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CULTURAL, MORPHOLOGICAL, PATHOGENIC VARIABILITY AND MYCELIAL COMPATIBILITY AMONG THE ISOLATES OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DE BARY CAUSE OF SCLEROTINIA ROT

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ABSTRACT
In the present study all the twelve *S. sclerotiorum* isolates collected from different hosts showed variations in cultural, morphological and pathogenic characteristics. Among different isolates ,*Calendula officinalis* and *Circium arvense* isolates were found significantly fast growing. Maximum number of sclerotia (25 no.) was produced in *Ocimum sanctum* isolate, while minimum (10 no.) in *Cajanus cajan*. Largest size of sclerotia (4.8x4.2 mm) was observed in *Cicer arietinum*, while smallest (3.9x3.2 mm) in *B. juncea* isolates. *B. rapa* and *Solanum melongena* isolates were found most virulent, while *Phaseolus vulgaris* was least virulent. On the basis of mycelial compatibility in dual culture study these isolates were classified in four groups viz. Group I- *B. juncea*, *B. rapa* and *B. campestris* var. *toria*; Group II- *P. vulgaris*, *Cicer arietinum* and *C. cajan*; Group III- *O. sanctum* and *Tegetes erecta*; Group IV- *Chrysanthemum coronarium*, *C. arvense, C. officinalis* and *S. melongena*. The present study could be exploited for the identification of resistant sources against *S. sclerotiorum* causing stem rot in rapeseed-mustard

INTRODUCTION

Rapeseed-mustard crop plays a significant role in the diet of Indian people as a source of edible oil and vegetable. The crop is attacked by several diseases such as Alternaria blight (*Alternaria brassicae*), white rust (*Albugo candida*), downy mildew (*Hyaloperospora brassicae*) and Sclerotinia rot (*Sclerotinia sclerotiorum*). Among these , Sclerotinia rot was of minor importance till few years back, but recently it has been assumed serious threats in major rapeseed-mustard growing areas in the country. Under favourable condition it causes 40-72 per cent yield loss in rapeseed-mustard.(Chattopadhyay *et al*., 2003 and Ghasolia *et al*., 2004). The pathogen is reported to have a wide host range and known to infect more than 400 plant species (Kolte, 1985). Still the disease is unmanageable economically. The knowledge of pathogen variability is essential for breeding resistant varieties which is one of the most economic ways to overcome this problem (Kohn *et al*., 1991). Considering the importance of pathogen diversity in the development of resistant varieties the present study was undertaken.

MATERIALS AND METHODS

Isolation of pathogen from disease samples

Diseased plant parts showing characteristic symptoms of Sclerotinia rot (*S. sclerotiorum*) were collected from 12 different hosts viz. Indian mustard (*Brassica juncea*), rapeseed (*B. rapa*), toria (*B. rapa* var. *toria*), french bean (*Phaseolus vulgaris*), chick-pea (*Cicer arietinum*), pigeon-pea (*Cajanus cajan*), calendula (*Calendula officinalis*), chrysanthemum (*Chrysanthemum coronarium*), marigold (*Tegetes erecta*), tulsi (*Ocimum sanctum*), brinjal (*Solanum melongena*) and katili weed (*Circium arvense)*.The diseased plant parts were cut into small pieces, thoroughly washed 3 to 4 times in sterilized distilled water and then surface sterilized by dipping in 0.1% $HgCl_2$ solution for 1 min., followed by washing with sterilized water 3-4 times which were then aseptically transferred into Petri plates containing PDA. These plates were incubated at $20 \pm 1^{o}C$ for seven days. The pathogen was identified as *S. sclerotiorum* by the presence of sclerotia with fluffy mycelial growth, The pathogen was purified by hyphal tip method and maintained on PDA slants for further studies.

Morphological variability

Twelve different isolates of *S. sclerotiorum* collected from 12 different hosts were inoculated separately on Petri plates containing PDA using mycelial disc of 5mm dia. of 3 days old actively growing culture.The plates were incubated at $20 \pm 1^{o}C$ for 4-7 days. The cultural characters *viz.*, colony colour, type of growth, radial growth and growth rate of the pathogen were examined at 24 hrs interval for 4 days. The morphological characters viz. sclerotia formation, size of sclerotia, shape and colour of sclerotia were examined at 4-7 days after incubation.

Pathogenic variability

The susceptible Indian mustard var. Varuna (*B. juncea*) was

sown in field, in 3m row length with 30 cm distance between row to row and 10 cm plant to plant. After germination 15 plants per row were maintained. Each isolate was inoculated 70 days after sowing in each row of 15 plants in three replications separately. Mycelial disc of 5.0 mm dia. cut from the margins of 7 days old culture of each isolate grown on PDA was placed on the middle of the stem at the junction of branching with the help of a forceps and were wrapped with transparent cello-tape to protect it from dehydration. The uninoculated plants was retained as control. The plants were examined periodically for the appearance of disease symptoms and final observations on disease incidence and severity were recorded 30 days after inoculation. Disease reaction was observed using rating scale (0-4).

Mycelial compatibility

Mycelial compatibility among different isolates was studied byinoculating 5 mm disc of two different isolates on PDA 10 mm apart from the periphery on opposite sides of Petri plates. A total of 78 combinations were made for the study.The plates were incubated at 20 ± 1ºC for 4-7 days. Mycelial reaction was recorded as incompatible when an apparent line of demarcation, a barrage zone, or a mycelial free zone was observed between the confronting paired isolates. The pairing was scored as compatible when the two isolates merged to form one colony, with no distinct interaction zone.

RESULTS AND DISCUSSION

Cultural and morphological variability

All the isolates of *S. sclerotiorum* viz. *B. juncea, B. rapa, B. rapa* var. *toria*, *P. vulgaris*, *C. arietinum*, *C. cajan*, *S. melongena*, *C. officinalis*, *C. coronarium*, *T. erecta*, *O. sanctum* and *C. arvense* were differed in their cultural and morphological characters. The cultural characters like type of growth, colony colour, radial growth, growth rate and morphological characters viz.sclerotia formation, number and size of sclerotia were recorded on PDA plates (Plate 1). The results shown in Table 1 revealed that after 72 hrs significantly maximum radial growth (90 mm) was observed in *C. officinalis* and *C. arvense* isolates followed by *B. campestris* var. *toria* and *S. melongena* isolates (80 mm) . However, minimum in *P. vulgaris* isolate (72 mm). Maximum growth rate was observed in *B. rapa* isolate (65 mm/day) followed by *C. arvense* (58 mm/day) and *C. officinalis* (49 mm/day) at 72 hrs after incubation. However, other isolates were at par with each other in their growth rates. Sparse-irregular growth pattern was observed in *B. juncea*, *P. vulgaris*, *C. arietinum*, *C. coronarium* and *T. erecta* isolates; circular-irregular in *B. rapa* var. toria, *B. rapa var.* yellow sarson; fluffy-irregular in *C. cajan, O. sanctum* and in *S. melongena*; sparse regular in *C. officinalis* and fluffy-regular type of growth pattern in *C. arvense*.

Sclerotia were commonly formed either at the periphery or at the center of the Petri Plate in circular or scattered manner. Maximum number of sclerotia were formed in the isolate *O. sanctum* (25.0) followed by *C. coronarium* (24.0), *B. rapa* var. *toria* (23.6) and *C. officinalis* (23.0) which were at par with each other but significantly different from other isolates. However, minimum (10.0) in *C. cajan* isolate. In all the isolates the maturation of sclerotia with blackening rind occurred at 7-9 days after incubation. In all the isolates the shape of the sclerotia was more or less spherical to semi spherical. Largest size of sclerotia was observed in *C. arietinum* isolate (4.8x4.2 mm) followed by *O. sanctum* (4.5x4.2 mm) and *S. melongena* (5.3x3.5 mm) isolates. However, smallest in *B. juncea* isolate (3.9x3.2 mm) (Table 2). Manjunatha *et al*. (2014) also reported different types of growth in different isolates like white fluffy , white suppressed and dull white suppressed. In the present investigation isolates were also different in colony colour and growth pattern. In the present study sclerotia were formed at the periphery of the Petri Plates at 4-6 days after incubation in most of the isolates.However, Thilagavathi *et al*. (2013) reported formation of sclerotia 8 days after inoculation during the study of 9 isolates collected from different host plants.Ghasolia *et al*. (2007) who studied morphological variability among 38 isolates and reported that all the isolates showed variation in their morphological traits i.e., colony colour, shape, and sclerotia number, size, position and pattern

Table 1: Cultural variability among *S. sclerotiorum* isolates

Isolate	Hrs after incubation								Colonycolour	Type ofgrowth
	24		48		72		96			
	*RG (mm)	*GR (mm/day)	*RG (mm)	*GR (mm/day)	*RG (mm)	*GR (mm/day)	*RG (mm)	*GR (mm/day)		
B.juncea	5.5	4.9	35	29.5	78	43.0	90	12.0	Creamy white	Sparse, irregular
B. rapa	5.5	5.5	30	24.5	75	65.0	90	15.0	Creamy white	Fluffy, irregular
B. Rapa var. *toria*	9.0	9.0	40	31.0	80	40.0	90	10.0	Creamy white	Fluffy, irregular
Phaseolus vulgaris	5.0	5.0	32	27.0	72	40.0	90	18.0	Creamy white	Sparse, irregular
Cicer arietinum	7.0	7.0	30	23.5	75	45.0	90	15.0	Creamy white	Sparse, irregular
Cajanus cajan	5.5	6.2	30	23.8	76	46.0	90	14.0	Creamy white	Circular, irregular
Calendula officinalis	11.0	11.0	41	30.0	90	49.0	90	0.00	Creamy white	Sparse, regular
Chrysanthimum coronarium	6.0	6.0	27	21.0	75	47.0	90	16.0	Creamy white	Sparse, irregular
Tegetes erecta	6.5	6.5	30	23.5	76	46.0	90	14.0	Creamy white	Sparse, irregular
Ocimum sanctum	7.0	7.0	32	25.0	78	46.0	90	12.0	Creamy white	Circular, irregular
Solanum melongena	7.0	7.0	37	30.6	80	42.6	90	10.0	Creamy white	Circular, irregular
Circium arvense	8.0	8.9	34	24.0	90	58.0	90	0.00	Creamy white	Fluffy, regular
CD (0.05)	1.82	1.67	4.34	4.17	2.55	5.12	-	2.55	-	-
CV (%)	15.5	14.3	7.80	9.50	1.92	6.42	-	13.3	-	-

* Mean of three replication; RG = Radial growth; GR = Growth rate

Table 2: Morphological variability among *S. sclerotiorum* isolates

Isolate	Initiation of sclerotia formation(DAI)	*No. of sclerotia(7 DAI)	** Size of sclerotia	
			Length(mm)	Width(mm)
B.juncea	5	19.6	3.9	3.2
B. rapa	6	13.0	4.8	3.8
*B. rapa*var. *toria*	4	23.6	3.9	3.0
Phaseolus vulgaris	6	19.0	3.7	3.5
Cicer arietinum	6	11.0	4.8	4.2
Cajanus cajan	6	10.0	4.6	4.0
Calendula officinalis	4	23.0	4.5	3.9
Chrysanthimum coronarium	4	24.0	5.2	3.5
Tegetes erecta	5	18.0	3.8	3.3
Ocimum sanctum	4	25.0	4.5	4.2
Solanum melongena	5	18.0	5.3	3.5
Circium arvense	6	13.0	5.0	4.0
CD (0.05)		3.66	0.51	0.34
CV (%)		11.9	6.87	5.59

* Mean of three replication;** Mean of five *sclerotia*

Table 3: Pathogenic variability among *Sclerotinia sclerotiorum* isolates affecting rapeseed-mustard

Isolates	Inoculated plants no.	Infected plants no.	Disease Incidence (5)	Severity(Length of infected stem) cm
Calendula officinalis	15	2	13.33 (17.64)	1.0 (2.72)
Solanum melongena	15	15	100.00 (90.00)	23.32 (28.83)
Tagetes erecta	15	4	26.67 (30.79)	5.07 (12.83)
Brassica rapa var. *toria*	15	15	100.00 (90.00)	13.77 (21.43)
Brassica juncea	15	15	100.00 (90.00)	19.86 (26.40)
Phaseolus vulgaris	15	1	6.67 (8.86)	0.27 (1.71)
Cirsium arvense	15	14	93.33(81.14)	10.95 (18.85)
*Brassica rapa var. y*ellow sarson	15	15	100.00 (90.00)	23.91 (28.86)
Ocimum sanctum	15	7	46.67 (43.08)	2.83 (7.82)
Pisumsativum	15	12	80.00 (68.07)	7.87 (15.69)
Cajanuscajan	15	12	80.00 (68.07)	6.75 (14.81)
Cicer arietinum	15	9	60.00(55.78)	4.35 (10.95)
CD at 5%			35.20 (31.137)	7.9 (8.184)
CV			31.451 (30.828)	47.108 (30.82)

Table 4: Mycelial compatibilty among *S. sclerotiorum* isolates

SR isolate	Bj	Brpy	Bcrpt	Pv	Ca	Cc	Cof	Cco	Te	Os	Sm	Car
B. juncea(Bj)	C	C	C									
B. rapa var. yellow sarson(Brpy)	C	C	C									
*B. rapa*var. *toria* (Brpt)	C	C	C									
Phaseolus vulgaris(Pv)				C	C	C						
Cicer arietinum(Ca)				C	C	C						
Cajanus cajan(Cc)				C	C	C						
Calendula officinalis(Cof)							C	C			C	C
Chrysanthimumcoronarium(Cco)							C	C			C	C
Tegeteserecta(Te)									C	C		
Ocimum sanctum(Os)									C	C		
Solanum melongena(Sm)							C	C			C	C
Circium arvense(Car)							C	C			C	C

when grown on PDA.

Pathogenic variability

On the basis of disease incidence and severity, twelve *Sclerotinia* isolates showed different pathogenic behavior. It was observed that isolates *B. juncea* (Pantnagar), *B. rapa* var. Toria, *B. rapa* var. yellow sarsonand *S. melongena* showed significantly higher severity and hundred per cent incidence indicating their high virulence as compared to other isolates followed by, *C. Arvense,P.sativum and C. Cajan* isolates. However *P. vulgaris* and *C. officinalis* isolate were less virulent. Ghasolia *et al.* (2007) also reported the pathogenic variability among 38 isolates of *S. sclerotiorum* and found that all the isolates were virulent on all the 10 genotypes.

Mycelial compatibility reaction

On the basis of mycelial compatibility (compatible and incompatible reactions) in dual culture study twelve selected

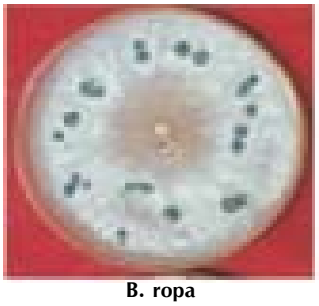

B. juncea


Tegetus erectus


Cojanos cojan


Colendulo officinalis


Cicer ariteeoum

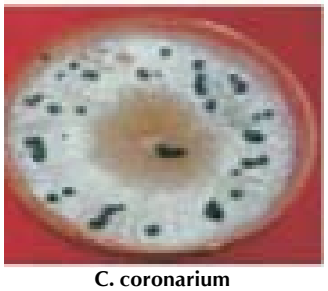

B. ropa


Solonum melongeno


Phaselous vulgoris


Circium orvense


B. Conpestris var toria


C. coronarium

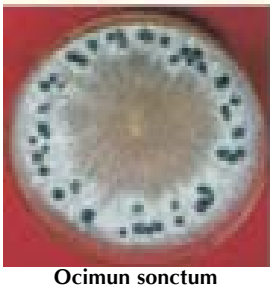

Ocimun sonctum

Plate 1: Cultural and morphological variability among S. selerotiorum isolates


Compatible reaction between two isolates


Incompatible reaction between two isolates

Plate 2: Mycelial compatibitity among different isolates of S. sclerotiorum

isolates were paired with each other to study mycelia compatibility. There were 78 pairings of the 12 isolates and out of all, 27 combinations showed a compatible reaction (34% of all the combinations) where mycelia of the two isolates intermingled at the zone of interaction, while 51 combinations (65% of all the combinations) showed incompatible reaction i.e. formation of zone between two isolates. On the basis of mycelial compatibility reactions these isolates were classified in four groups viz. Group I- *B. juncea, B. rapa* and *B. rapa* var. *toria*; Group II- *P. vulgaris* and *C. arietinum* and *C. cajan*; Group III- *O. sanctum* and *T. erecta* ; Group IV-*C. coronarium*, *C. arvense, C. officinalis and S. melongena*. The isolates within a group are similar with each other in most of the characteristics while differ with the isolates of other groups

The findings of the present investigation were also supported by earlier workers (*Kohn et al., 1991; Durman et al., 2003;* Kull *et al.*, 2004; *Zandoki et al., 2005; Akram et al., 2008;* Banik and Sharma (2009)) who studied variability on the basis of compatible and incompatible reactions among *S. sclerotiorum* isolates collected from different hoststhe Indian isolates of *S. sclerotiorum*. This variability can be further used for molecular study to determine genetic variability among sclerotinia isolates. The present study could be exploited for the identification/selection of resistant varieties against *S. sclerotiorum* causing stem rot in mustard.

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