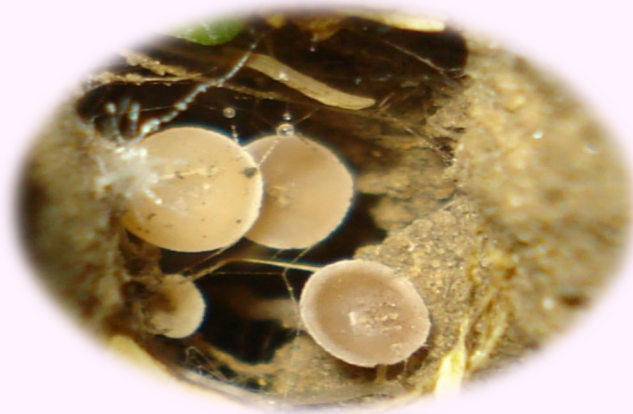


# Epidemiology and management of Sclerotinia stem rot in Indian mustard (*Brassica juncea*)



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# *Sclerotinia sclerotiorum*



- ✓ *Sclerotinia* disease of rapeseed-mustard, caused by fungal pathogen *Sclerotinia sclerotiorum*, is the most destructive disease worldwide.
- ✓ *Sclerotinia* rot is also a serious threat to oilseed rape production with substantial yield losses worldwide including Australia, Europe, India and North America.
- ✓ This disease gained importance particularly in areas where farmers practiced mono-cropping of Indian mustard, which led to complete crop failure
- ✓ 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.

# Sclerotinia stem rot infected mustard field

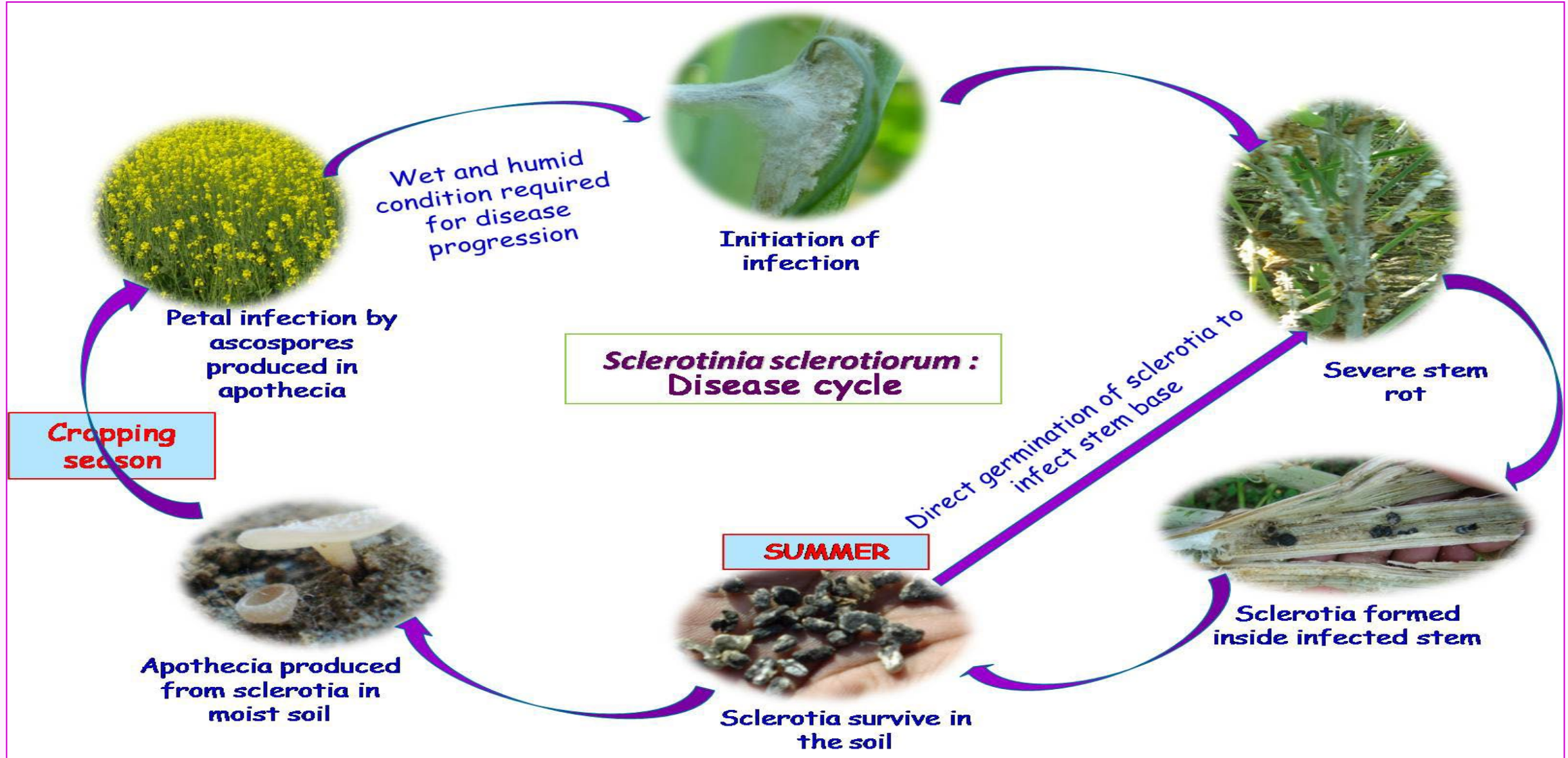


# Symptoms of Sclerotinia rot on stem, leaf and pod

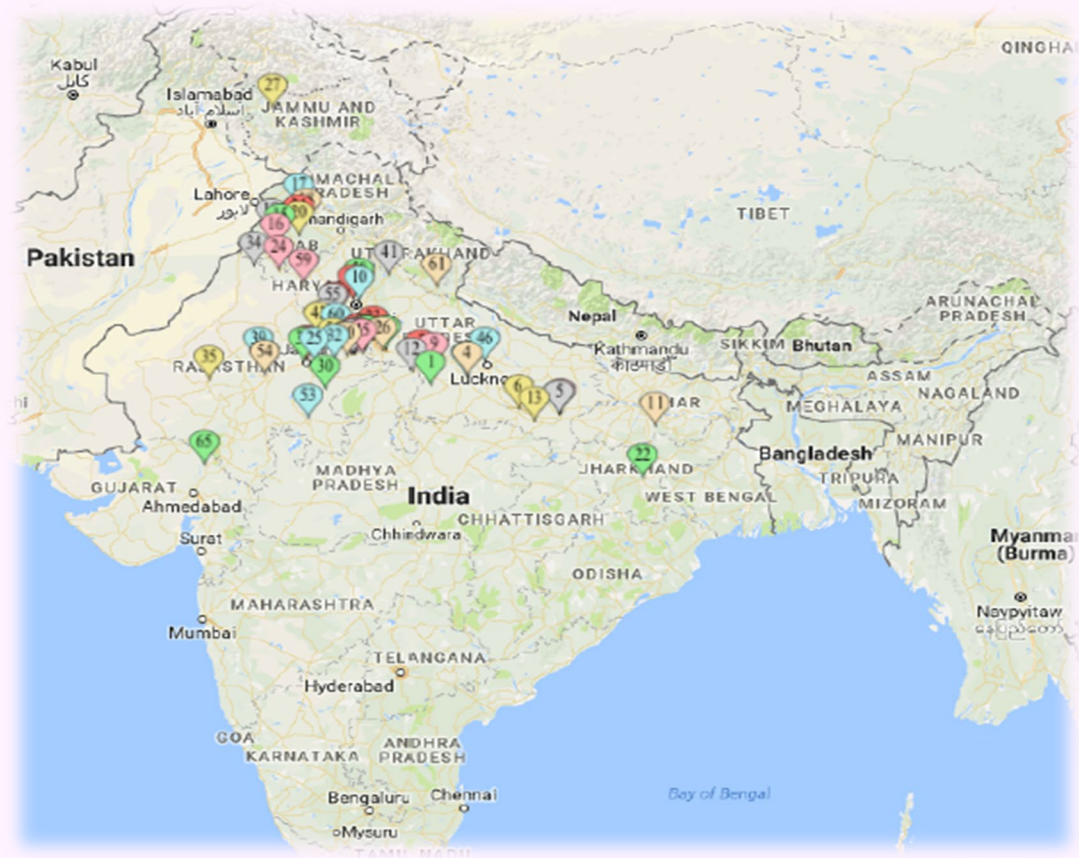


Sclerotia formed on stem, in pith & on root

# Sclerotinia sclerotiorum : Disease cycle

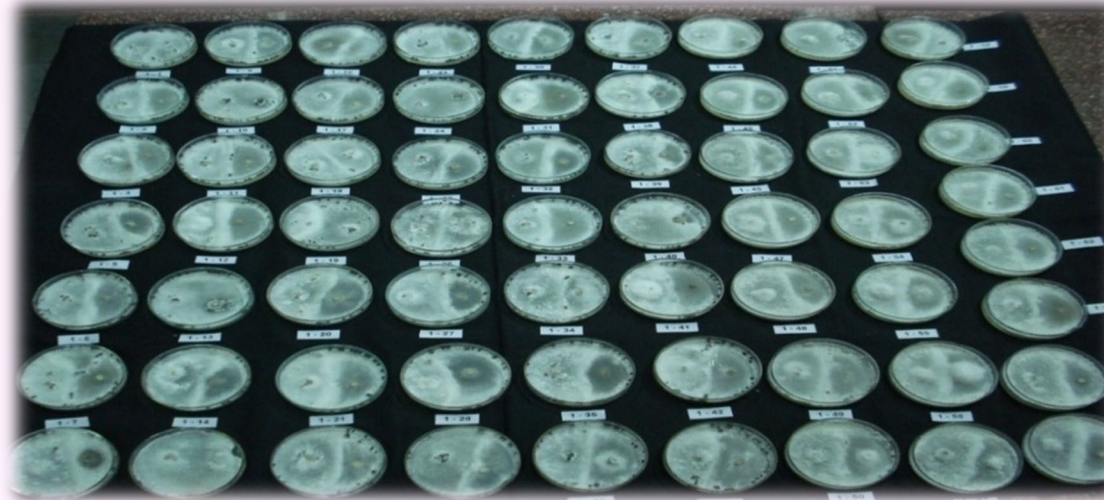
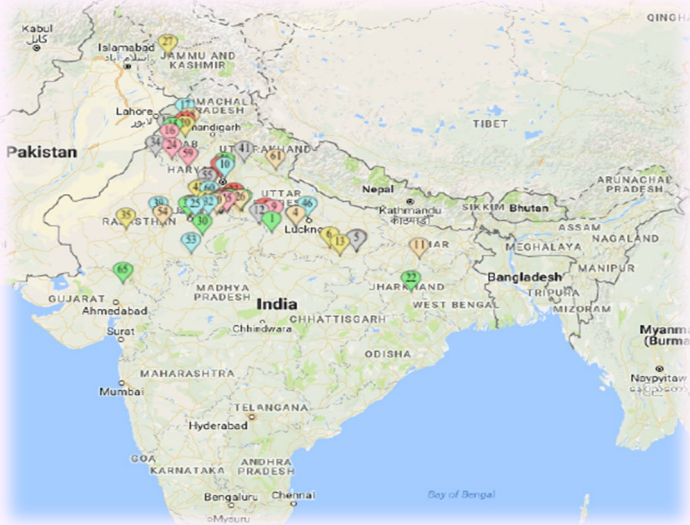


# About Pathogen: Morphological variability



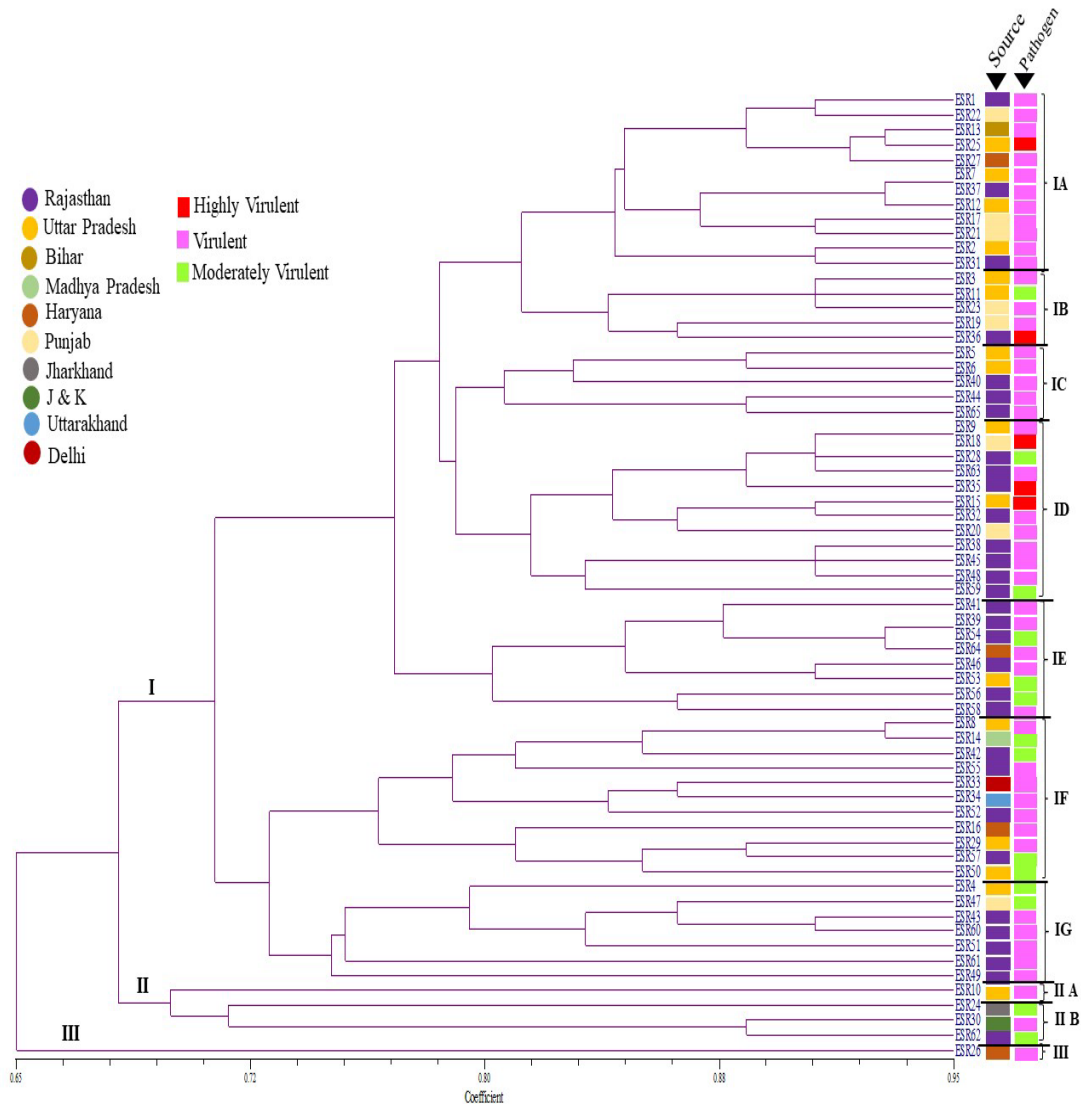
75 different geographical isolates of *S. sclerotiorum* studied for their morphological variability including mycelial growth, no. of days to form sclerotia, no. of sclerotia per Petri plate, size of sclerotia.

# About Pathogen: Mycelial Compatibility Groups (MCG)



- Mycelial compatibility is the ability of two strains of filamentous fungi to anastomosis and form one continuous colony.
- There were **5625 pairings of 75 isolates**, of these **439 combinations** showed **compatible reaction** (7.8% of all combinations), where mycelia of the two isolates intermingled at the interaction zone. The isolates, ESR-1, ESR-2 and ESR-3 were most compatible with other isolates.
- 18 isolates among 75 were completely incompatible with other isolates. This **strongly indicated predominant factor(s) of incompatibility** at the whole population level of *S. sclerotiorum*.

# About Pathogen: Genetic Diversity



Dendrogram derived from the morphological of the 65 different *S. sclerotiorum* isolates, based on growth, color, texture, shape and size of the mycelia and sclerotia and their respective pathogenicity over Brassica species.

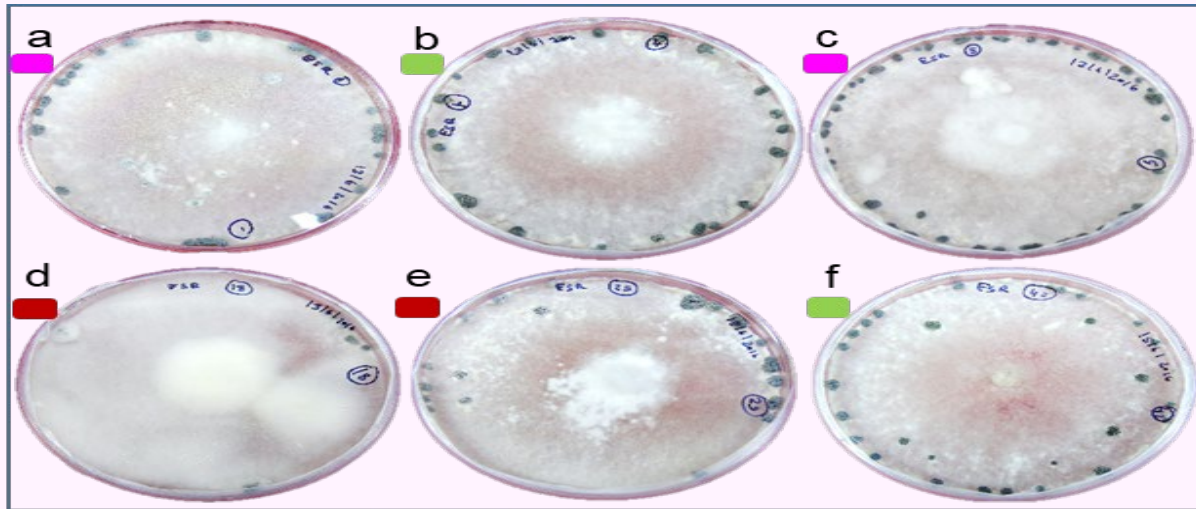
# About Pathogen: PAN India collection

❖ SS isolates for secondary metabolite Profiling

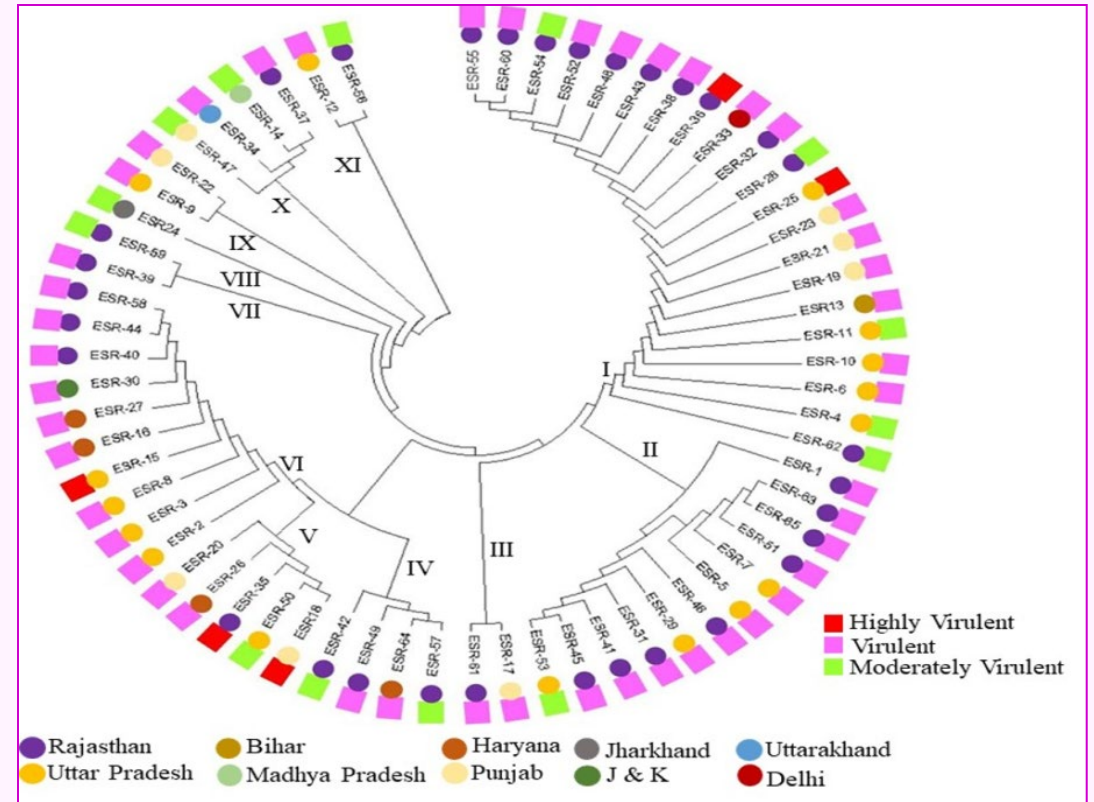
**Highly Virulent (HV):** ESR 18, ESR 25

**Virulent (V):** ESR 01, ESR 05

**Moderately V (MV):** ESR 04, ESR42

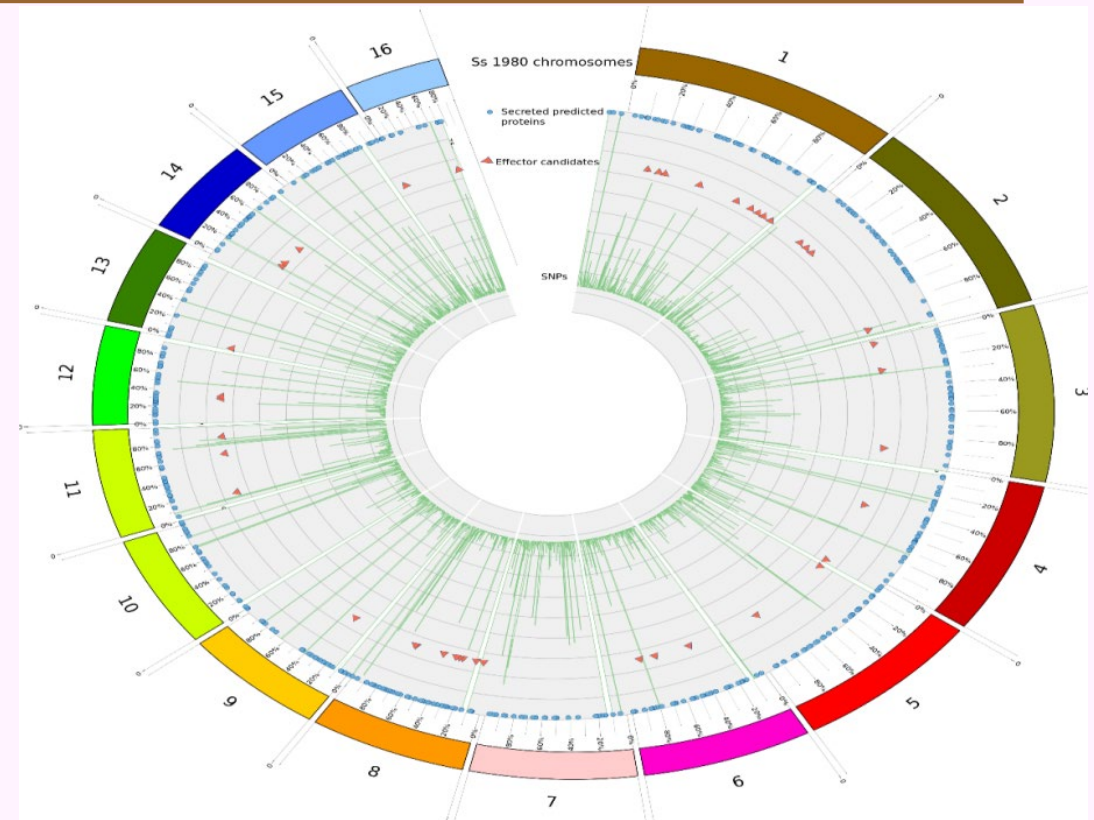


**HV** **V** **MV**



# About Pathogen: Genome sequencing and secretome analysis

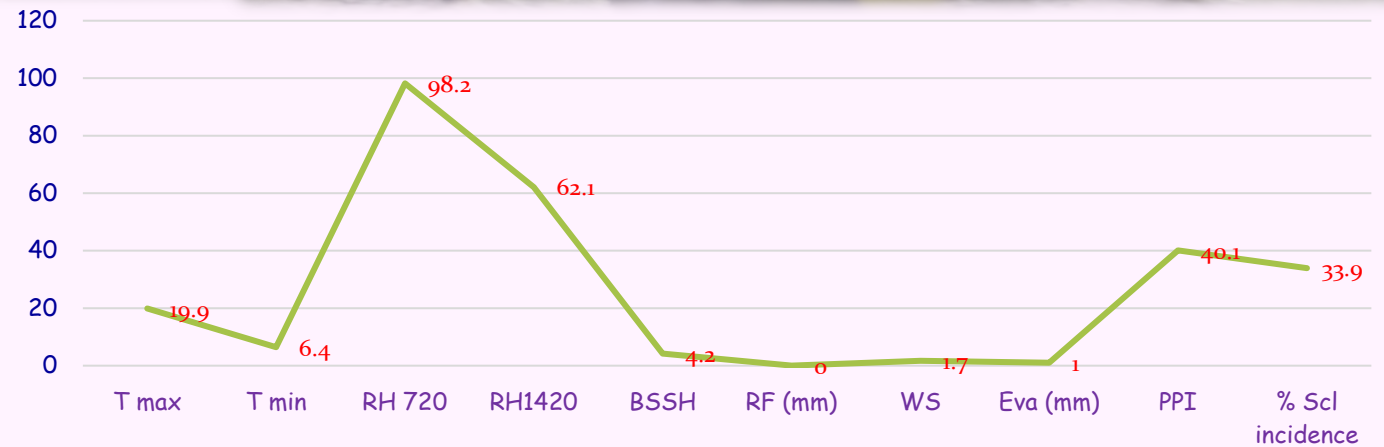
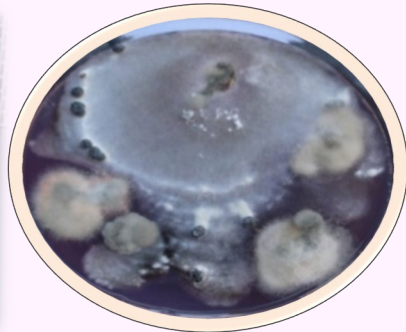
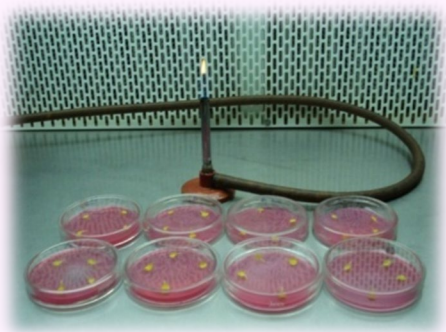
*S. sclerotiorum* isolate ESR-01 was sequenced and an assembly size of **~41 Mb** with 328 scaffolds having N50 of 447,128 was obtained through a *de novo* assembly of the high quality reads. A total of 9469 protein-coding genes were predicted from genome assembly. The average **gene length** and its density in the *S. sclerotiorum* genome were **1,587 bp** and **230.95 genes per Mb**, respectively.



CIRCOS plot of the assembled scaffolds of ESR-01 against "1980" isolate of *S. sclerotiorum* displaying comparative genomic and secretory proteins features. Schematic representation of the total SNPs, secretory proteins and the effector proteins of the secretome data of the *S. sclerotiorum* 'ESR-01' genome shown as histogram (different colors and types) and inner circle (blue) is total SNPs and scattered circle is secretory proteins and scattered red triangle are the effector proteins predicted in ESR-01 isolate.



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

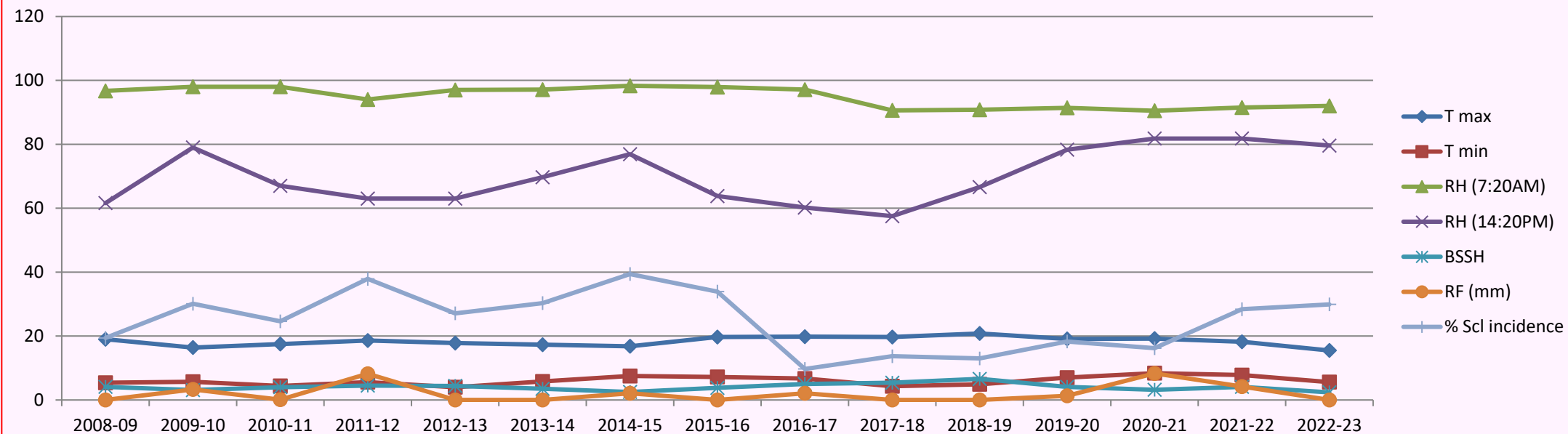


Effect of different weather variables on per cent Sclerotinia incidence (1-3 standard week)

# Weather data and SR incidence (last 15 years)

Year	T max	T min	RH (7:20AM)	RH (14:20PM)	BSSH	RF (mm)	% Scl incidence
2008-09	19.0	5.4	96.7	61.6	4.1	0.0	19.4
2009-10	16.4	5.7	98.0	79.0	3.1	3.3	30.1
2010-11	17.5	4.4	98.0	67.0	4.0	0.1	24.6
2011-12	18.6	5.6	94.0	63.0	4.5	8.2	37.9
2012-13	17.8	4.0	97.0	63.0	4.4	0.0	27.1
2013-14	17.3	5.8	97.1	69.7	3.5	0.0	30.3
2014-15	16.8	7.5	98.3	76.9	2.5	2.1	39.4
2015-16	19.7	7.2	97.9	63.8	3.8	0.0	33.9
2016-17	19.8	6.7	97.1	60.2	5.0	2.1	9.7
2017-18	19.7	4.3	90.6	57.5	5.4	0.0	13.7
2018-19	20.8	4.9	90.8	66.6	6.6	0.0	13.0
2019-20	19.1	7.0	91.4	78.3	4.1	1.3	18.3
2020-21	19.2	8.4	90.5	81.8	3.2	8.3	16.2
2021-22	18.2	7.8	91.5	81.8	4.1	4.2	28.4
2022-23	15.5	5.6	92.0	79.6	2.4	0.0	29.9
<b>AV</b>	<b>18.3</b>	<b>6.0</b>	<b>94.7</b>	<b>69.9</b>	<b>4.0</b>	<b>2.0</b>	<b>24.8</b>

# Correlations



Correlations		Tmax	Tmin	RH72	RH142	RF	Sunshi	SR
Spearman's rho	Tmax	1	0.076	-.304**	-.602**	-0.096	.573**	-.111*
	Tmin	0.076	1	.158**	.355**	.316**	-.389**	.127*
	RH72	-.304**	.158**	1	.135**	0.076	-.372**	.187**
	RH142	-.602**	.355**	.135**	1	.202**	-.544**	.143**
	RF	-0.096	.316**	0.076	.202**	1	-.199**	0.033
	Sunshi	.573**	-.389**	-.372**	-.544**	-.199**	1	-.150**
	SR	-.111*	.127*	.187**	.143**	0.033	-.150**	1

\*\*Correlation is significant at the .01 level (2-tailed).

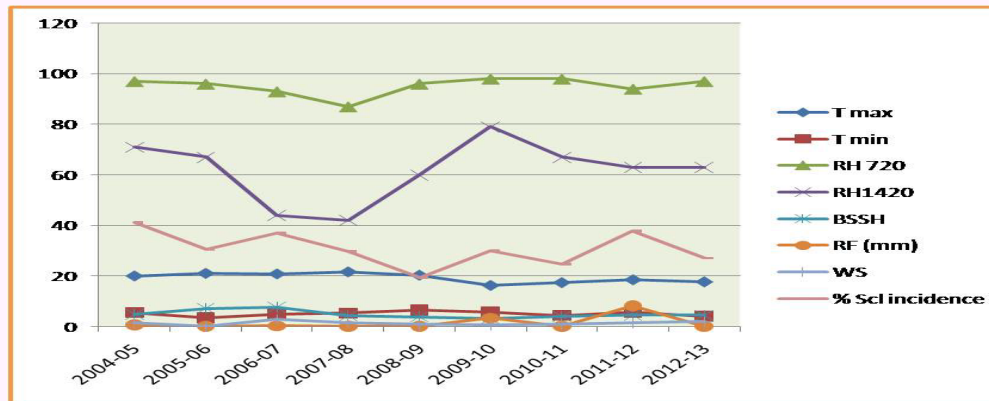
\* Correlation is significant at the 0.05 level (2-tailed).

# Forecasting Models

Date of planting	Models	R <sup>2</sup>	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<sup>2</sup> value of the regression analysis between observed and estimated SR prevalence was 0.98.



# Disease management: Difficulties

## 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

## Cultural Control

- ✓ Deep ploughing of field during summer.
- ✓ Sowing of healthy, certified and clean seeds free from the sclerotial bodies.
- ✓ Use of recommended dose of fertilizers @ N: P: K: S- 60:40:40:40 kg/ha.
- ✓ Maintain optimum plant population in the field (45x20 RxP).
- ✓ Avoidance of overcrowding of plants in a row to minimize plant to plant contact through root and stem to avoid disease spread by mycelial means.
- ✓ Irrigation management (no irrigation during 25 Dec-15 Jan) of mustard field.





# Management

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## Mechanical Control

- ✓ Destruction of diseased crop debris of previous crop.
- ✓ Based on symptom of early ripening of *Sclerotinia* rot affected plants, they could be rogue out before formation of sclerotia in order to reduces soil inoculum.
- ✓ At the time of harvesting and threshing sclerotia mixed with seed. These sclerotia could be removed with the help of different size sieves.

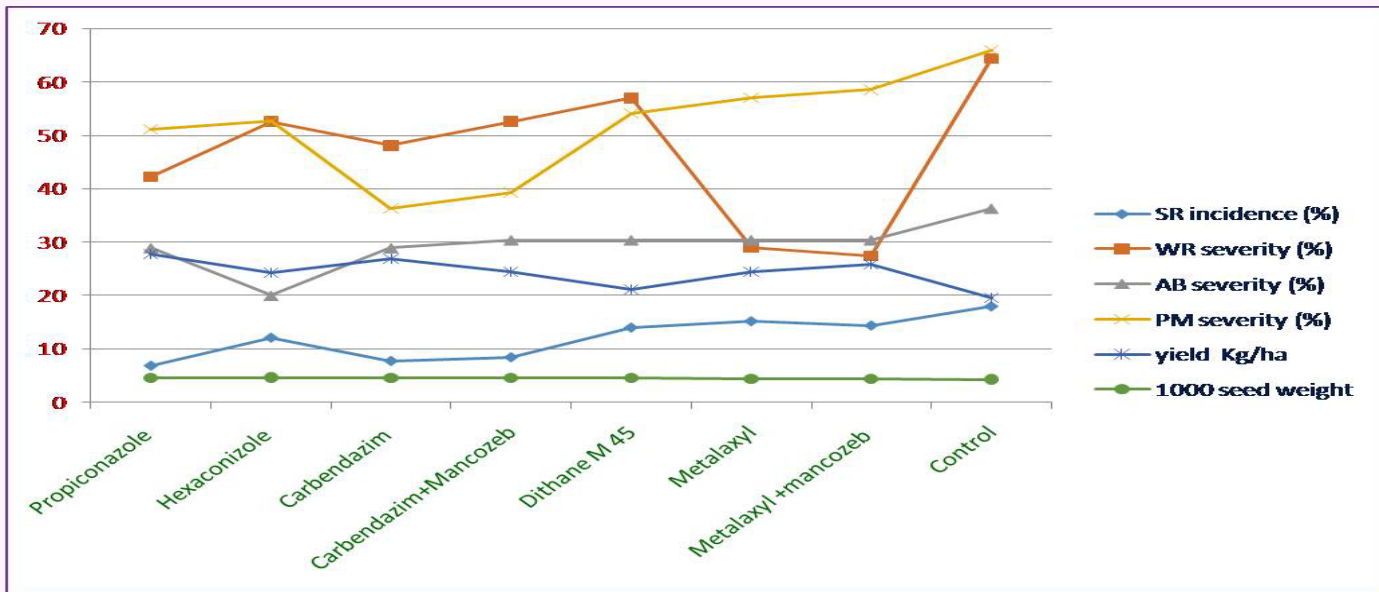
## Bio-agents

- ✓ Seed treatment with *Trichoderma* @10g/kg seed is effective.



# Fungicides

- 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.



- Sclerotinia rot incidence was low in propiconazole (6.8%) followed by carbendazim (7.7%).
- Propiconazole treatment given maximum seed yield (27.7 q/ha) as compared to control (19.6 q/ha).

The module was having combination of :

- Seed treatment with *T. harzianum* @10g/ kg seed,
- Soil application of Trichoderma (1 kg/ 50kg FYM),
- Basal application of zinc sulphate @ 15 kg/ ha +
- S (dose location specific)
- Boron (10 kg borax/ ha),
- line sowing 45X20 cm (RxP),
- no irrigation during 25th Dec to 15th Jan.
- ❖ control was maintained with farmer practices.

IDM module given maximum seed yield and 1000 seed weight (24.9 q/ha; 4.8g) as compared to control (18.8 q/ha; 4.2g).

Disease severity in IDM module was 8.9% (SR), 25.9% (WR), 21.4% (ABL) as compared to control 19.8, 60.0 and 28.8% respectively.  
(2019-20)

# 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.



# Resistance





# Screening for Sclerotinia rot resistance



✓ >5000 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 ICAR-DRMR.

# Mycelial growth in Sclerotinia sick plot



# Susceptible reactions



# Tolerant reactions



## Tolerant/resistant sources registered

Three Brassica germplasm first time (2022) registered with ICAR-NBPGR for Sclerotinia rot tolerance/resistance,

- ✓ *Brassica juncea* RH 1222-28 (IC0645775; INGR22166)
- ✓ *B. napus* NIPB-Bnap 114 (IC 0646858; INGR 22167)
- ✓ *B. Carinata* NIPB-Bcar 115 (IC 0646859; INGR 22168)





## WHERE WE ARE TODAY ??



1. Epidemiological studies provide the growers with information about a well-timed application of fungicides to manage SR.
2. SR can be effectively and economically managed by foliar application of fungicides viz., carbendazim/propiconazole or bio-agent *T. harzianum*.
3. Cultural practices, wider row spacing (45 cm) and no irrigation during 25 December to 15 January, also reduce the microclimate favourable for disease development.
4. >5000 Brassica germplasm were screened for resistance, tolerant sources were identified and registered in *B. juncea*, *B. carinata* and *B. napus*.





## Acknowledgment

- Director, ICAR-DRMR
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