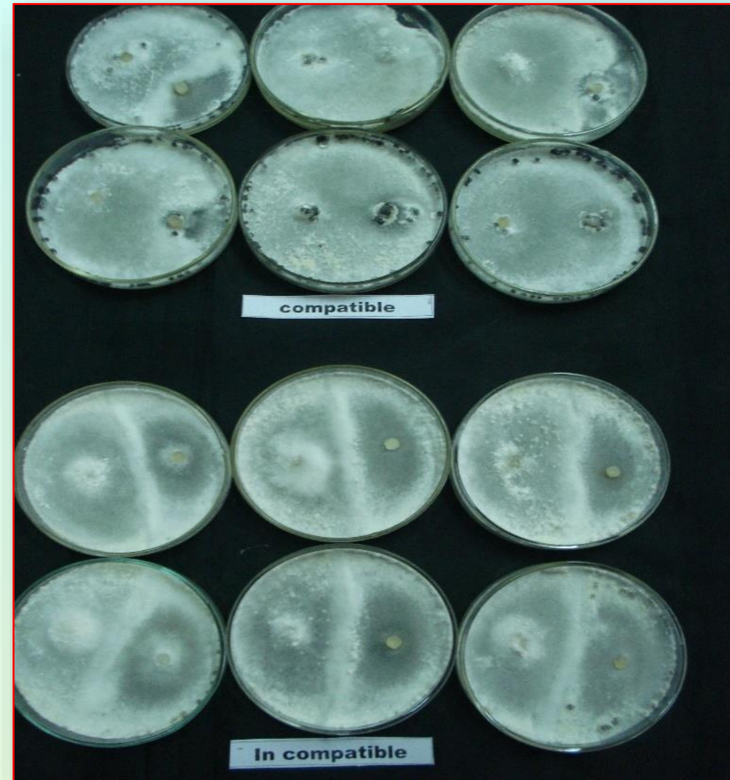
Morphological variability among 65 geographical isolates of *S. sclerotiorum*



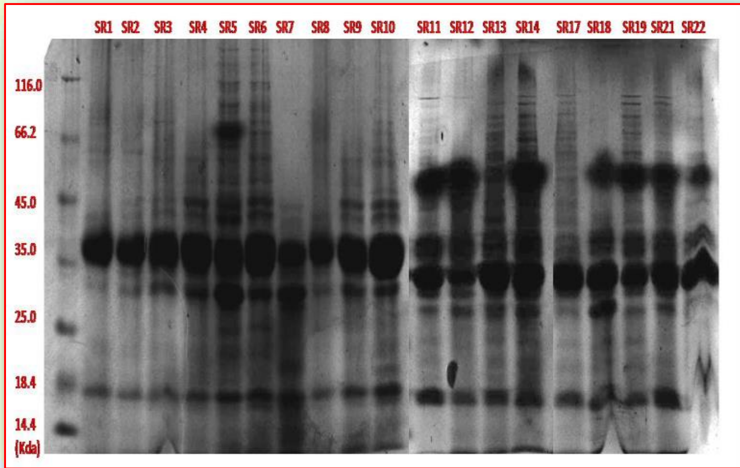
65 different geographical isolates of *S. sclerotiorum* studied for their morphological varability including mycelial growth, no. of days to form sclerotia, no. of sclerotia per Petri plate, size of sclerotia.

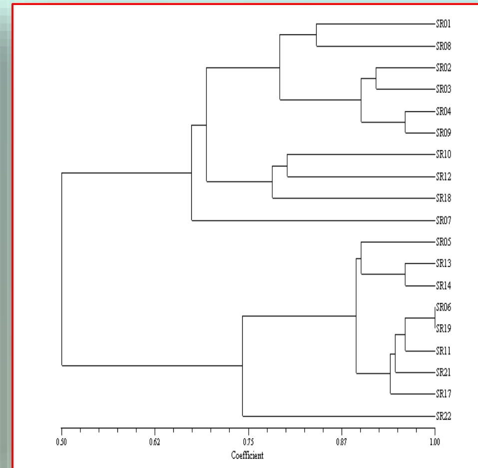
Mycelial Compatibility Groups (MCG)

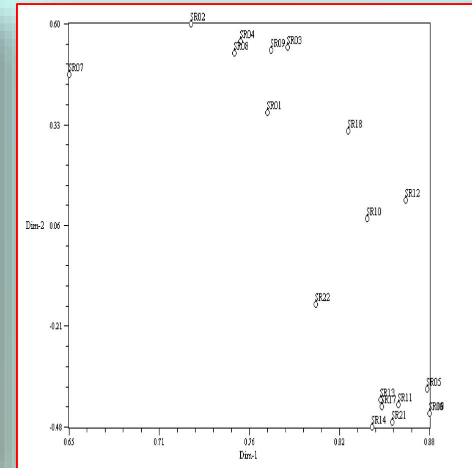


- Mycelial compatibility is the ability of two strains of filamentous fungi to anastomosis and form one continuous colony.
- The compatibility and incompatibility between 65 geographical isolates were considered as 0 and 1 and data recorded were used for cluster analysis.

Protein profile based variability

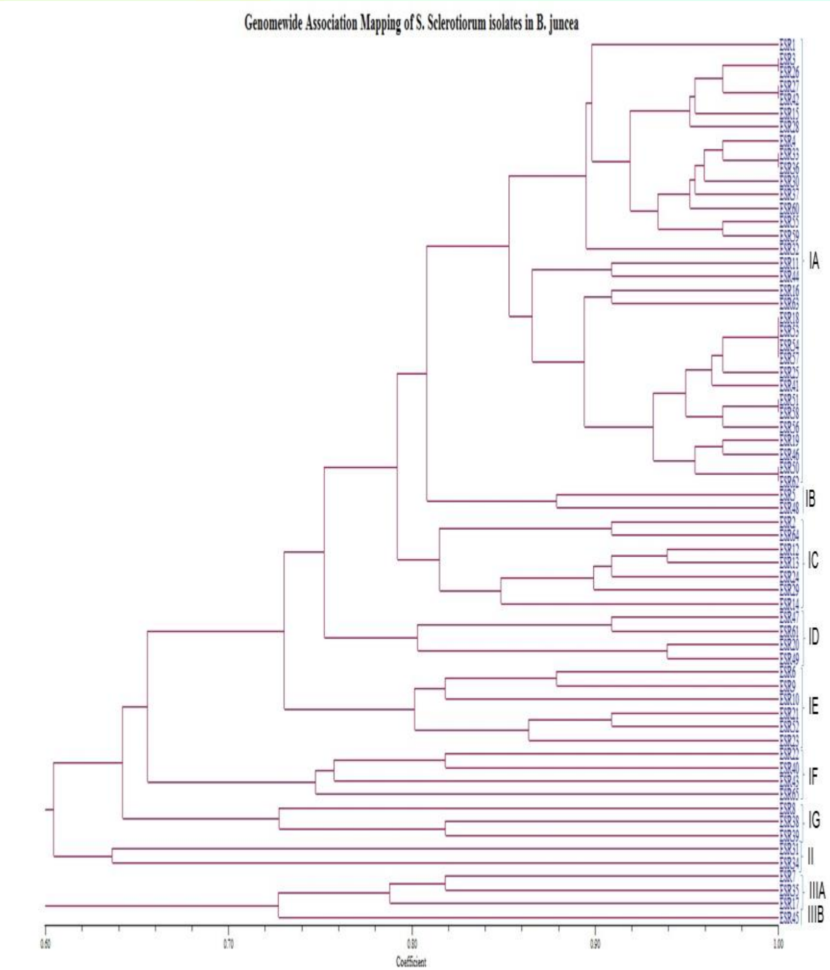






- Total 25 bands were observed having relative mobility (Rm) value ranging from 0.14 to 0.72 and all bands were obtained only in isolate SR 14.
- The similarity indices for different isolates ranged from 0.32 to 1.0 indicating high variability among the different geographical isolates studied.

Genetic Diversity



- 65 isolates divided into three major groups. Group I consisted of 59 isolates from distinct locations with 61% of genetic similarity. It was divided into seven subgroups.
- Group II comprised of 2 isolates exhibited 64% genetic similarity whereas
- Group III consisted of 4 isolates with 73% similarity. It divided into two subgroups with 3 isolates in subgroup IIIA and 1 isolate in subgroup IIIB at 73% of similarity coefficient.

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African Journal of Microbiology Research

Full Length Research Paper

Genetic diversity and morphological variability of *Sclerotinia sclerotiorum* isolates of oilseed Brassica in India

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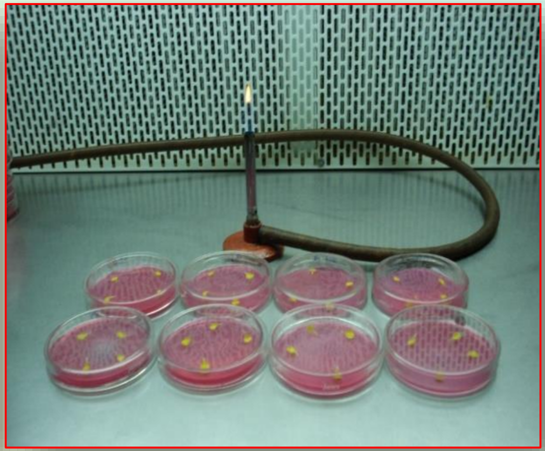
Directorate of Rapeseed - Mustard Research (ICAR), Bharatpur 321 303, India.

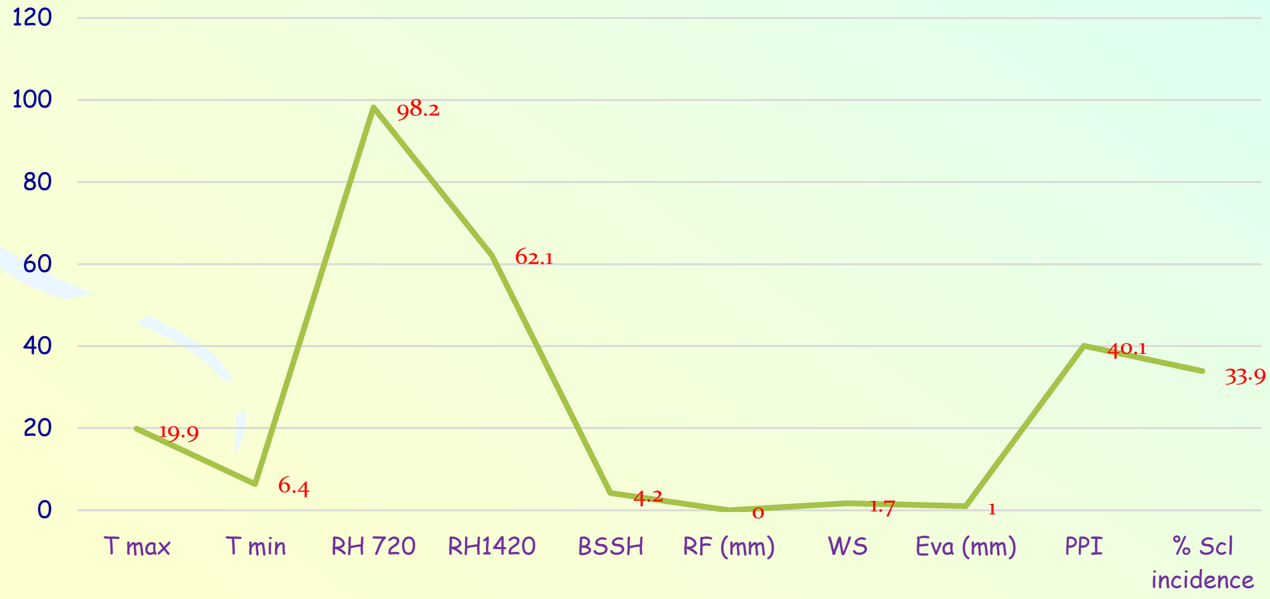
Epidemiology

Per cent petal infection

Date of Sowing	1 Oct	8 Oct	15 Oct	22 Oct	29 Oct	5 Nov	12 Nov	19 Nov
Date of petal inoculation	23.12.15	23.12.15	28.12.15	30.12.15	30.12.15	4.1.16	11.1.16	18.1.16
No. of petals inoculated	312	312	264	244	244	192	120	48
No. of petals observed with *S. sclerotiorum*	62	74	74	74	98	28	5	0
Per cent Petal infestation	**19.8**	**23.7**	**28.0**	**30.3**	**40.1**	**14.5**	**4.1**	**0.0**

Combination of high RH, low BSSH and high soil moisture during the critical stage of crop (60-70 DAS) favour higher Sclerotinia rot incidence



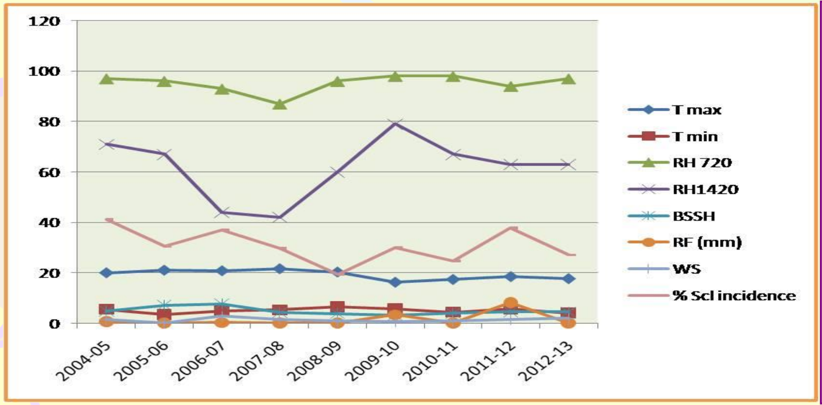
Effect of different weather variables on per cent Sclerotinia incidence (1-3 standard week)

Forecasting Models (Sharma *et al.*, 2015)

Date of planting	Models	R^2	Forecast	
			observed	forecast
8 Oct.	$Y=3.93 + 0.002 * Z_{341}$	0.97	28.0	24.3
29 Oct.	$Y= 51.296 + 0.958 Z_{50} + 0.289 Z_{31} -0.164 Z_{41}$	0.98	37.9	32.9
19 Nov.	$Y= 8.276 + 1.6217 Z_{21} + 0.013 Z_{451}$	0.98	18.8	19.6

Soil moisture along with RH and bright sunshine hour were most significant variable responsible for disease development in crop.

- The R^2 value of the regression analysis between observed and estimated SR prevalence was 0.98.



Phytoparasitica (2015) 43:509–516
DOI 10.1007/s12600-015-0463-4

Forewarning models for Sclerotinia rot (*Sclerotinia sclerotiorum*) in Indian mustard (*Brassica juncea* L.)

Pankaj Sharma · P. D. Meena · Amrender Kumar · Vinod Kumar · D. Singh

Disease management difficulties in Sclerotinia rot

Cultural Control :

•Persistent nature of sclerotia

•Wide host range

Chemical Control:

• Difficulty in foliar application at the time of ascospores release.

Resistant varieties:

• Potential and economic sustainable method of control.

Management Options

Resistant varieties

Offer the only economic/sustainable control.

BUT:

- Need high level resistance
- Need a reliable and appropriate screening technique
- Must relate to field stem infection and associated losses
- Must be repeatable- as much as that is possible with Sclerotinia
- Must be able to handle genotypes of differing maturity that will be inoculated at different times.

Cultural Control :

- ✓ Cultural practices including wider row spacing or lower plant populations that reduce the microclimate favorable for disease development are effective.

- ✓ A significant positive relationship between SR incidence and seeding rate was found.

- ✓ The type of soil and amount of frequency of irrigation significantly affect sclerotial germination and apothecial development.

- ✓ The persistent nature of sclerotia and the wide host range of this pathogen generally render cultural practices ineffective.

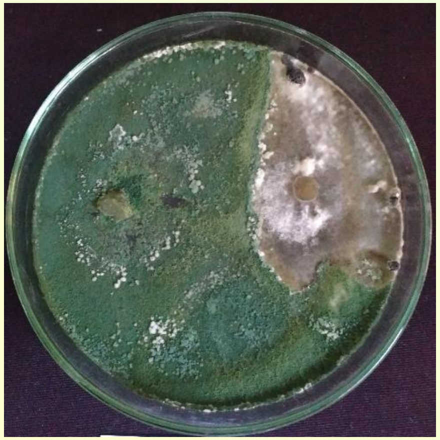
Bio-control

In the light of present day concern about the environment, human health and development of resistance to fungicides, biological control is an attractive alternative for plant disease management.

Fungal and Bacterial antagonists:

The mycoparasitic fungi parasitizing sclerotia include *Coniothyrium minitans*, *Trichoderma* spp., *Gliocladium* spp. etc.

Bacillus subtalis, *Pseudomonas fluorescens* 132, and *P. maltophila* reported effective. *Pseudomonas* spp. (DF41) and *P. chlororaphis* (PA23) inhibited the germination of ascospores of *S. sclerotiorum* (Savchuk and Fernando, 2004).



- For successful control of SR in mustard and potential mycoparasite must therefore, be applied both aerially and in the soil.

Fungicides

- *Sclerotinia* continues to be a very difficult pathogen to control. Therefore, fungicides have been extensively used for the control of *S. sclerotiorum*.
- Fungicides are applied at the full bloom stage to prevent infection of the senescing petals, which can fall on the leaf axils leading to infection of the stem.
- Foliar spray of carbendazim at bloom provided significant disease reduction and highest seed yield (Sharma *et al.*, 2011).

Treatment	% Sclerotinia infection*	% Sclerotinia reduction over control*	Pooled mean Seed yield (kg/ha)
SA Zinc (25kg/ha)	22.5[2]	44.9	2024
SA Boron (1kg/ha) + FS (0.1 %)	23.6[2]	42.2	2316
FS Boron (0.1 %)	21.2[1]	48.1	2302
FS K_2SO_4 (0.1%)	12.5[1]	69.4	2374
SA Mustard cake (2 tonne/ha)	19.4[3]	52.5	2194
SA Zinc (25kg/ha) + Boron (1kg/ha)	20.1[2]	52.7	2299
SA Zinc (25kg/ha) + Mustard cake (2 tonne/ha)	16.0[2]	60.8	2312
SA Boron (1kg/ha) + Mustard cake (2 tonne/ha)	15.8[2]	61.3	2241
SA Zinc (25kg/ha) , Boron (1kg/ha) + Mustard cake (2 tonne/ha)	17.4[2]	57.4	2274
ST +FS Trichoderma	19.9[2]	51.3	2095
FS P. fluorescens (10^8 cfu/ml)	14.6[2]	64.3	2238
FS Bacillus subtalis	18.3[1]	55.2	2110
ST Iprodione+ Carbendazim (0.2%) + FS (0.1%)	9.8[2]	76.0	2405
ST+FS Carbendazim (0.2%)	4.9[5]	88.0	2439
ST Carbendazim (0.2%) + FS Tebuconazole (0.1%)	5.1[2]	87.5	2430
Control (no treatment)	40.9[5]	0.0	1688
CD (5%)	4.03		142.6

- Carbendazim ST+FS provided significant (P= 0.05) disease reduction (88.0%) and highest seed yield (44.4%) followed by ST carbendazim + FS tebuconazole.

- *Pseudomonas fluorescens*, *Bacillus subtalis* and *Trichoderma* were also minimize disease incidence (51.3-64.3%).

Popularization of Sclerotinia rot management technology

✓ Carpogenic infection initiated in 52 standard week and maximum pressure of SR continued during 1-2 standard weeks when crop is in full bloom stage.

✓ Soil moisture is one of the important factor for development of SR.

✓ A management strategy including seed treatment with Carbendazim (2g/kg seed), no crop irrigation during 25 Dec to 15 Jan and foliar spray of carbendazim (0.2%) during first week of January was formulated and well tested through experiments, on farm testing and demonstrations.


TRIAL: SCREENING OF AUSTRALIAN,
CHINESE & INDIAN BRASSICA
GERMPLASM FOR SCLEROTINIA ROT
RESISTANCE
DATE OF SOWING: 27 Oct 2009
REPLICATION: ONE PLOT SIZE: SINGLE ROW 3m
SUS.CHECKS: ROHINI LINES: 84

Resistance

Screening for Sclerotinia rot resistance

✓>4300 germplasm from core collection, exotic, National Gene Bank and mutagenic plants of *B. juncea*, *B. carinata* screened under sick plot with artificial stem inoculation at DRMR during 2016-17 season.

Mycelial growth in sick plot

Mycelial growth in sick plot



Sclerotinia sick plot



Sclerotinia sick plot


Susceptible reactions

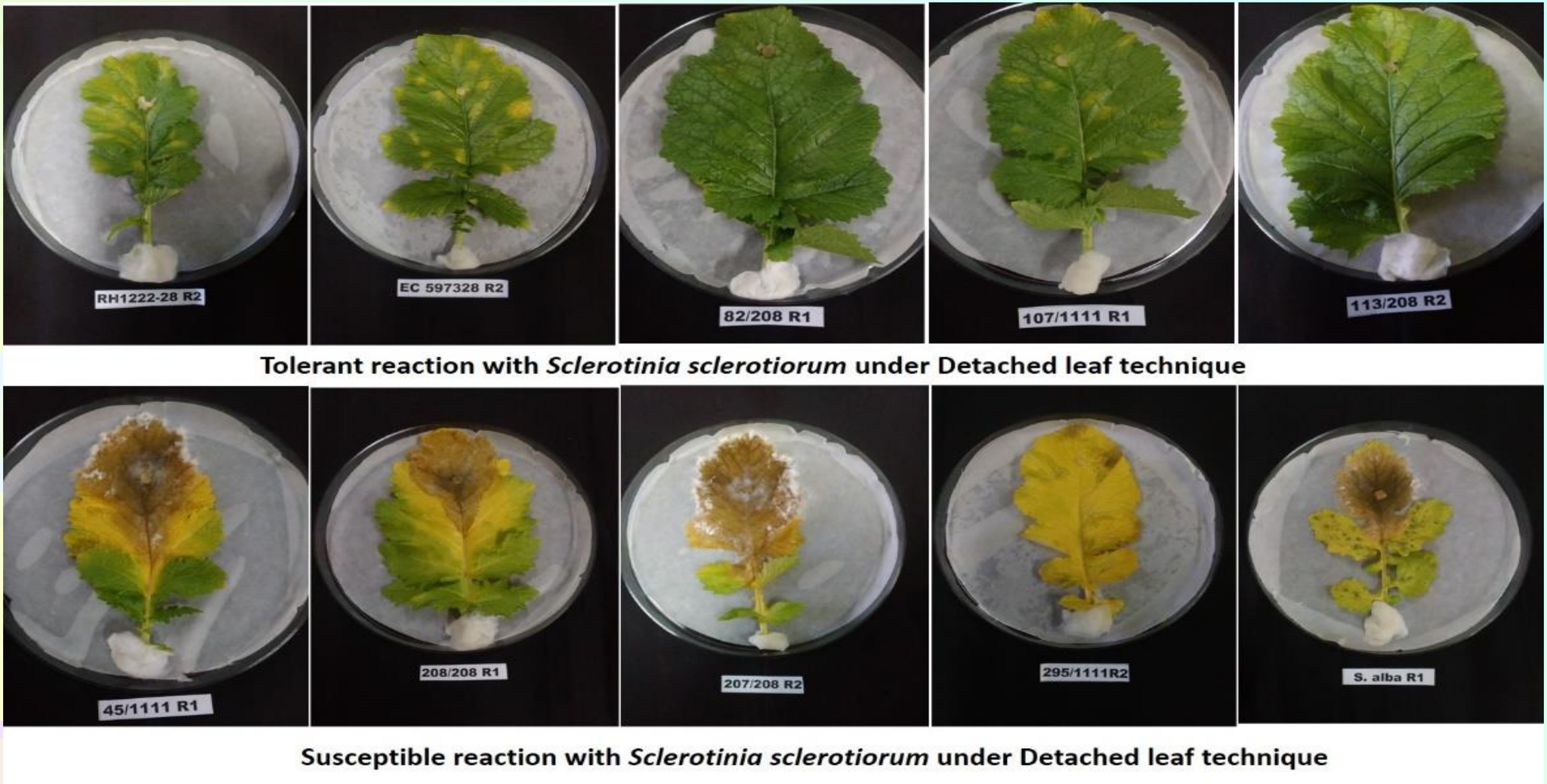
Tolerant reactions





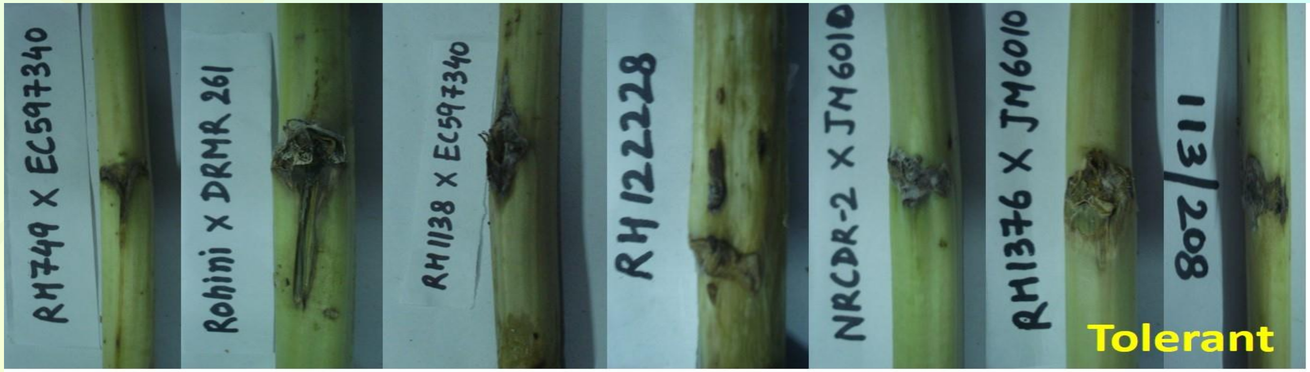
- EC 597317, EC 597328, RH 1222-28, DRMR 360, DRMR 1034, DRMR 1493, WR 2035, IC 50316, IC 492724, DRMR 2585 *B. juncea* and DRMR 261 *B. carinata* germplasm showed tolerant reaction (lesion size <3.0 cm and disease incidence<10%).

Promising genotypes with detached leaf technique (*in vitro*)



B. juncea germplasm IC 570316, IC 492724, DRMR 2585, 205/208, RH-1222-28 and *B. carinata* DRMR-261 were the most tolerant.

Promising genotypes and F1 crosses with detached stem technique (*in vitro*)



19: NRCDR-02 x DRMR-261, NRCDR-02 x EC-597340, NRCDR-02 x EC-597343, RH-1117 x EC-597343, RH-1138 x EC-597328, RH-1231 x Ec-597328, RH-1231 x EC-597343, RH-555 x EC-597328, RH-555 x EC-597344, RH-1372 x EC-552576, RH-1372 x EC-597340, RH-1372 x EC-597343, RH-1372 x DRMR-261, RH-045 x EC 597343, RH-749 x EC-552576, RH-749 x EC-597328, RH-749 x DRMR-261, RH-749 x JM6010.

Tolerant reactions in F1 crosses


RH 749 x DRMR 261


Rohini x DRMR 261

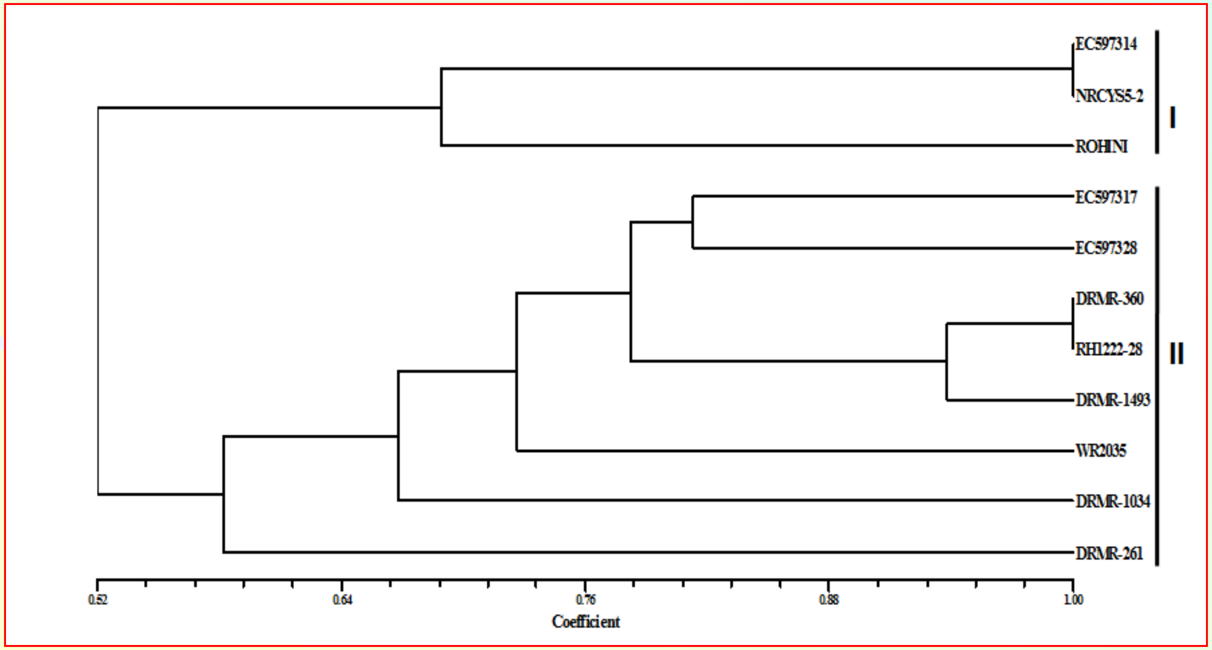
RH 1117 x EC 597328

RH 1117 x EC 597343

RH 555 x JM6010

Genetic diversity among tolerant and susceptible genotypes

- Eight tolerant and 3 susceptible (Rohini, NRCYS 5-2 and EC 597314) were used for diversity analysis and the dendrogram constructed gave two distinct groups.
- The cluster analysis evidently discriminated and differentiated the 11 genotypes into tolerant and susceptible.
- The similarity coefficients varied between 0.38 to 1.0 thus revealing the presence of maximum diversity between these genotypes.



Female	Donor (male)
RH 749	RH 1222-28
RH 406	DRMR 2585
RH 555	IC 576316
RH 1138	IC 766097
RH 1231	EC 597328
	IC 492724
	IC 206751

- Crosses were attempted for Sclerotinia resistance as well as multiple disease resistance.

J Mycol Plant Pathol, Vol. 42, No.3, 2012 297

Full Paper

Evaluation of Indian and Exotic *Brassica* Germplasm for Tolerance to Stem Rot caused by *Sclerotinia sclerotiorum*

Pankay Sharma, JS Chauhan and Arvind Kumar
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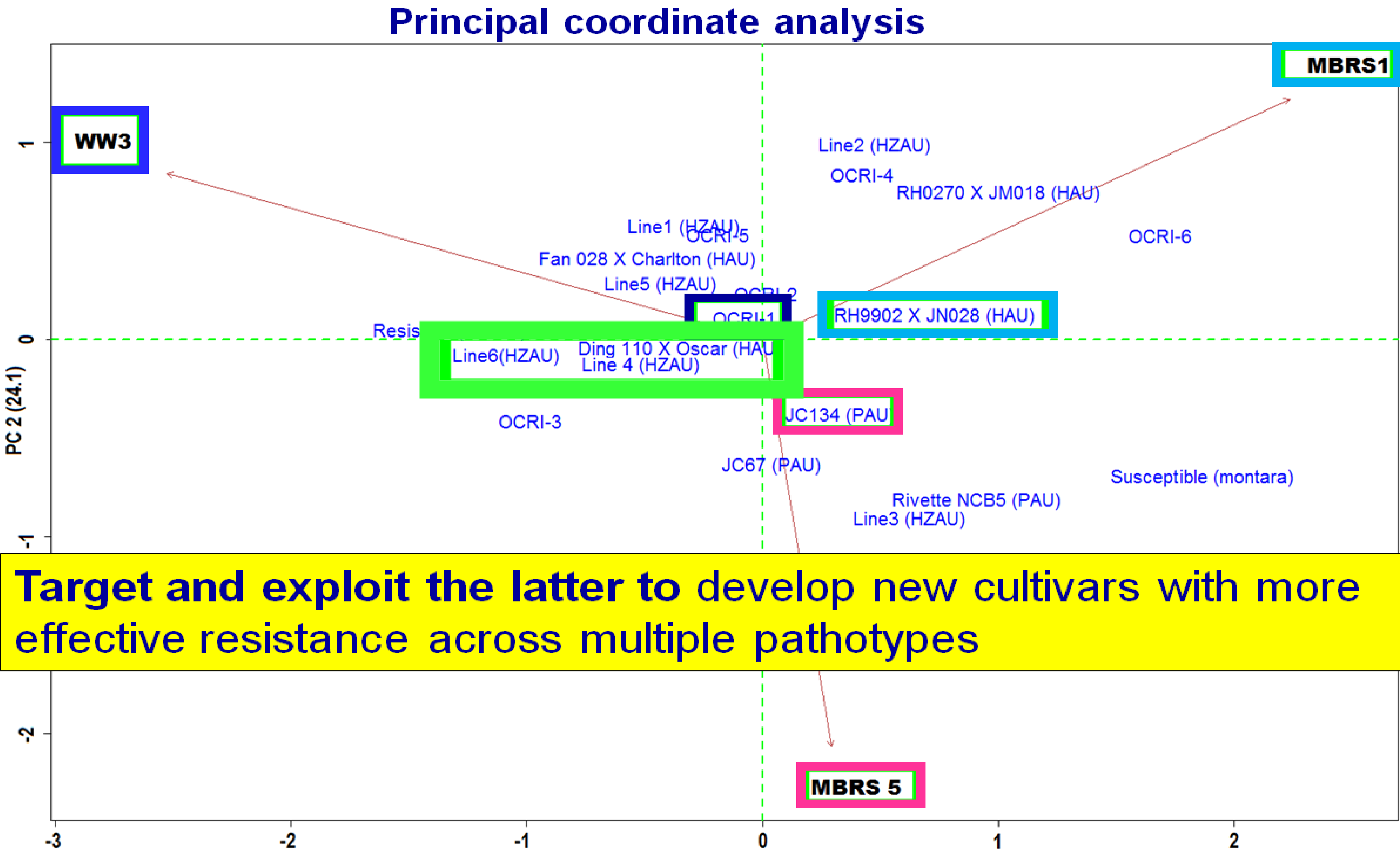
Resistance to sclerotinia is a desirable but rare trait.

- More recently, higher levels of resistance have been identified in *B. napus* germplasm from Pakistan, South Korea and Japan (IRC 2015).
- Numerous major and minor quantitative resistance loci (QRL) were identified through quantitative trait loci analysis.
- In addition, QRL were identified by association mapping using a set of resistant and susceptible accessions in a world collection of *B. napus*.

5. Breeding for resistance – different resistance types



Target and exploit the latter to develop new cultivars with more effective resistance across multiple pathotypes

Barbetti, Banga, Fu, Li, Singh, Liu, Ge, Banga 2014 *Euphytica* 197: 47–59

1. Appropriate screening methodology

STEM INOCULATION chosen for screen genotypes under field conditions at flowering stage (single agar plug disc bearing actively growing mycelium according to Buchwaldt et al, (2004, 2005).

2. Characterize Pathogenic sub-specific variation

BUT there was a greater challenge

- Genotypes responses differ across regions and countries
- High levels of sub specific pathogen variations
- Needed symptoms to characterize it

3. Locate host resistance

Excellent resistance/tolerance to prevailing isolates

B.juncea

RH 1222-28,
IC 570316,
IC 492724,
DRMR 2585,
DRMR 1034,
DRMR 1493
WR 2035
(lesion 0.0 to 1.2 cm)

B.carinata

DRMR 261

***Best* =**

B. napus ZY006 (**China**)
(stem lesion length <0.45cm)

***Others excellent* =**

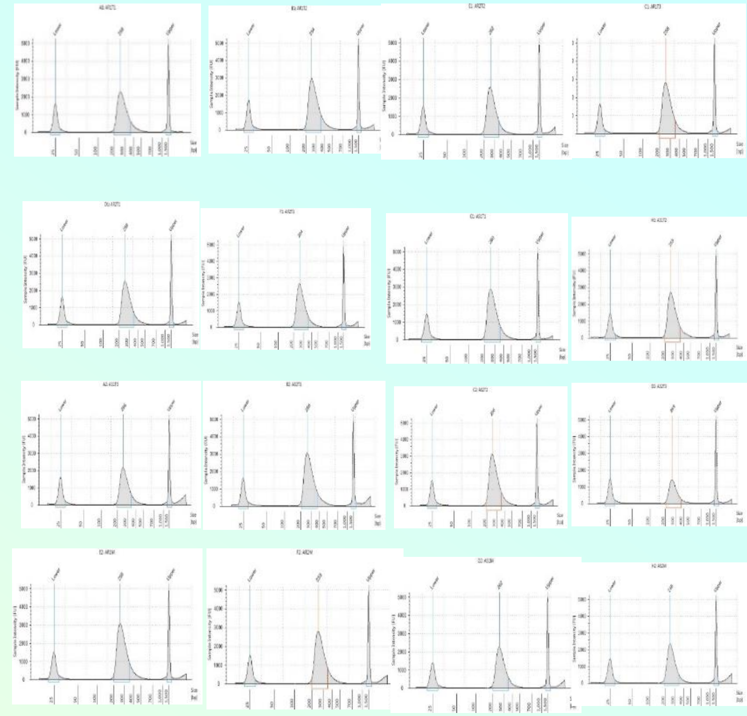
B. napus
06-6-3792 & ZY004 (**China**)
RT108 (**Australia**)

B. juncea
JM06018 & JM06006 (**Australia**)
B. juncea-2 (**China**)

Dr. M. Barbetti, IRC 2015

Transcriptome

- ✓ RH122-28 has been found most promising tolerant germplasm amongst all the screened population and NRCHB 101 as susceptible check.
- ✓ To know the basic molecular mechanism behind its potential tolerance and also to identify the potential target gene (s) RH 1222-28 (R) and NRCHB 101 (S) were chosen for the comparative RNASeq analysis by transcriptome profiling.
- ✓ RNA sample was analyzed by Bioanalyzer. Rin value of all the RNA samples were very good >/= 8.5
- ✓ All the 16 samples, 8 from each R and S line have been used for RNAseq library preparation and all the QC qualified libraries were processed for the run on Illumina HiSeq 4000 platform.



RNASeq Library prepared from all the pooled 16 samples by Tru-seq RNA LT kit and their TAPE Station profile.

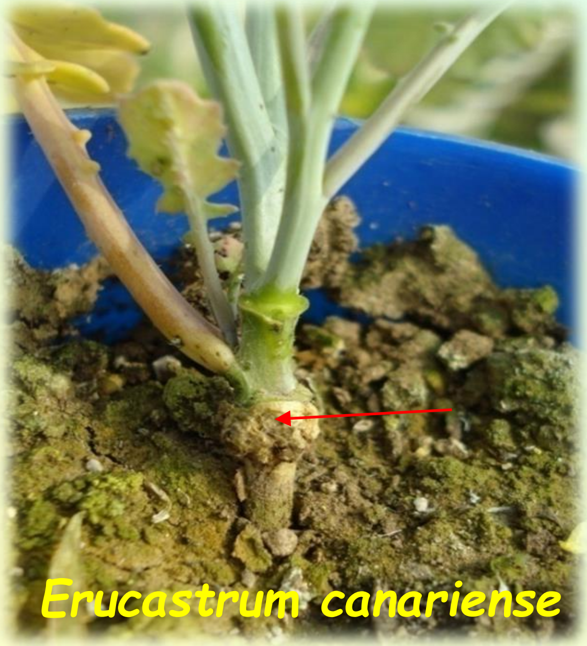
- ✓ After complete bioinformatics analysis we will be able to predict the potential target genes and after validation of the target genes by QRT PCR, pathways will be revealed for further investigation and application.

Quantitative Trait Locus (QTL)

- ✓Results from mapping and genetic analysis of *Sclerotinia* resistance show that QTLs in rapeseed would be very useful for marker-assistant selection and durable resistance cultivar breeding.
- ✓Zhao and Meng (2003) identified three quantitative trait loci (QTLs) on the linkage groups, N3, N12 and N17 of the *A*- and *C*-genomes of *B. napus* involved in the control of resistance to SR at the seedling stage, although, three QTLs on N7, N10 and N15 control resistance at the adult plant stage.
- ✓On the other hand, Zhao *et al*. (2006) identified eight regions on N2, N3, N5, N12, N14, N16 and N19 affecting resistance to this disease.
- ✓A total of 12 QTL for leaf resistance and six QTL for stem resistance were identified (Mei et al, 2013).

5. Breeding for resistance – Introgressions from weeds

- Lack of effective resistance in cultivated species has stimulated interest of the researchers towards exploitation of wild crucifer species to diversify the existence gene pool.
- Thirteen wild *Crucifers* were screened with artificial stem inoculation technique under pot house condition.



- ✓ Reaction to the disease was observed which indicated *Diplotaxis gomezcampoi*, *D. settiana*, *Brassica fruticulosa* and *Arabidopsis thaliana* showed resistant reaction.

WHERE WE ARE TODAY ??

Finally- effective management can be a reality

1. Good disease management strategy- irrigation management and foliar spray of carbendazim/bio-agent.

2. Host resistance in *B. juncea* (India), *B. napus (China & Australia)* reported.

AND more to come

1. Now need to have a host differential set; so can:

- Monitor current and new pathotype distributions
- Identify resistance(s) against the predominant pathotype(s)
- Combine resistances to different pathotypes in future cultivars.

My sincere Thanks to:

- GCIRC
- Organizers of Technical meeting 2017
- Indian Council of Agricultural Research (ICAR), New Delhi
- Director, ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur
- Team members of Extra-mural research project Dr. N.C. Gupta, NRCPB; Dr. L.Prasad, IARI