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Morphological variability and mycelial compatibility among the isolates of sclerotinia sclerotiorum associated with stem rot of chickpea

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Abstract

Variability among 16 isolates of *Sclerotinia sclerotiorum* associated with the stem rot of chickpea is being reported. The isolates varied in colony morphology, mycelial growth rate, sclerotium formation, and its size and color. Variability among the isolates on the basis of their mycelial compatibility was also observed, and out of 120 combinations, 70 showed compatible reactions. Based on mycelial compatibility, 58% vegetative compatibility groups (VCG) were identified among the isolates.

Keywords: *Sclerotinia sclerotorum*, isolates, variability

Introduction

Stem rot also known as *Sclerotinia* wilt or white mold, caused by *Sclerotinia sclerotiorum* is a serious disease of chickpea. It infects all the economically important food and feed legumes (Pratt and Knight, 1984). This fungus has a wide host range and has a worldwide distribution on numerous crops (Purdy, 1979; Boland and Hall, 1994). It is one of the pathogens associated with root rot/wilt complex of chickpea and its occurrence is increasing in both incidence and severity on chickpea grown in the Mediterranean region (Anonymous, 1996). The initial infection occurs in the late winter or early spring, and the fungal mycelia grow within and between plants. Patches like symptoms of dead plant parts enlarge and coalesce through spring and cause major losses in stands (Gilbert and Bannett, 1917). The fungus produces many black fleshy structures called sclerotia, which survive from one cropping season to the next. Over-wintered sclerotia may germinate during the summer or may stand dormant for many years (Adams and Ayers, 1979). The etiology, biology and epidemiology of the fungus have been studied extensively by several workers (Philips, 1987; Purdy, 1979; Roberts *et al*., 1984).

Cultivation of resistant varieties is the ideal and feasible control of the disease and no resistant variets against this disease have been identified so far. Erect type cultivars can better withstand against the disease and management can also minimize crop losses. Stable resistance could not be achieved due to the prevalence of virulent isolates of *S. sclerotiorum* (Sharma *et al*., 2002). Variability among *S. sclerotiorum* populations was demonstrated by earlier workers geographical around the world (Harlton *et al*., 1995; Nalim *et al*., 1995; Okabe *et al*., 1998). Studies of variability within the population in a geographical region are important because these also document the changes occurring in the population. The purpose of the present study was to understand the variability in cultural morphology, sclerotium formation, mycelial compatibility and sensitivity of mycelia to fungicides for the isolates of *S. sclerotiorum* collected from different infected chickpea plants in various locations of Pakistan.

Materials and Methods

Fungal isolates and culture maintenance

Sixteen isolates of *Sclerotinia sclerotiorum* causing stem rot of chickpea used in this study. These isolates were collected from the major chickpea growing areas of Pakistan included districts of Rawalpindi, Chakwal, Attock and Sialkot (Punjab), and Dera Ismail Khan, Karak, Kohat and Peshawar (NWFP) Capital Islamabad. These were collected from infected plant samples and designated (Table 1). The isolates were further purified by growing single sclerotia from each colony on Cornmeal agar medium (CAM) (cornmeal 20 g, dextrose 20 g, agar 20 g, distilled water 1 L) slants.

Morphological variability

Single sclerotial cultures of these isolates were preserved on CMA. Isolates were subjected to detailed morphological and cultural characteristics viz. radial colony growth on medium (mm), number of sclerotia developed in petri dishes, size of sclerotia (µm) and weight of sclerotia (mg). Inoculation of single sclerotia was

made on cornmeal agar medium and five replicates were kept for each isolate. These petri dishes were incubated at 25˚C. Data of radial colony growth were taken 5 days after inoculation while number of sclerotia and size of sclerotia for each isolate was recorded 25 days after inoculation.

Mycelial compatibility

Mycelial discs (5 mm diameter) taken from the edge of an actively growing colony (3 to 4 day old) of each isolate were placed approximately 40 mm apart on opposite sides of petri dishes (90 mm dia) and incubated at 25 ± 2 ^{o}C. Two isolates were paired on one dish and the test was repeated at least twice. The pairings were examined macroscopically after 10–15 day for the presence of an antagonistic (barrage or aversion) zone in the region of mycelial contact as described by Punja and Grogan (1983).

Results and Discussion

Variability in growth characters

Differences in morphological characteristics of *S. sclerotiorum* such as radial colony growth on medium (mm), number of sclerotia, size of sclerotia (µm) and weight of sclerotia (mg) were observed. There was considerable variation among the isolates for morphological characters. In case of the radial colony growth of sixteen isolates of the fungus, a significant difference was recorded. Based on the radial growth, isolates were classified into three groups; very fast growing, intermediate and slow growing. Data after 5 days incubations revealed that the isolates SSC-1, SSC-2, SSC-3, SSC-4, SSC-5, SSC-6, SSC-7 and SSC-8 represented significantly fast growing, isolates SSC-10 and SSC-11 intermediate, while SSC-9, SSC-12, SSC-13, SSC-14, SSC-15 and SSC-16 showed slow radial colony growth (Table 1). Similar variability among the various isolates of *S. sclerotiorum* has already been reported by Ansari and Agnihotri, (2000). On the basis of number of sclerotia produced by the isolates of *S. sclerotorum*, SSC-1, SSC-10, SSC-14, SSC-15 and SSC-16 ranked as higher producers of sclerotia, SSC-2, SSC-4, SSC-8, SSC-9, SSC-11, SSC-12 and SSC-13 were intermediate, and SSC-3, SSC-5, SSC-6 and SSC-7 showed least number of sclerotial formation. As far as size of sclerotia is concerned, isolates SSC-4, SSC-5, SSC-7 and SSC-14 were larger in size because their diameter was 0.31 to 0.50 um, respectively which is greater than the others. Isolates SSC-1, SSC-2, SSC-3, SSC-6, SSC-8, SSC-10, SSC-12, SSC-13 and SSC-15 were intermediate while SSC-9, SSC-11 and SSC-16 showed least size of sclerotia (Table 1). Anil and Sastry, (1980) and Hooda and Grover, (1982) recorded in cultural character and growth rates among different isolates obtained from different host species. Based on sclerotial diameter several workers recorded variation in size of sclerotia among different isolates of the fungus (Haigh, 1930. Dhingra and Sinclair, 1973; Gupta and Kolte, 1982). According to the weight of sclerotia, they are categorizes in two groups; isolates having heavy sclerotia (sclerotial weight more than 0.01 mg) and isolates with low weight sclerotia (sclerotial weight less than 0.01 mg). Thus isolates SSC-1, SSC-2, SSC-5, SSC-6, SSC-8, SSC-12, SSC-13, and SSC-14and SSC-16 were heavy weight isolates while SSC-3, SSC-4, SSC-7, SSC-9, SSC-10, SSC-11 and SSC-15 were considered low weight isolates.

Mycelial compatibility

There were 120 pairings of the 16 isolates and out of these, 70 combinations showed a compatible reaction (58% of all the combinations) where mycelia of the two isolates intermingled at the zone of interaction (Table 2). The remaining combinations showed antagonistic reactions with each other, forming a thin band of living or dead mycelia (Fig. 1). Based on mycelial compatibility, 50 vegetative incompatibility groups were found among the isolates. In all the antagonistic reactions, sclerotia were not formed at the interaction zone. Sclerotia were formed only in the border of the lytic zones of the two isolates. However, a few sclerotia produced later on such lytic zone in some combinations failed to develop to the full size as those produced on the border of such barrages. On prolonged incubation, the antagonistic site, in some combinations, was broadened at the interaction zone either parallel to both sides traversing to almost 2/3 of the mycelial growth, or in some cases lysis occurred completely in one isolate only (Fig. 2). Interestingly, sclerotia were not formed in such combinations. However, in some combinations, the interacting zone did not widen even after prolonged incubation.

The results of the present study revealed wide variation among isolates of *S. sclerotiorum*. Since the sexual stage of *S. sclerotiorum* is rare in nature and its role in the life cycle of the fungus is unknown, genetic exchange between mycelia of *S. sclerotiorum* isolates is largely thought to be limited by mycelial compatibility (Nalim *et al*., 1995). However, consistent production of the teleomorph stage in four isolates of *S. sclerotiorum* on CRMA medium may strengthen the claim that genetic exchange may occur through normal genetic recombination, i.e., meiosis. The absence

of the teleomorph stage in most of the isolates may be because they have lost the ability to produce basidiospores during the course of evolution or they require specific conditions. Alternatively, the genetic factor responsible for sexual reproduction may be triggered in some isolates by components in CRMA medium. However, according to Nalim *et al* (1995), nuclear exchange through anastomosis in hyphae may be responsible for normal genetic recombination in this fungus.

The high rate of antagonistic reactions in the mycelial compatibility test further shows the extent of the diversity among these isolates of *S. sclerotiorum.* This is an important observation that distinguishes the stem rot causing isolates from others. The death of mycelia at the interaction zone is attributed to the heterokaryotic condition of the nuclei but the involvement of toxin(s) cannot be ruled out. A detailed study in this regard may reveal more information about the cause of mycelial death in the incompatible reactions.

Table 1: Isolates of *S. sclerotiorum* collected from different chickpea growing areas of Pakistan**.**

S. No	Isolates	Locations	Districts	Province	Year of collection
1	SSC-1	NARC, Chak Shezad	Islamabad	Punjab	2005
2	SSC-2	Jatli	Rawalpindi	"	"
3	SSC-3	BARI, Thoa	Chakwal	"	"
4	SSC-4	ARS, Bhun	"	"	"
5	SSC-5	GRS	Attock	"	"
6	SSC-6	BARS, Fatehjang		"	"
7	SSC-7	PRS, Sahowali	Sialkot	"	"
8	SSC-8	AZRI	Bhakkar	"	"
9	SSC-9	Hudali	Khushab	"	"
10	SSC-10	Chaubara	Layyah	"	"
11	SSC-11	GRS, Kaluukot	Mianwali	"	"
12	SSC-12	AARI	Faisalabad	"	"
13	SSC-13	NIFA,	Peshawar	NWFP	"
14	SSC-14	BARS, Jarma	Kohat	"	"
15	SSC-15	ARS, Ratta Kalachi	DI Khan	"	"
16	SSC-16	AZRI, Dera Baluchan	DI Khan	"	"

Table 2: Morphological characteristics of the isolates of *Sclerotinia sclerotiorum*, the stem rot of chickpea collected from various locations of Pakistan

S. No.	Isolates	Radial growth (mm)	No. of Scleortia	Size of sclerotia (mm)	Ave. weight of a sclerotium (mg)
1	SSC-1	90.0 a	71.40 abc	0.236 ef	0.010 abcd
2	SSC-2	90.0 a	55.00 cdefdg	0.220 f	0.013ab
3	SSC-3	90.0 a	45.20 efg	0.248 ef	0.009 abcd
4	SSC-4	90.0 a	59.00 bcde	0.322 ab	0.070 cd
5	SSC-5	90.0 a	49.60 defg	0.344 a	0.014 a
6	SSC-6	90.0 a	39.00 g	0.248 ef	0.013 ab
7	SSC-7	90.0 a	40.60 fg	0.350 a	0.008 bcd
8	SSC-8	84.0 a	64.20 bcd	0.234 ef	0.012 abc
9	SSC-9	30.0 c	56.60 bcdef	0.174 g	0.007 cd
10	SSC-10	52.0 b	73.80 ab	0.296 bcd	0.006 d
11	SSC-11	54.6 b	64.80 bcd	0.130 h	0.007 cd
12	SSC-12	34.6 c	57.20 bcdef	0.244 ef	0.011 abcd
13	SSC-13	44.4 b	60.20 bcde	0.278 cde	0.010 abcd
14	SSC-14	32.8 c	81.80 a	0.310 abc	0.010 abcd
15	SSC-15	27.4 c	82.40 a	0.256 def	0.008 bcd
16	SSC-16	32.6 c	73.40 ab	0.174 g	0.010 abcd

* Figures sharing the same letters are non-significant at 0.05% level of probability.

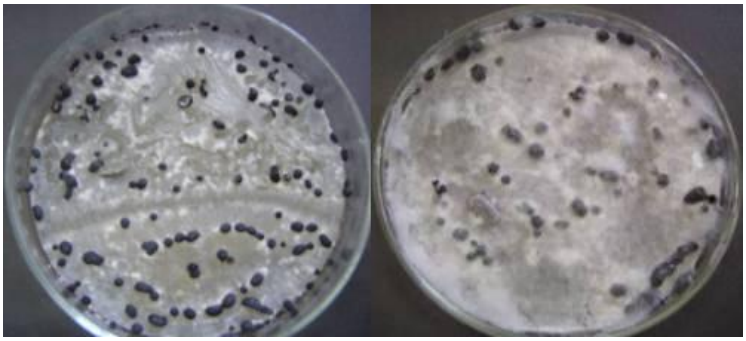

Fig. 1: Mycelial compatibility reactions between isolates of *Sclerotia sclerotiorum*. Incompatible (left) isolates and Compatible (right).

Table 3: Mycelial compatibility among the isolates of *Sclerotinia sclerotiorum* associated with stem rot disease of chickpea

Isolates	**SS-1**	**SS-2**	**SS-3**	**SS-4**	**SS-5**	**SS-6**	**SS-7**	**SS-8**	**SS-9**	**SS-10**	**SS-11**	**SS-12**	**SS-13**	**SS-14**	**SS-15**	**SS-16**
SS-1		N	C	C	N	C	C	C	C	C	N	N	C	C	N	C
SS-2			C	C	C	N	N	N	C	C	C	N	N	C	N	N
SS-3				N	C	N	N	C	C	C	C	N	C	C	C	N
SS-4					C	N	N	C	C	C	C	C	C	C	C	C
SS-5						C	C	C	N	C	C	C	N	C	C	N
SS-6							N	N	C	N	N	C	C	N	C	N
SS-7								N	C	C	N	C	C	N	C	N
SS-8									C	N	N	C	N	N	C	N
SS-9										N	C	C	N	N	N	N
SS-10											C	C	N	C	N	N
SS-11												C	N	C	C	C
SS-12													C	C	C	N
SS-13														N	C	N
SS-14															C	N
SS-15																C
SS-16																

C = compatibility, N = incompatibility

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