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Variability among the isolates of *sclerotium rolfsii* associated with collar rot disease of chickpea in Pakistan

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Abstract

Variability among 12 isolates of *Sclerotium rolfsii* Sacc collected from various localities of chickpea growing areas of Punjab province was studied. The isolates varied in colony morphology, mycelial growth rate, sclerotium formation, and sclerotial size and color. Variability among the isolates of *S. rolfsii* was determined on the basis of their sensitivity to different fungicides. Mycelial incompatibility among the isolates was also studied, and out of 66 combinations, only 26 combinations (39%) showed compatible reactions. Based on mycelial compatibility, 39% vegetative compatibility groups (VCG) were identified among the isolates.

Keywords: *Sclerotium rolfsii*, isolates, variability

Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume crop sown under rainfed conditions in Pakistan. It is a rich and cheap source of vegetable protein for human nutrition (Hules, 1991). Average yield of chickpea in Pakistan is very low than almost half of the world and Asia (Malik and Tufail, 1984). A number of biotic and abiotic factors are involved in low production of chickpea. Among the biotic factors, collar rot disease caused by *Sclerotium rolfsii* Sacc. [teleomorph *Athelia rolfsii* (Curzi) Tu and Kimbrough] is a serious threat, which under conducive conditions caused 55-95% mortality of the crop at seedling stage (Gurha and Dubey, 1982). Prevalence of collar rot of chickpea has been recorded in many countries (Nene *et al*., 1984). *S. rolfsii* is a devastating soil-borne plant pathogenic fungus with a wide host range (Aycock, 1966, Punja, 1988), has prolific growth and ability to produce persistent sclerotia contributing in high degree of economic losses (Mahen *et al*., 1995).

This fungus can overwinter as mycelium in infected tissues or plant debris. Sclerotia serve as the principal over wintering structure and primary inoculum for disease persisting near the soil surface, sclerotia may exist free in soil or in association with plant debris (Aycock, 1966; Punja, 1985). Those buried deep in the soil may survive for a year or less, whereas those at surface remain viable and may germinate in response to alcohols and other volatile compounds released from decomposing plant material. Sclerotia disseminates by cultural practices (infected soil and contaminated tools), infested transplant seedling, water (especially through irrigation), wind and possibly seeds. The fungus forms differentiated sclerotia and sterile mycelia like other sclerotium-producing fungi. Those characterized by small tan to dark-brown or black spherical sclerotia with internally differentiated rind, cortex, and medulla were placed in the form genus *Sclerotium* (Punja and Rahe 1992). However, the teleomorphic state was discovered later (Punja 1988), confirming that the fungus was a basidiomycete. *Sclerotium rolfsii* usually causes collar rot (Singh and Pavgi 1965).

Cultivation of resistant varieties is the ideal and feasible management of the disease and resistant sources against this disease had been identified in various countries (Sugha *et al*., 1991; Gurha *et al*., 1982; Gurha and Dubey, 1982) but stable resistance could not be achieved due to the prevalence of virulent isolates of *S. rolfsii* (Sharma *et al*., 2002). Geographical variability among *S. rolfsii* populations was demonstrated by earlier workers (Harlton *et al*., 1995; Nalim *et al*., 1995; Okabe *et al*., 1998). Investigations on variability within the population in a geographical region are important because these also document the changes occurring in the population. Objectives of the present study were to understand the variability in cultural morphology, sclerotium formation, mycelial compatibility and sensitivity of

fungicides to mycelia of the isolates of *S. rolfsii* collected from different infected chickpea plants from various locations in Pakistan.

Materials and Methods

Fungal isolates and culture maintenance

Twelve isolates of *S. rolfsii* causing collar rot of chickpea were used in this study. These were collected from various localities of chickpea growing area of Pakistan from infected plants samples (Table 1). The isolates were further purified by growing single sclerotia from each colony on cornmeal agar (CA) medium (cornmeal 20 g, dextrose 20 g, agar 20 g, distilled water 1 L) slants (Azhar *et al*., 2006).

Cultural variation

Radial growth (colony diameter in mm), colony morphology, and sclerotial production (total number per plate and size) were evaluated on CA medium (Prithiviraj *et al* 2000). At least five plates each of CA medium were inoculated with a 5 mm diameter mycelial disc taken from the margin of an actively growing colony (5 to 7 days) of each isolate. The inoculated plates were incubated at 25 ± 2^0C under diffuse light. The colony diameter was measured after 5 day by taking two measurements at right angles. The number of sclerotia per colony was counted after 25 days of incubation. Diameter of 10 sclerotia was measured. The data from the replicated plates were averaged.

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 25 to 35 mm apart on opposite sides of petri dishes of 9 cm diameter and incubated at 25 ± 2^0C. Two isolates were paired on one dish and the test was repeated at least twice. The pairings were examined macroscopically after 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).

Sensitivity to fungicides

The sensitivity of *S. rolfsii* isolates to nine fungicides viz; antracol, benlate, captan, cobox, dithane M-45, pentachloro nitro benzene (PCNB), ridomil, sancozeb and trimiltox-forte (Table 2) at the rate of 100 ppm was studied using poison food technique (Nene and Thapliyal, 1982). A weighed quantity of each fungicide was amended in the Corn meal agar medium after autoclaving. Twenty ml of amended and non-amended medium was poured into each of the five 9-cm diameter petri dishes. After solidification 6 mm agar plugs containing *S. rolfsii* mycelium were cut from 7 days old culture plates using a sterile cork borer and were placed in the center of each petri dish. The inoculated petri dishes were incubated at 28^0C and radial growth (cm) of *S. rolfsii* was recorded after 7 days of incubation. Data were analyzed statistically by applying Duncan's Multiple Range Test (Steel and Torrie, 1980). Isolates were grouped as non-sensitive "N" with radial growth of the fungus more than 35 mm and sensitive "S" with growth less than 35 mm.

Results

Variability in growth characters

The isolates of *S. rolfsii* varied in all of the test parameters, e.g., colony morphology, mycelial growth rate, colony colour, sclerotial production, number and sclerotial size of sclerotia. Out of 12 isolates, colonies of 7 isolates were fluffy, whereas 5 were compact. The growth rate of the isolates varied substantially, the isolates SRC-1, SRC-18, SRC-19 and SRC-112 were fast growing (76.7- 90 mm dia) while the isolate SRC-2, SRC-4, SRC-5, SRC-11 were slow growing (16.0 – 30.6mm dia). Others were medium in growth and varied from 40.8 to 61.7 mm dia. Production of sclerotia among isolates varied significantly. Most of the isolates produced a very large number of sclerotia (>300 sclerotia/plate), while isolates SRC-2, SRC-8 and SRC-9 produced fewer (<300 sclerotia/plate). Similarly, the size of sclerotia varied in different isolates. The average size of sclerotia for most of the isolates were >40 µm in dia, whereas some isolates SRC-6, SRC-7, SRC-8 and SRC-11 produced the small sclerotia of <40 µm in diameter. The color of sclerotia was generally dark to reddish brown at maturity (Table 3).

There were 66 pairings of the 12 isolates and out of all, only 26 combinations showed a compatible reaction and the 40 combinations were incompatible where mycelia of the two isolates intermingled at the zone of interaction (Table 4). For combinations which showed antagonistic reactions with each other, a thin band of living or dead mycelia was formed (Fig. 1). Based on mycelial compatibility, 39% vegetative compatibility groups (VCG) 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 zone 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.

Sensitivity to fungicides

Variability among the isolates of *S. rolfsii* was also determined on the basis of their sensitivity to different fungicides. It was observed that there was a significant variability in this regard. All the isolates were sensitive to benlate. It means that benlate was found to be the most effective in suppressing the growth of all the test isolates. It was followed by ridomil and sancozeb with respect to efficacy. Captan and PCNB were the least effective where all the isolates showed non-sensitivity. Trimiltox forte, antracol, dithane M-45 and copper oxychloride exhibited intermediate response in efficacy (Table 5).

Discussion

The results of the present study reveal wide variation among isolates of *S. rolfsii*. Since the sexual stage of *S. rolfsii* is rare in nature and its role in the life cycle of the fungus is unknown, genetic exchange in mycelia of *S. rolfsii* isolates is largely thought to be limited to mycelial compatibility (Nalim *et al*., 1995). However, consistent production of the teleomorph stage in the isolates of *S. rolfsii* on CA medium may strengthen the claim that genetic exchange may occur through normal genetic recombination, i.e., meiosis discernible in the progeny. 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. rolfsii*. Interestingly, all of the isolates exhibited mycelial compatibility with each other but antagonistic reactions with collar rot- or foot rot-causing isolates. This is an important observation that distinguishes the isolates from one another. The death of mycelia at the interaction zone is attributed to the heterokaryotic condition of the nuclei (Punja, 1985), but the involvement of toxin 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.

El-Tobshy *et al*. (1981) found that *Fusarium oxysporum* was very sensitive to fungicides during *in vitro* studies in which this fungus was equally inhibited by benomyl, thiabendazole and thiophenate methyl. Thus, the efficacy of benlate in the present study had been confirmed in the light of previous research. Similarly, benlate had been used against a wide spectrum of fungal diseases such as grey mold, powdery mildew and black spot of roses, scab and powdery mildew of apples, powdery mildew of cucurbits and strawberries (Scot *et al*., 1979). Similarly, it was effective for the control of rice blast (Kamerwar-Row, 1976). The results obtained with benlate are in agreement with those of Bashir *et al*. (1985) who recommended it for the control of mungbean anthracnose (*Colletotrichum lindemuthianum*). Bashir and Ilyas (1983) had also reported benlate as the most effective fungicide in reducing the seed-borne inoculum of *Ascochyta rabiei*. The sensitivity of mycelia to benlate has also been reported for *Ascochyta lentis* (Ilyas *et al*., 1992). Benlate is a systemic fungicide which is used against the fungal diseases as spray, soil drenching and seed treatment for air-borne, soil-borne and seed-borne diseases, respectively. Its systemic fungitioxicity in many plants had been reported by Erwin *et al*. (1969). The significant difference between growth of fungus on agar medium containing benlate may be due to conversion to methyl-1, 2-benzimedazole carbamate (MBC) and its uptake by the fungus (Nene and Thapliyal, 1982).

Table I: Isolates of *S. rolfsii* collected from different chickpea growing areas of Pakistan.

S.No.	Isolates	Locations	Host tissues	Year of collection
1	SRC-1	Islamabad	Roots	2004
2	SRC-2	"	"	"
3	SRC-3	Chakwal	"	"
4	SRC-4	Khushab	"	"
5	SRC-5	Mianwali	"	"
6	SRC-6	D.I. Khan	"	"
7	SRC-7	"	"	"
8	SRC-8	Attock	"	"
9	SRC-9	Sialkot	"	"
10	SRC-10	Narowal	"	"
11	SRC-11	"	"	"
12	SRC-12	Rawalpindi	"	"

Table 2: Fungicides used for the determination of variability in *Sclerotium rolfsii* Sacc.

Fungicide	Chemical Name	Mode of action	Formulation	Manufacturer
Antracol	Propineb	Contact	70 wp	Bayer (Pvt) Ltd.
Benlate	Benomyl	Systemic	50 wp	R.B. Avari Entreprises Ltd.
Captan	Orthocide	Contact	50 wp	ICI (Pvt) Ltd.
Cobox	Copper oxychloride	"	50 wp	Agricide (Pvt) Ltd.
Dithane M-45	Mancozeb	"	80 wp	Rohm & Hass Ltd.
PCNB	Pentachloronitro- benzene	Systemic	100 wp	ICN Biomedicals
Ridomil	Matalaxyl	Contact	68 wp	Novartis (Pvt) Ltd.
Sancozeb	Mancozeb	"	80 wp	Pak Agro (Pvt) Ltd.
Trimiltox-forte	Copper compounds & Mancozeb	"	41 wp	Novartis (Pvt) Ltd.

Table 3: Morphological characteristics of the Isolates of *S. rolfsii* collected from different chickpea growing areas of Pakistan.

Isolates	Radial growth (mm)	No. of slerotia	Diameter of sclerotia (um)	Colony appearance	Colony colour
SRC-1	76.7 c	438 b	48.5 b	Compact	Dark brown
SRC-2	30.6 g	293 e	40.9 cde	Fluffy	"
SRC-3	61.7 d	547 a	45.7 bcd	Fluffy	"
SRC-4	16.0 i	444 b	44.2bcde	Compact	"
SRC-5	20.2 hi	367 cd	47.1bc	Fluffy	"
SRC-6	40.8 f	423 b	39.6def	Fluffy	"
SRC-7	50.2 e	386 c	34.3 f	Compact	"
SRC-8	98.3 a	260 e	37.7 ef	Compact	"
SRC-9	84.6 b	264 e	45.6 bcd	Fluffy	"
SRC-10	24.7 h	334 d	49.0 b	Fluffy	"
SRC-11	17.3 i	547 a	37.6ef	Fluffy	"
SRC-12	90.0 b	428 b	57.8 a	Compact	"

*Figures sharing the same letters are non-significant at 5% level of significance.

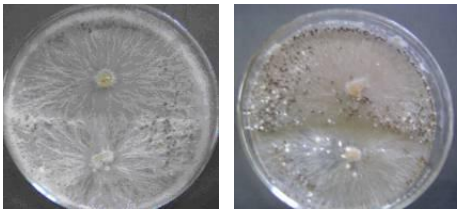

Fig.1: Mycelial compatibility reactions between isolates of *Sclerotium rolfsii*. Compatible (left) and Incompatible (right) isolates

Table 4: Mycelial compatibility among the isolates of *Sclerotium rolfsii* associated with collar rot disease of chickpea

S.No	Isolates	SRC-1	SRC-2	SRC-3	SRC-4	SRC-5	SRC-6	SRC-7	SRC-8	SRC-9	SRC-10	SRC-11	SRC-12
1	**SRC-1**		C	N	N	N	N	N	C	N	C	C	N
2	**SRC-2**			N	N	N	N	C	C	N	N	C	N
3	**SRC-3**				C	N	N	N	N	N	N	N	C
4	**SRC-4**					N	N	N	N	N	N	N	N
5	**SRC-5**						C	N	C	C	C	C	C
6	**SRC-6**							C	N	C	N	N	C
7	**SRC-7**								C	N	N	C	N
8	**SRC-8**									C	C	C	C
9	**SRC-9**										N	N	N
10	**SRC-10**											C	N
11	**SRC-11**												C
12	**SRC-12**												

Table-5: Determination of the variability of radial growth in different isolates of *Sclerotium rolfsii* Sacc. At 100 ppm concentration of nine fungicides.

Isolates	Benlate	Dithane M-45	Captan	PCNB	Antracol	Ridomil	Sancozeb	Trimiltox forte	Copper oxychloride
SRC-1	S	N	N	N	S	S	S	S	N
SRC-2	S	N	N	N	S	N	N	N	N
SRC-3	S	S	N	N	S	S	S	S	N
SRC-4	S	N	N	N	N	N	S	S	N
SRC-5	S	N	N	N	N	N	S	N	S
SRC-6	S	S	N	N	S	S	N	S	S
SRC-7	S	N	N	N	S	S	S	N	N
SRC-8	S	N	N	N	S	S	S	S	N
SRC-9	S	S	N	N	S	S	S	N	N
SRC-10	S	N	N	N	N	S	S	N	N
SRC-11	S	S	N	N	S	S	N	N	N
SRC-12	S	N	N	N	N	S	S	S	S

S= Sensitive (radial growth <3.5 cm), N= Non-sensitive (radial growth > 3.5 cm)

References

Aycock R, 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. *NC Agric Exp Stn. Tech. Bull.*, **174**: 202 p.

Bashir M, Alam SS, Qureshi SH, Malik BA, 1985. Control of mungbean anthracnose by foliar fungicides. *Pak. J. Agric. Res.*, **6**: 173-175.

El-Tobshy ZM, El-Sayed EI, Sattar MA, Rahman A, 1981. Studies on the control of damping off and root rot diseases of Egyptian clover. *Research Bulletin No*, 178. Faculty of Agriculture, Ain Shams University. 11 pp.

Erwin DC, Wang MC and JJ Sinsx JJ, 1969. Translocation of 2-(2-thiazolyl) benzimidazole in cotton. *Phytopathology*, **60**: 1291.

Gurha SN, Kamthan KP, Misra DP, 1982. Reaction of some exotic and indigenous cultivars of chickpea (*Cicer arietinum* L.) against *Sclerotium rolfsii* Sacc. *Madras Agric. J.*, **69**: 471-473.

Gurha SN, Dubey RS, 1982. Occurrence of possible sources of resistance in chickpea (*Cicer arietinum* L.) against *Sclerotium rolfsii* Sacc. *Madras Agric. J.*, **70**: 63-64.

Harlton CE, Lévesque CA, Punja ZK, 1995. Genetic diversity in *Sclerotium (Athelia)*

rolfsii and related species. *Phytopathology*, **85**:1269-1281

Hulse JH, 1991. Nature, composition and utilization of grain legumes. P. 11-27. In: Uses of tropical legumes: Proceedings of a Consultants' meeting, 27-30 March 1989, ICRISAT Patancheru, AP 502 324, India.

Hussain A, Iqbal SM, Ayub N, Zahid MA, 2006. Factors affecting the development of collar rot disease in chickpea. *Pak. Journal of Botany* **38**: 211-216.

Ilyas MB, Khan MSA, Bhatti MAR, 1992. Chemical control of *Ascochyta* blight of lentil. In: R.H. Qureshi, I. Hussain, S.H. Shah, M. Ibrahim and M. Ahmad (eds). Proc. Of National Seminar on the role of Plant Health and Care in Agriculture Production 29-29 December 1988, UA, Faisalabad. pp 112-117.

Kamerwar Row KVSR, 1976. Efficacy of organic fungicides for the control of rice blast. *Pesticides*, **10**: 25-29.

Mahen VK, Mayer CD, Brennemen TB, McDonald D, 1995. Stem and pod rots of groundnut. *Information Bulletin No. 4*, ICRISAT, India, pp 28.

Malik BA, Tufail M, 1984. Control of Ascochyta blight of chickpea through clean seed. In: Ascochyta Blight and Winter Sowing of Chickpea: Proceeding of the workshop, 4-7, May 1981. Saxena, MC and Singh, KB (eds.). Aleppo, Syria. 229-235.

Nalim FA, Starr JL, Woodard KE, Segner S, Keller NP, 1995 Mycelial compatibility groups in Texas peanut field populations of *Sclerotium rolfsii*. *Phytopathology*, **85**:1507-1512

Nene YL, Thaplyal PN, 1982. Fungicides in plant disease control. Oxford and IBH Publishing Company, New Delhi, 507 pp.

Nene YL, Sheila VK, Sharma SB, 1984. A world list of chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajnus cajan* (L.) Millsp.) pathogens. ICRISAT Pulses Pathology Progress Report 32, pp19.

Okabe I, Morikawa C, Matsumoto N, Yokoyama K, 1998. Variation in *Sclerotium rolfsii* isolates in Japan. *Mycoscience*, **39**:399-407.

Prithiviraj B, Sara BK, Srivastava JS, Singh UP, 2000. Effect of Ca^{2+}, Mg^{2+}, light, temperature and pH on athelial stage formation in *Sclerotium rolfsii* Sacc. *J. Pl. Dis. Prot.*, **107**:274-278.

Punja ZK, 1985. Biology, ecology and control of *Sclerotium rolfsii*. *Ann Rev Phytopathol*., **23**:97-127.

Punja ZK, 1988 *Sclerotium (Athelia) rolfsii*, a pathogen of many plant species. In: Sidhu GS, ed. Genetics of plant pathogenic fungi. Vol. 6. London: Academic Press. p 523–534.

Punja ZK, Grogan RG, 1983. Basidiocarp induction, nuclear condition, variability and heterokaryon incompatibility in *Athelia (Sclerotium) rolfsii*. *Phytopathology* **73**:1273-1278

Punja ZK, Rahe JE, 1992. *Sclerotium*. pp. 166–170. In: Singleton, LL, Mihail JD, Rush CM, eds. Methods for research on soilborne phytopathogenic fungi. St. Paul: APS Press.

Scot DH, Draper AD, Maos JI, 1979. Benomyl for control of powdery mildew on strawberry plants in greenhouse. *Plant Dis. Rept*. **54**: 362-363.

Singh UP, Pavgi MS, 1965. Spotted leaf rot of plants, a new sclerotial disease. *Plant Dis. Reptr*. **49**:58-59.

Sharma D, Jodha NS, 1984. Pulse production in Semi-arid region of India. Proceedings of Pulses Production, Constraints and Opportunities. p 241-265.

Steel RGD, Torrie JH, 1980. Principles and procedures of Statistics. 2nd Ed. McGraw Hill Book Co. Singapore.

Sugha, SK, Sharma BK, Tyagi PD, 1991. A modified technique for screening chickpea (*Cicer arietinum*) varieties against collar rot caused by *Sclerotium rolfsii*. *Indian J. Agric. Sci.*, **61**: 289-290.