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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 collected from various localities of Pakistan is reported. The isolates varied in colony morphology, mycelial growth rate, sclerotium formation, sclerotial size and color. Variability among the isolates on the basis of their mycelial compatibility was also observed and out of 120 combinations more than half showed compatible reactions between either two isolates. Based on mycelial compatibility, 58% vegetative compatibility groups (VCG) were identified among all the isolates. Sixteen isolates were grouped into two clusters at 50% dissimilarity and both the clusters consisted 8 isolates in each case. The members of same cluster were compatible in most of the cases, whereas it was not true for the isolates with different background on the basis of cultural and morphological characteristics. A detailed study to investigate molecular and genetic basis of diversity in relation to antagonistic activity is suggested.

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 & Knight, 1984). This fungus has a wide host range and has a worldwide distribution on numerous crops (Purdy, 1979: Boland & Hall, 1994). It is one of the pathogen 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 (Anon., 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 (Bolton *et al*., 2006). 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 & Ayers, 1979). The etiology, biology and epidemiology of the fungus had been studied extensively by several workers (Philips, 1987; Purdy, 1979; Roberts *et al*., 1982).

Cultivation of resistant varieties is the ideal and feasible control of the disease and no resistant varieties against this disease has been identified so for. Erect type cultivars can better withstand against the disease and management can also minimize the 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 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 and mycelial compatibility of 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 were collected from the major chickpea growing areas of Pakistan included districts of Islamabad, Rawalpindi, Chakwal, Attock and Sialkot (Punjab), and Dera Ismail Khan, Karak, Kohat and Peshawar (NWFP). These were collected from infected plant samples and designated as SS-1 to SS-16 (Table 1). The isolates were purified by growing single sclerotia from each colony on Cornmeal agar (CMA) medium (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 medium. 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 5 replicates were kept for each isolate. These Petri dishes were incubated at 25 ± 2˚C. Data of radial colony growth were taken 5 days after inoculation while number of sclerotia and size of sclerotia of each isolates were 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 at 40 mm apart on opposite sides of Petri dishes (90 mm dia) and incubated at 25 ± 2^0C. Two isolates were paired on one dish and the test was repeated twice (Fig. 1). 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 & Grogan (1983).

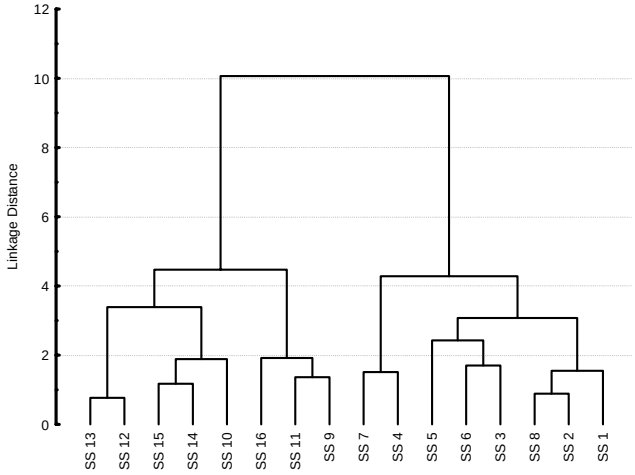

Fig. 1. Cluster diagram of 16 isolates of *Sclerotinia sclerotiourm* for cultural and morphological characteristics

Result and Discussion

Variability in growth characters: Differences in all the morphological characters of *S. sclerotiorum* were observed. Based on radial growth, the isolates were classified into three groups; very fast growing, intermediate and slow growing. Data after 5 days incubations revealed that the isolates SS-1, SS-2, SS-3, SS-4, SS-5, SS-6, SS-7 and SS-8 represented significantly fast growing, isolates SS-10 and SS-11 were intermediate, while SS-9, SS-12, SS-13, SS-14, SS-15 and SS-16 showed slow radial colony growth (Table 1). Variability among the isolates of *S. sclerotiorum* has already been reported by Carpenter *et al*., (1999). On the basis of number of sclerotia produced by the isolates of *S. sclerotorum*, SS-1, SS-10, SS-14, SS-15 and SS-16 ranked as higher producer of sclerotia, SS-2, SS-4, SS-8, SS-9, SS-11, SS-12 and SS-13 were intermediate and SS-3, SS-5, SS-6 and SS-7 showed least number of sclerotial formation. As for size of sclerotia is concerned, isolates SS-4, SS-5, SS-7 and SS-14 were bigger with diameter 0.31 to 0.50 um. Isolates SS-1, SS-2, SS-3, SS-6, SS-8, SS-10, SS-12, SS-13 and SS-15 were intermediate while SS-9, SS-11 and SS-16 showed least size of sclerotia.

On the basis of cultural and morphological characteristics, 16 isolates were grouped into two clusters at 50% dissimilarity (Fig. 1). Both the clusters consisted 8 isolates in each case. It was observed that the members of same cluster were compatible in most of the cases, whereas it was not true for the isolates with different background on the basis of cultural and morphological characteristics. Punja & Damiani (1996) and Zarani & Christensin, (1997) recorded differences in growth rates among different isolates obtained from various host species. Based on sclerotial diameter several workers recorded variation in size of sclerotia among different isolates of the fungus (Dhingra & Sinclair, 1973; Mirza *et al*., 1985). 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 SS-1, SS-2, SS-5, SS-6, SS-8, SS-12, SS-13, SS-14 and SS-16 were heavy weight isolates while SS-3, SS-4, SS-7, SS-9, SS-10, SS-11 and SS-15 were considered low weight isolates.

Mycelial compatibility: The combinations with antagonistic reactions with each other formed a thin band of living or dead mycelia (Fig. 2). There were 120 pairings of the 16 isolates and out of all, 70 combinations showed a compatible reaction (58% of all the combinations) where mycelia of the two isolates intermingled at the zone of interaction. Based on mycelial compatibility, 50 vegetative incompatibility groups (VCG) were found among all the isolates. The isolates, SS- 1, SS- 3, SS- 4, SS- 5 SS- 11 and SS- 13 were compatible with most of the isolates, and among these SS- 4, SS- 5 and SS- 11 were the highest compatible (Fig. 3). The isolate SS- 15 collected from D.I. Khan was the lowest for compatibility that was compatible for SS1, SS- 4, SS- 10 and SS- 126 only. In all the antagonistic reactions, sclerotia were not formed at the interaction zone. Sclerotia were formed only in the border of the lytic zone of the two isolates. However, a few sclerotia produced later on such lytic zone in some combinations but failed to develop to 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. Interestingly, sclerotia were not formed in such combinations. However, in some combinations, the interacting zone did not widen even after prolonged incubation.

Table 1. Morphological characteristics of the isolates of *Sclerotinia sclerotiorum*, the stem rot of chickpea collected from various locations of Pakistan during 2005.

Isolates	Locations	Radial growth (mm)	No. of Scleortia	Diam. of sclerotia (mm)	Ave. wi. of sclerotium (mg)
SS-1	National Agricultural Research Ccentre, Islamabad	90.0 a	71.4 abc	0.24 ef	0.010 abcd
SS-2	Farmer's field, Jatli, Rawalpindi	90.0 a	55.0 cdefdg	0.22 f	0.013ab
SS-3	Barani Agricultural Research Institute, Chakwal	90.0 a	45.2 efg	0.25 ef	0.009 abcd
SS-4	Agricultural Research Station, Ckawal	90.0 a	59.0 bcde	0.32 ab	0.070 cd
SS-5	Groundnut Research Station, Attock	90.0 a	49.6 defg	0.35 a	0.014 a
SS-6	Barani Agricultural Research Station, Fatehjang	90.0 a	39.0 g	0.25 ef	0.013 ab
SS-7	Pulses Research Station, Sialkot	90.0 a	40.6 fg	0.35 a	0.008 bcd
SS-8	Arid Zone Research Institute, Bhakkar	84.0 a	64.2 bcd	0.24 ef	0.012 abc
SS-9	Farmer's field, Hudali Khushab	30.0 c	56.6 bcdef	0.18 g	0.007 cd
SS-10	Farmer's field,Chaubara, Layyah	52.0 b	73.8 ab	0.30 bcd	0.006 d
SS-11	Gram Research Station, Kaluukot	54.6 b	64.8 bcd	0.13 h	0.007 cd
SS-12	AyubAgricultural Research Institute, Faisalabad	34.6 c	57.2 bcdef	0.25 ef	0.011 abcd
SS-13	Nuclear Institute for Food & Agriculture, Peshawar	44.4 b	60.2 bcde	0.28 cde	0.010 abcd
SS-14	Barani Agricultural Research Station, Kohat	32.8 c	81.8 a	0.31 abc	0.010 abcd
SS-15	Agricultural Research Station, D.I. Khan	27.4 c	82.4 a	0.26 def	0.008 bcd
SS-16	Arid Zone Research Insitute, D.I. Khan	32.6 c	73.4 ab	0.18 g	0.010 abcd

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

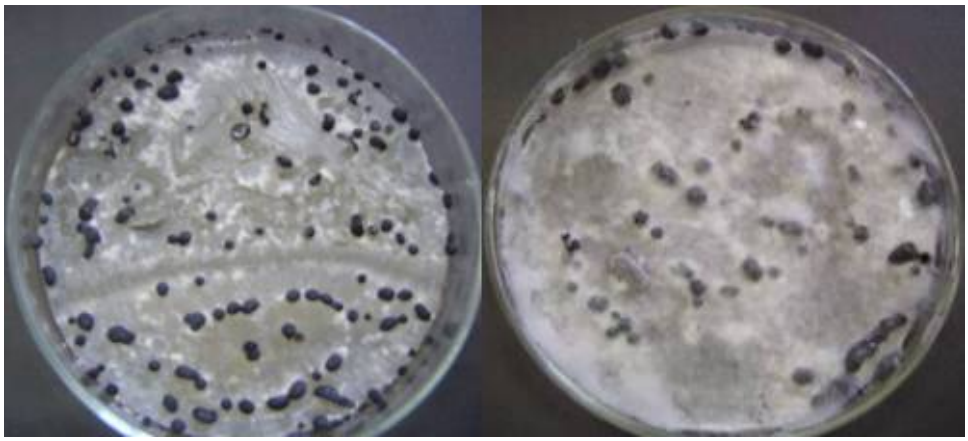

Fig. 2. Mycelial compatibility reactions between isolates of *Sclerotinia sclerotiorum*. Incompatible (left) isolates and Compatible (right).

Isolates	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		■	■		■	■	■	■	■			■	■		■
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													■		■

Fig. 3. Mycelial compatibility (indicated by shaded blocks) among 16 isolates of *Sclerotinia sclerotiorum* associated with stem rot disease of chickpea.

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 in mycelia of *S. sclerotiorum* isolates is largely thought to be limited to mycelial compatibility (Nalim *et al*., 1995). However, consistent production of the teleomorph stage in four isolates of *S. sclerotiorum* on CMA 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 CMA 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 (Punja 1985), but the involvement of toxin(s) cannot be ruled out (Punja 1985). A detailed study in this regard may reveal more information about the cause of mycelial death in the incompatible reactions.

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