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Research Articles

Morphological and pathogenic variability among Indian populations of *Sclerotinia sclerotiorum* causing stem rot of chickpea (*Cicer arietinum*)

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ABSTRACT

Stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is one of the most important diseases of chickpea (*Cicer arietinum* L.). Irrespective of hosts of origin, isolates were proved to be pathogenic on chickpea cultivar BDG 1005. Majority of the isolates (20) produced white to dull white and fluffy growth while, only 4 isolates, namely, SS5 (Delhi), SS14 (Uttar Pradesh), SS20 (Haryana) and SS24 (West Bengal) produced suppressed growth without sclerotia which is the rare evidence in this pathogen. Isolates were grouped into three categories on the basis of their growth rate as slow, medium and fast growing. Sclerotia formed in different isolates were highly variable in number as well as in size. Isolate SS6 (Delhi) produced maximum number (50/plate) of smaller size (2.16 mm) sclerotia whereas, isolate SS15 (Himachal Pradesh) produced 21 sclerotia/plate with largest size (4.54 mm). Isolates were also variable in respect of their virulence. They were grouped into 7 pathotypes based on differential reactions on a set of 10 chickpea differential cultivars, namely, DCP 92-3, Pusa 212, Vishal, JG 74, KRW 108, GNG 469, JG 62, GPF 2, Pusa 1073 and WR 315. In the present study, cultivars DCP 92-3, Pusa 212, Vishal, JG 74, GPF 2 and WR 315 were considered to be host differentials for grouping of *S. sclerotiorum* into 7 pathotypes. Pathotype groups were not followed the continuity with that of geographical origin of isolates. It indicated that each region had mosaic of pathotypes. This is the first study in which the pathotypes of *S. sclerotiorum* populations were determined.

Key words: Chickpea, Differentials, Pathotypes, *Sclerotinia sclerotiorum*, Stem rot, Virulence

Sclerotinia sclerotiorum (Lib.) de Bary is the most cosmopolitan and versatile pathogen and it infects more than 400 species of plants, including field crops, vegetables and weeds. Sclerotinia stem rot was reported in Australia (Fuhlbohm *et al.* 2003), Canada (Hilton 2000) and United States (Chen *et al.* 2006). During 1986, its occurrence was reported in Himachal Pradesh with considerable yield losses of chickpea (Grewal and Pal 1986, Sharma 1995). In India, chickpea (*Cicer arietinum* L.) is prone to many diseases, amongst them stem rot caused by *S. sclerotiorum* is one of the most devastating diseases and causes significant yield loss in cool and moist areas. The disease causes total crop failure where chickpea is grown in the same paddock in successive years. Dense crops are likely to be the most severely affected by the disease, particularly under moist condition. It is also frequently found to be associated with the root rot complex in chickpea. Besides chickpea, it also causes damage to vegetables and oilseed crops. *S. sclerotiorum* is a cosmopolitan, homothallic and necrotrophic, ascomycetous fungus dispersed by air borne ascospores or soil borne sclerotia (Hambleton *et al.* 2002).

The different isolates of *S. sclerotiorum* were found to have different growth characteristics, growth rate as well as sclerotia size (Sun *et al.* 2005). Physiological specialization was not yet reported in *S. sclerotiorum*, but strains can differ in their reactions to various hosts and also in different cultivars of the same host (Kim *et al.* 2000, Yli-Mattila *et al*. 2010). High level of genetic and phenotypic diversity of *S. sclerotiorum* on a small geographic scale, present challenges in managing the diseases it causes (Attanyake *et al.* 2011).

Soil borne nature of the pathogen makes the crop rotation as well as chemical methods for disease management ineffective. Host plant resistance is an economical approach, but only a few sources of moderate resistance to stem rot are available (Vaid *et al*. 2005). Therefore, commercial cultivars with genetic resistance to the disease have not yet been developed. The information is not available in respect of virulence/pathotypes of *S. sclerotiorum* prevalent across the country. The knowledge on variability in respect of virulence and aggressiveness of a pathogen is considered to be an important aspect for disease management especially for development of area specific resistant cultivars. Therefore, the present investigation was undertaken to determine the

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morphological and pathogenic variability in the Indian populations of *S. sclerotiorum*.

MATERIALS AND METHODS

Twenty four isolates of *S. sclerotiourm* (12 from chickpea and 12 from other host crops) collected from different states of India were selected for morphological and pathogenic characterization (Table 1). The purified (by single sclerotia) cultures of various isolates were identified on the basis of mycelial and sclerotial characters. The isolates were maintained in potato dextrose agar (PDA) slant at 4°C for further studies. The pathogenicity of the cultures of *S. sclerotiorum* was tested on highly susceptible chickpea cultivar BDG 1005 to establish Koch's postulates under artificially inoculated condition by detached shoot-screening technique described by Melouk *et al.* (1992).

Table 1 Isolates of *Sclerotinia sclerotiorum* collected from different states of India and their pathogenicity on chickpea cultivar BDG 1005 during 2008-09

Isolate number	Location	State	Host	Lesion size (mm)*
SS1	IARI, New Delhi	Delhi	Chickpea	106.33
SS2	IARI, New Delhi	Delhi	Chickpea	75.00
SS3	IARI, New Delhi	Delhi	Pea	86.67
SS4	ITCC (6094), New Delhi	Delhi	Pea	51.67
SS5	ITCC (4042), New Delhi	Delhi	Mustard	36.33
SS6	Delhi University, New Delhi	Delhi	Mustard	70.00
SS7	Ureeka, Jhunjhunun	Rajasthan	Chickpea	72.00
SS8	ARS, Sriganganagar	Rajasthan	Chickpea	83.33
SS9	Jhunjhunun	Rajasthan	Mustard	71.66
SS10	Muktsar	Punjab	Chickpea	52.33
SS11	Gidderanwali, Firozpur	Punjab	Chickpea	70.33
SS12	BHU, Varanasi	Uttar Pradesh	Chickpea	77.33
SS13	Bhagwanpur, Varanasi	Uttar Pradesh	Rajma	106.67
SS14	Aligarh	Uttar Pradesh	Menthol	52.00
SS15	Dholaquan	Himachal Pradesh	Chickpea	63.33
SS16	Ranichauri	Uttarakhand	Chickpea	72.00
SS17	Ranichauri	Uttarakhand	Pea	97.00
SS18	Ranichauri	Uttarakhand	Pea	56.00
SS19	Pantnagar	Uttarakhand	Mustard	92.67
SS20	Hisar	Haryana	Coriander	37.67
SS21	Hisar	Haryana	Chickpea	63.00
SS22	Srinagar	Jammu & Kashmir	Chickpea	86.83
SS23	Samastipur	Bihar	Chickpea	87.67
SS24	Coochbehar	West Bengal	Tobacco	40.33
SEm ±				0.81
CD (P=0.05)				2.50

*Mean of three replications.

The isolates of *S. sclerotiorum* were grown on PDA medium (HiMedia, India). Colony diameter was measured on PDA medium poured into 90 mm petridishes (20 ml/plate) with 3 replications. The inoculated plates were incubated at 24±1°C for 48 h under 12 h alternate light and dark conditions. Colony characters such as growth pattern were recorded along with colony diameter. The isolates were grouped into 3 categories based on growth rate as slow (<1 mm/h), medium (1-1.5 mm/h) and fast (>1.5 mm/h) growing. The plates were incubated for 14 days for sclerotia development. Sclerotia formation pattern, number and size were recorded by using electronic digital calliper.

A set of 10 probable resistant and susceptible cultivars of chickpea, namely, DCP 92-3, Pusa 212, Vishal, JG 74, KRW 108, GNG 469, JG 62, GPF 2, Pusa 1073 and WR 315 were evaluated in the glass house by detached shoot technique (Melouk *et al.* 1992) to determine pathogenic variability. The chickpea plants were grown in pots in the greenhouse for 6 weeks and 3 shoots of 15 cm long were detached from each genotype for inoculation. Individual shoot was supported by a cotton plug and the cut end was immersed individually in 1×14 cm test tube containing Hoagland solution. Each shoot was inoculated by placing a 4 mm mycelial plug of *S. sclerotiorum* from 2-day-old culture grown on PDA at the axil between the stem and the petiole at about the mid portion of the shoot. Inoculated shoots were put on metal racks and placed in a growth chamber (24±1°C and 85-90% relative humidity). Shoots were randomized after inoculation before placement on the racks. Lesion development on the shoots was measured after 8 days of inoculation and scored for the disease on 0-5 scale **(**Asoufi *et al.* 2007**)**. Control without inoculation was also maintained.

Completely Randomized Design (CRD) was followed for these experiments and data were analysed statistically in CRD factorial using Windostat version 7.0 (Indostat Services, Hyderabad, India). The statistical significance was assessed at P<0.05 and Fisher's least significant difference test was used to separate means. This work was done in the year 2010.

RESULTS AND DISCUSSION

Pathogenicity test

All the 24 isolates of *S. sclerotiorum* collected from different states of India irrespective of hosts were proved to be pathogenic on chickpea cultivar BDG 1005 (Table 1). Thus, this finding convincingly proved the non-existence of host specialization in *S. sclerotiorum*. The re-isolated pathogens from the infected stems of chickpea were similar in their morphological characters with original isolates.

Morphological variability

The isolates were variable in respect of their colony characters. Most of the isolates (20) produced white to dull

white and fluffy growth while only 4 isolate, namely, SS5 (Delhi), SS14 (Uttar Pradesh), SS20 (Haryana) and SS24 (West Bengal) produced dull white and suppressed growth. The highest growth diameter was recorded in isolate SS13 (Uttar Pradesh) followed by isolates SS19 (Uttarakhand) and SS9 (Rajasthan) at 24 h of incubation. Similar growth was recorded in all these isolates at 48 h of incubation. The least growth was measured in SS18 (Uttarakhand) at both the durations of incubation. In the present study, based on the growth rate, the isolates were grouped into three categories. Six isolates, namely, SS14 (Uttar Pradesh), SS15 (Himachal Pradesh), SS16, SS17 and SS18 (Uttarakhand) and SS20 (Haryana) were slow growing (<1 mm/h) while other 6 isolates, namely, SS8 and SS9 (Rajasthan), SS10 and SS11 (Punjab), SS13 (Uttar Pradesh) and SS19 (Uttarakhand) were fast growing (>1.5 mm/h) and remaining 12 isolates were medium (1-1.5 mm/h) growing. It is evident that each group had the isolates from different areas of the country and different host. Earlier worker while working on *S. sclerotiorum* associated with different crop plants also grouped the isolates into different categories based on colony diameter on PDA medium (Sun *et al.* 2005).

Sclerotia formed in different isolates were also highly variable in number, size as well as in formation pattern. The sclerotial size ranged from 1.57-5.89 mm among the isolates. SS6 (Delhi) produced maximum number (50 sclerotia/plate) of sclerotia with smallest size (2.16 mm), whereas SS15 (Himachal Pradesh) produced 21 sclerotia/plate with largest size (4.54 mm). Two types of sclerotia formation patterns (scattered and periphery) were common. Twelve isolates produced scattered while 8 isolates produced sclerotia in periphery. The cultural and morphological characters were not correlated with geographical origin of the isolates.

Interestingly, four isolates, namely, SS5 (Delhi), SS14 (Uttar Pradesh), SS20 (Haryana) and SS24 (West Bengal) isolated from other than chickpea hosts did not produce any kind of sclerotia. Such altered debilitated traits of *S. sclerotiorum* might be due to infection of mycovirus/ hypovirus, uncapsidated dsRNA molecule (Allen *et al*. 2003, Xie *et al*. 2006) which needs further confirmation. Therefore, these 4 isolates may be correlated with hypovirulence isolates of *S. sclerotiorum.* But, this needs further confirmation for association of mycovirus with these 4 isolates of *S. sclerotiorum.*

Pathogenic variability and host differentials

The isolates were highly variable in their virulence by producing 9.8 to 90.3 mm lesion size on 10 chickpea cultivars during artificial inoculation. The results of mean lesion size of 10 cultivars revealed that SS4 (Delhi) isolate produced the largest lesion size (90.3 mm) and considered to be highly virulent isolate followed by SS23 (Bihar) and SS21 (Haryana) isolates with similar sizes (72.4 mm). The lowest lesion size was produced by SS5 (Delhi) followed by SS14 (Uttar Pradesh) isolates with statistically similar results. Therefore, these isolates were considered to be least virulent. The cultivars of chickpea varied in their reactions response against different isolates of *S. sclerotiorum*. Amongst the cultivars evaluated, JG 74 was proved to be highly susceptible by providing the largest lesion size by all the isolates of *S. sclerotiorum* and the smallest lesion size was recorded in cultivar Pusa 1073. Among the cultivar and isolate interactions, SS23 (Bihar) × DCP 92-3 showed the largest lesion size followed by SS4 × Pusa 212 with statistically similar sizes (Table 2). Highly resistant reaction was observed only in few cases. Vaid *et al*. (2005) observed that none of the cultivars of chickpea were found to be resistant against *S. scleortiorum.* Only a few cultivars of chickpea showed moderately resistant reaction.

On the basis of disease reactions (Table 3) on 10 cultivars of chickpea, 24 isolates of the pathogen were classified into 7 pathotype groups and differential cultivar of each group was identified (Table 4). The isolate SS19 (Uttarakhand) belonging to first pathotype was differentiated by the cultivar DCP 92-3 showing resistant reaction. The second pathotype consisting of two isolates SS1 (Delhi) and SS13 (Uttar Pradesh) were differentiated by cultivars DCP 92-3 and Pusa 212. The isolate SS6 (Delhi) produced resistant reaction only on cultivar Vishal was grouped into the third pathotype. Four non-sclerotia forming isolates , namely, SS5 (Delhi), SS14 (Uttar Pradesh), SS20 (Haryana) and SS24 (West Bengal) were placed into the fourth pathotype as they were found to be least virulent and showed resistant reaction on the most of the cultivars of chickpea. The cultivar JG 74 was considered to be differential for this pathotype. Two isolates SS16 (Uttarakhand) and SS17 (Uttarakhand) showed resistant reaction only on cultivars GPF 2. Therefore, these two isolates were placed into the fifth pathotype and the cultivar GPF 2 was considered as differential for this pathotype. Similarly, SS3 (Delhi) and SS18 (Uttarakhand) were grouped into the sixth pathotype with WR 315 as differential cultivar showing resistant reaction. The remaining 12 isolates, namely, SS2 (Delhi), SS4 (Delhi), SS7 (Rajasthan), SS8 (Rajasthan), SS9 (Rajasthan), SS10 (Punjab), SS11 (Punjab), SS12 (Uttar Pradesh), SS15 (Himachal Pradesh), SS21 (Haryana), SS22 (Jammu and Kashmir) and SS23 (Bihar) were placed into the seventh pathotype as they proved to be highly virulent on all the cultivars included in the present study and caused susceptible reaction.

In the present study, the cultivars, namely, DCP 92-3, Pusa 212, Vishal, JG 74, GPF 2 and WR 315 were considered to be differentials for grouping of 24 isolates of *S. sclerotiorum* into 7 pathotypes. Pathotype groups were not followed the continuity with that of geographical origin of isolates. It indicated that each region had mosaic of pathotypes. Interestingly, out of 12 chickpea isolates, 10 isolates were in the seventh pathotype.

Physiological specialization has not been reported in *S. sclerotiorum*, but strains can produce different reactions in various hosts and in different cultivars of the same host (Kim *et al.* 2000, Yli-Mattila *et al*. 2010). Variability in the virulence of the isolates was due to variation in the production of cellulase (Asoufi *et al.* 2007), oxalate (Xu *et al.* 2011)

Table 2 Lesion size (mm) in different chickpea cultivars screened under artificially inoculated condition against different isolates of *Sclerotinia sclerotiorum* by detached shoot technique

Cultivar	Lesion size (mm)* produced by different isolates																							
	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13	SS14	SS15	SS16	SS17	SS18	SS19	SS20	SS21	SS22	SS23	SS24	Mean
DCP 92-3	0.0	54.0	87.5	96.0	70.0	64.5	89.0	47.5	42.5	54.5	42.5	98.0	0.0	15.0	67.0	59.0	35.0	27.5	0.0	0.0	21.0	64.5	109	0.0	47.7
Pusa 212	2.5	76.0	50.0	105	0.0	45.0	52.5	27.5	45.0	60.5	55.5	62.0	0.0	8.5	50.5	76.5	25.5	39.0	75.0	60.0	55.0	47.0	65.5	5.0	45.4
Vishal	7.5	65.5	50.0	77.5	0.0	0.0	75.0	32.5	32.5	83.0	50.5	75.0	57.0	0.0	58.5	20.0	49.0	0.0	45.0	0.0	85.5	72.5	81.0	5.0	42.6
JG 74	19.0	97.5	87.5	97.5	0.0	65.5	46.0	40.0	37.5	50.5	92.5	97.5	59.0	0.0	97.5	76.5	58.5	38.0	40.5	0.0	99.0	109	60.0	7.5	57.4
KRW 108	62.5	57.5	49.0	83.0	2.5	23.0	29.5	37.5	82.5	60.0	54.5	74.5	25.0	20.0	68.0	23.0	69.5	0.0	49.0	2.5	69.5	72.5	101	67.5	49.1
GNG 469	87.0	95.0	37.5	80.0	7.5	85.5	69.5	20.0	12.5	89.5	29.5	80.0	36.0	0.0	60.0	68.0	32.0	7.5	78.5	0.0	75.5	51.5	65.0	32.0	50.0
JG 62	78.0	31.0	50.0	77.0	0.0	44.0	56.5	87.5	50.0	75.5	88.5	33.0	67.5	0.0	53.0	100	50.0	10.5	52.0	65.5	101	57.5	104	2.5	55.6
GPF 2	37.0	77.5	55.0	96.0	5.0	65.0	64.5	72.5	75.5	55.5	75.0	29.0	66.5	14.5	80.0	7.5	0.0	0.0	50.0	0.0	35.0	57.5	55.5	78.5	48.0
Pusa 1073	29.0	70.0	12.5	93.0	10.0	14.5	60.0	65.0	19.0	77.0	75.5	58.5	74.5	0.0	45.5	38.0	49.0	32.0	36.5	0.0	90.0	36.5	22.5	2.5	42.1
WR 315	50.5	52.0	7.5	97.5	2.5	54.0	60.0	70.5	17.5	58.5	80.5	56.0	62.5	46.5	60.5	45.0	59.0	2.5	36.0	33.5	92.0	65.0	60.0	18.0	49.5
Mean	37.3	67.6	48.7	90.3	9.8	46.1	60.3	50.1	40.5	66.5	64.5	66.4	44.8	10.5	64.1	51.4	42.8	15.7	46.3	16.2	72.4	63.4	72.4	21.9	48.7

SEm ± for isolate = 0.58, cultivar = 0.37 and isolate × cultivar = 1.83; CD (P=0.05) for isolate = 1.61, cultivar = 1.04 and isolate × cultivar = 5.10. *Mean of two replications. SS1- SS6: Delhi ; SS7- SS9: Rajasthan; SS10 and SS11: Punjab; SS12- SS14: Uttar Pradesh; SS15- SS19: Uttarakhand; SS20 and SS21: Haryana; SS22: Jammu & Kashmir; SS23:Bihar and SS24: West Bengal.

Table 3 Disease reaction of different chickpea cultivars evaluated under artificially inoculated conditions against different isolates of *Sclerotinia sclerotiorum* by detached shoot technique

Cultivar	Disease reaction produced by different isolates																							
	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10	SS11	SS12	SS13	SS14	SS15	SS16	SS17	SS18	SS19	SS20	SS21	SS22	SS23	SS24
DCP 92-3	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R	R	S	S	S	R
Pusa 212	R	S	S	S	R	S	S	S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S	R
Vishal	R	S	S	S	R	R	S	S	S	S	S	S	S	R	S	S	S	R	S	R	S	S	S	R
JG 74	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R	S	S	S	R
KRW 108	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	R	S	S	S	S
GNG 469	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S	R	S	R	S	S	S	S
JG 62	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	R
GPF 2	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	R	R	R	S	R	S	S	S	S
Pusa 1073	S	S	S	S	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	R	S	S	S	R
WR 315	S	S	R	S	R	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S

SS1-SS6: Delhi; SS7-SS9: Rajasthan; SS10 and SS11: Punjab; SS12-SS14: Uttar Pradesh; SS15- SS19: Uttarakhand; SS20 and SS21: Haryana; SS22: Jammu & Kashmir; SS23: Bihar and SS24: West Bengal. R = Resistant (0-2 grade), S = Susceptible (3-5 grade); Grade 0 = No infection/symptom, 1 = >1-5 mm water soaked lesion, 2 = >5-10 mm water soaked lesion, 3 = >10-25 mm water soaked lesion, 4 = >25-50 mm water soaked lesion with/without mycelium and 5 = >50 mm water soaked lesion with mycelium, stem collapsed.

Table 4 Pathotypes of *Sclerotinia sclerotiorum* isolates based on differential reactions on chickpea cultivars

Pathotype	Isolate	Differential cultivar	Reaction
1	SS19 (Uttarakhand)	DCP 92-3	Resistant
2	SS1 (Delhi) and SS13 (Uttar Pradesh)	DCP 92-3 and Pusa 212	Resistant
3	SS6 (Delhi)	Vishal	Resistant
4	SS5 (Delhi), SS14 (Uttar Pradesh), SS20 (Haryana) and SS24 (West Bengal)	JG 74	Resistant
5	SS16 and SS17 (Uttarakhand)	GPF 2	Resistant
6	SS3 (Delhi) and SS18 (Uttarakhand)	WR 315	Resistant
7	SS2 and SS4 (Delhi); SS7, SS8 and SS9 (Rajasthan); SS10 and SS11 (Punjab); SS12 (Uttar Pradesh); SS15 (Himachal Pradesh); SS21 (Haryana); SS22 (Jammu & Kashmir) and SS23 (Bihar)	All 10 cultivars under study	Susceptible

and association of mycovirus (Zhang *et al.* 2009). Earlier to this, it was also observed that association of mycovirus could change the morphology of the isolates as well as virulence of *S. sclerotiorum*. In the present study also, 4 isolates were clearly distinct from the rest of the isolates by lack of sclerotia, comparatively slow growing and showing less virulence.

All isolates of *S. sclerotiorum* were found to be pathogenic on chickpea. The isolates were variable in their morphological characters and virulence. The isolates were also variable in their growth rate, size, number and pattern of sclerotia formation. On the basis of reactions on a set of 10 cultivars of chickpea, the isolates were grouped into 7 pathotypes and differential cultivar of each pathotype was identified. Morphological characters and pathotype groups did not correlated with geographical origin of the isolates.

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