The Bioscan 8(4): 1425-1430, 2013 (Supplement on Genetics & Plant Breeding)
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

MORPHOLOGICAL AND GENOMIC VARIABILITY AMONG *SCLEROTIUM ROLFSII* POPULATIONS

THILAGAVATHI RASU*, NAKKEERAN SEVUGAPPERUMAL, RAGUCHANDER THIRUVENGADAM AND SAMIYAPPAN RAMASAMY
Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, INDIA - 641 003
e-mail: rthilagaphd@gmail.com

KEYWORDS
Morphological and genomic variations
RAPD analysis
S. rolfsii isolates

Received on :
07.07.2013

Accepted on :
28.09.2013

***Corresponding author**

ABSTRACT
Isolates of *Sclerotium rolfsii* collected from different host plants and geographic locations in Tamil Nadu, India were examined for their morphological and genomic variations. Out of 17 isolates tested for their cultural morphology, most of them were observed with compact colonies and few were fluffy colonies. Based on growth rate, they were categorized into three groups such as slow growing, fast growing and intermediate. Number of sclerotia and dry weight of 100 sclerotia also varied among isolates appreciably. Mostly dark to light brown sclerotia was observed for the isolates. RAPD banding patterns were established for 10 isolates of *S. rolfsii* using five random primers. Size of DNA fragments amplified by all five primers ranged from 100 bp to > 1 kb indicating polymorphism among *S. rolfsii* isolates. The result from UPGMA based dendrogram generated for the *S. rolfsii* isolates revealed that they were divided into two main clusters which were further divided into sub-clusters. Overall, all the isolates have about 54% similarity coefficient indicating that they were genetically varied by their unique banding patterns however, they shared more number of common bands and clustered together. Hence this study clearly indicated that morphological and genetic variability existed among *S. rolfsii* isolates.

INTRODUCTION

Sclerotium rolfsii is an omnivorous soil borne fungal pathogen causes disease on a wide range of agricultural and horticultural crops. The fungus being a well known type member of the genus *Sclerotium* as it forms differentiated sclerotia, usually causes collar rot diseases. Sclerotia are considered to be extremely hardy and relatively resistant survival structures (Singh *et al*., 2003), principle means of dispersal (Okabe *et al*., 2000). Profuse mycelial growth and sclerotial production contribute to the considerable crop losses associated with *S. rolfsii* (Kokub *et al*., 2007). Because of its basidial stage which is not easily observed, this fungus is characterized by the morphology of sclerotia in most cases (Punja and Damiani, 1996), consequently little has been studied on its genetic background and variation. Studies of variability within the population in a geographical region are important because these also document the changes occurring in the population. Variability among *S. rolfsii* populations from different geographical regions was demonstrated by earlier workers (Harlton *et al.,*1995, Okabe *et al.,* 1998, Sarma and Singh, 2002). Molecular markers play a major role in analyzing genetic basis of genotypic variation among fungal population. Welsh and McClelland (1990) described a modification of the PCR procedure referred to as the randomly amplified polymorphic DNA (RAPD) marker technique that can be used to detect genetic polymorphisms in fungi. This technique can overcome the limitations of RFLPs such as they are relatively slow, expensive and laborious, as generally only a single locus can be analyzed with each RFLP reaction. RAPD technique differs from conventional PCR in that only a single primer which is derived from an arbitrary sequence is used for amplifying DNA (Perez Moreno *et al*., 2002). Hence the RAPD analysis being used as a powerful tool for the investigation of genetic relatedness and diversity among closely related strains and was found to be a valuable method for differentiating the genetic variability of *S. rolfsii* isolates (Saude *et al*., 2004). To understand the ecology and pathogenicity aspects of the *S. rolfsii,* it is essential to study about the phenotypic and genetic variability among isolates. The objective of the present study was to study the morphological and genetic relationships among isolates of *S. rolfsii* from different host plants and geographic locations of India.

MATERIALS AND METHODS

Study on morphological characters of *S. rolfsii* isolates

The morphological characters of 17 isolates of *S. rolfsii* from different host plants and diverse geographic origins were studied by growing on the PDA medium. Nine mm mycelial discs from the margin of an actively growing colony of each isolate were placed in the centre of the Petri Plates. The inoculated plates were incubated at normal room temperature (27 ± 2ºC) and replicated four times. The colony diameter (growth rate) was measured every day until three days, colony morphology was observed after five days, number of days taken for sclerotial formation was calculated, number of sclerotia per Petri Plate was counted after 20 days, diameter of 25 sclerotia was measured in Alpha Imager (PCS technology, Amli, Silvassa), 100 sclerotial dry weight and colour were also measured for all the isolates (Sarma and Singh, 2002).

Statistical analysis

Whole experiment was repeated twice with four replications. The data were analyzed using the IRRISTAT version 92-1 programme developed by the biometrics unit, International Rice Research Institute, Philippines. Data were subjected to analysis of variance (ANOVA). The treatment means were compared by Duncan's multiple range test (DMRT) (Gomez and Gomez, 1984).

RAPD-PCR analysis of *S. rolfsii* isolates

Genomic DNA extraction

Ten *S. rolfsii* isolates were grown in potato dextrose broth. Mycelial mats were harvested after 10 days, subsequently dried in the sterilized blotter papers. The CTAB extraction procedure (Zolan and Pukkila, 1996) was followed with some modifications. About 100 mg of mycelium was macerated in liquid nitrogen until a powdered mycelium was obtained. The powder was then transferred into a test tube containing 5mL extraction buffer (700 mM of NaCl, 50 mM of Tris HCl with pH 8.0, 10 mM of EDTA, 2% CTAB, and 1% mercapto-ethanol). Samples were incubated for 1 h at 60ºC followed by two consecutive extractions with 5 mL chloroform-isoamylalcohol (24:1). The emulsions were centrifuged at 3500 rpm for 15 min and the aqueous phase was recovered and taken to another tube. DNA was precipitated by adding ice cold isopropanol and chilled at -20ºC for at least 30 min. DNA was collected by centrifugation at 10000 rpm at 4ºC for 15 min. The supernatant was discarded and the pellet was dried at room temperature. The dried pellet was dissolved in 500 μL of TE buffer (10 mM of Tris HCl with pH 8.0, 1mM of EDTA with pH 8.0) and treated with 5 μL of RNAse A (10 mg/mL) at 37ºC for 1h. The isolated total DNA was tested in the 0.8% agarose gel.

RAPD analysis

The 20 μL PCR reaction mixture contains DNA template 25 ng, 10x Taq buffer, 2.5mM of each of dNTP mixture, 2.5mM of $MgCl_2$, 30 picomole of random primer, and 2 U of Taq DNA polymerase (Bangalore Genei, India). PCR amplification was performed in a thermocycler (Eppendorf Mastercycler gradient, Westbury, Newyark) using the following conditions: initial denaturation at 94ºC for 5min, 30 cycles of denaturation at 94ºC for 1min, annealing at 36ºC for 1min, extension at 72ºC for 2 min, and a final elongation at 72ºC for 5 min (Gaitan *et al.,* 2002). A set of 5 random primers used in this study; C3 (CGG CTT GGG T), OPA02 (TGC CGA GCT G), OPC20 (ACTT CGC CAC), OPF01 (ACG GAT CCT G) and OPX07 (GAG CGA GGC T). The amplified PCR products were tested on 1.5% agarose gel. The gel was visualized with a UV transilluminator and photographed in the gel documentation system (Alpha Innotech Corporation, California). The experiment was repeated twice and only the RAPD bands which appeared consistently were evaluated.

Analysis of RAPD results

The banding patterns were scored for RAPD primers in each *S. rolfsii* isolate starting from the large size fragment to small sized one. Presence and absence of each band in each isolate was coded as 1 and 0 respectively. The scores were used to create a data matrix to analyze genetic relationship using the NTSYS-pc program version 2.02 (Exeter Software, New York, USA) described by Rohlf (1993). A dendrogram was constructed based on Jaccard's similarity coefficient (Jaccard, 1908) using the marker data from *S. rolfsii* isolates with Unweighted Pair-Group Method on Arithmetic Average (UPGMA) cluster analysis to group the isolates based on their overall similarities. Each RAPD pattern was compared with the other patterns and Euclidean distance matrix was calculated. The relationships among the isolates examined were represented as dendrogram by using UPGMA.

RESULTS AND DISCUSSION

Morphological variations

Variability in cultural morphology, mycelial growth rate, sclerotium formation, sclerotial size and colour among *S. rolfsii* isolates were observed by many researchers (Akram *et al.*, 2008; Okereke and Wokocha, 2007; Almeida *et al.,* 2001). In the present study, the isolates of *S. rolfsii* varied in all of the morphological characters *viz*, mycelial growth rate, colony

Table 1: Isolates of *S. rolfsii* and their morphological characters in terms of mycelial growth rate, type and duration for formation of sclerotia

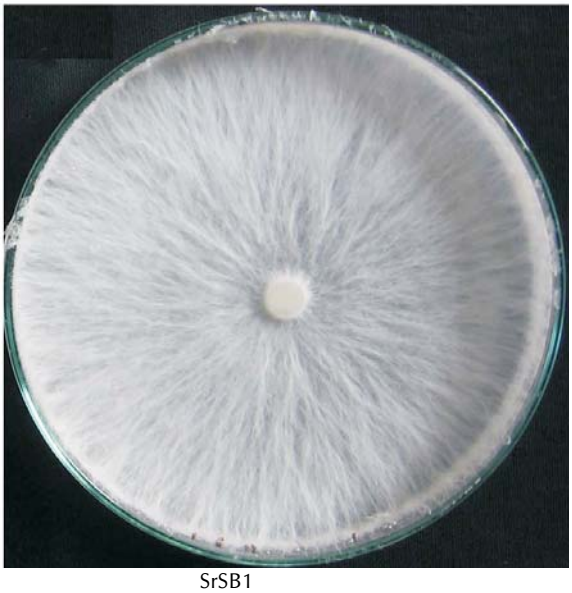
Isolate name	Host	Mycelial growth rate (dia in cm) DAI*			Mycelial type	Sclerotial formation(no. of days)
		1	2	3		
SrSB1	Sugarbeet	2.8^{c}	7.2^{c}	C^{a}	Compact	9.0^{c}
SrSB2	Sugarbeet	1.4^{j}	5.4^{i}	7.8^{bc}	Compact	7.0^{de}
SrSB3	Sugarbeet	3.4^{a}	7.6^{a}	C^{a}	Compact	6.0^{ef}
SrTO1	Tomato	2.0^{g}	6.0^{h}	C^{a}	Compact	6.0^{ef}
SrST1	Stevia	1.6^{i}	5.0^{jk}	7.6^{cd}	Compact	7.0^{de}
SrJ1	Jasmine	3.4^{a}	7.6^{a}	C^{a}	Fluffy	15.0^{a}
SrJ2	Jasmine	2.8^{c}	7.2^{c}	C^{a}	Compact	6.0^{ef}
SrGL1	*Gloriosa* sp.	2.4^{e}	6.6^{f}	C^{a}	Fluffy	8.0^{cd}
SrBA1	Banana	3.2^{b}	7.4^{b}	C^{a}	Compact	12.0^{b}
SrGN1	Groundnut	2.4^{e}	6.8^{e}	C^{a}	Compact	8.0^{cd}
SrSB4	Sugarbeet	2.2^{f}	6.4^{g}	7.9^{b}	Compact	6.0^{ef}
SrON1	Onion	1.8^{h}	5.3^{ij}	7.9^{b}	Compact	7.0^{de}
SrSB5	Sugarbeet	1.9^{gh}	5.2^{j}	7.8^{bc}	Fluffy	6.0^{ef}
SrTO2	Tomato	1.6^{i}	5.1^{jk}	7.7^{c}	Compact	5.0^{f}
SrGN2	Groundnut	2.6^{d}	7.0^{d}	C^{a}	Fluffy	7.0^{de}
SrAL1	Aloevera	2.3^{ef}	6.7^{ef}	C^{a}	Fluffy	9.0^{c}
SrBA2	Banana	2.7^{cd}	7.0^{d}	C^{a}	Compact	5.0^{f}

C- Complete growth; *DAI-Days after incubation; Values are mean of four replications,Means followed by a common letter are not significantly different at 5% level by DMRT

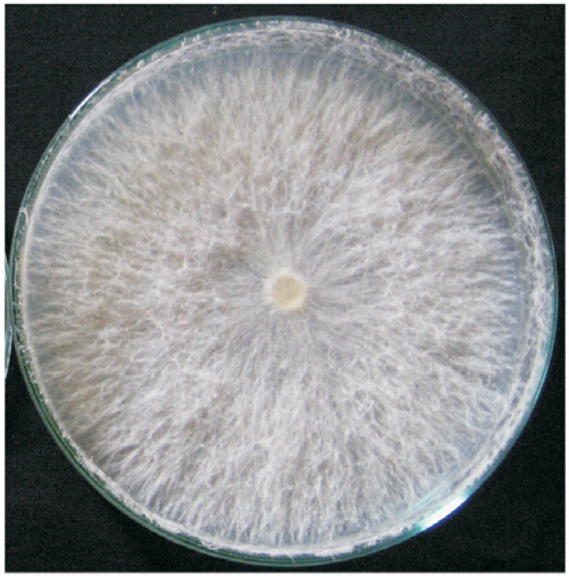
Table 2: Isolates of *S. rolfsii* and their morphological characters in terms of sclerotial production, dry weight, diameter and colour

Isolate name	Host	Total no.of sclerotia (plate^{-1})	Sclerotial dry weight (mg)(100 sclerotia^{-1})	Sclerotial dia (mm)	Colour of sclerotia
SrSB1	Sugarbeet	720^{g}	43^{hij}	0.9^{f}	DB
SrSB2	Sugarbeet	1242^{d}	28^{l}	1.0^{e}	RB
SrSB3	Sugarbeet	780^{f}	47^{gh}	1.1^{d}	DB
SrTO1	Tomato	954^{e}	40^{ijk}	1.2^{c}	DB
SrST1	Stevia	1782^{a}	37^{jk}	1.0^{e}	DB
SrJ1	Jasmine	242^{j}	86^{a}	1.6^{a}	LB
SrJ2	Jasmine	1764^{b}	38^{jk}	0.9^{f}	LB
SrGL1	*Gloriosa* sp.	468^{i}	61^{cd}	1.2^{c}	LB
SrBA1	Banana	1420^{c}	57de	1.0^{e}	DB
SrGN1	Groundnut	548^{h}	30^{l}	0.9^{f}	LB
SrSB4	Sugarbeet	186^{k}	36^{jk}	1.1^{d}	DB
SrON1	Onion	114^{n}	26^{l}	0.8^{g}	DB
SrSB5	Sugarbeet	140^{l}	63^{bc}	1.5^{b}	LB
SrTO2	Tomato	110^{n}	67^{b}	1.6^{a}	DB
SrGN2	Groundnut	125^{m}	53^{ef}	1.5^{b}	LB
SrAL1	Aloevera	720^{g}	43^{hij}	1.1^{d}	LB
SrBA2	Banana	145^{l}	51^{fg}	1.2^{c}	DB

LB: Light brown; DB: Dark brown; RB: Reddish brown; Values are mean of four replications; Means followed by a common letter are not significantly different at 5% level by DMRT



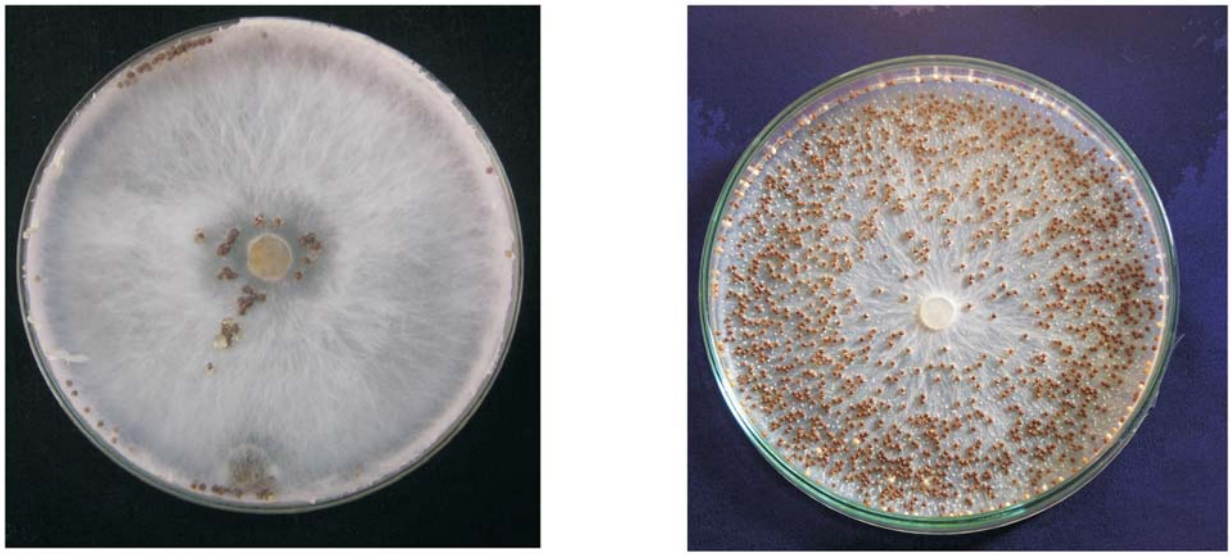
SrSB1



SrGN2

Figure 1: Compact and fluffy mycelium of *S. rolfsii* on PDA

morphology, sclerotial production, sclerotial numbers, sclerotial size, weight and colour. Out of 17 isolates, colonies of 5 isolates were fluffy, 12 isolates were compact (Fig. 1). Kokub *et al*. (2007) reported that the equilibrium of enzymes â-1, 3 glucanase and glucane synthetase which controlled the hyphal growth and branching in *S. rolfsii*. Mycelial growth rate also varied considerably upto 3 days. Data on first day after inoculation revealed that the isolates, SrSB3, SrJ1 and SrBA1 were fast growing (3.2 - 3.4 cm) as against the isolates SrSB2, SrST1, SrON1, SrSB5 and SrTO2 which were slow growing (1.4 - 1.9 cm). Medium growth rate of 2.0 to 2.8 cm was observed for other isolates. The isolates SrTO2 and SrBA2 formed sclerotia in a minimum of five days as against the isolates SrBA1 and SrJ1 which were taken a maximum of 12 and 15 days respectively. Other isolates varied from 6 to 9 days (Table 1). Considerable variability among *S. rolfsii* isolates in relation to the number, size and location of sclerotia on the medium surface was observed by Almeida *et al*. (2001). In the present study production of sclerotia among isolates varied appreciably. The isolates SrSB2, SrBA1, SrJ2 and SrST1 produced more number of sclerotia (1200 to 1800), while the isolates SrTO2, SrON1, SrGN2, SrSB5, SrBA2, SrSB4 and SrJ1 produced less number (100 to 250). Other isolates produced sclerotia in a medium range (400 to1000) (Table 2; Fig. 2). Similarly, the dry weight of 100 sclerotia varied among isolates considerably, the isolate SrJ1 recorded a maximum of 86 mg as against the isolates SrON1, SrSB2, and SrGN1 recorded a very less dry weight (< 30 mg). Other isolates recorded as 36 to 67 mg. Most of the isolates varied with the average sclerotial size of 1 to 1.2 mm in dia, whereas the largest sclerotia were



SrTO2 SrST1

Figure 2: *S. rolfsii* isolates with less and more number of sclerotia on PDA

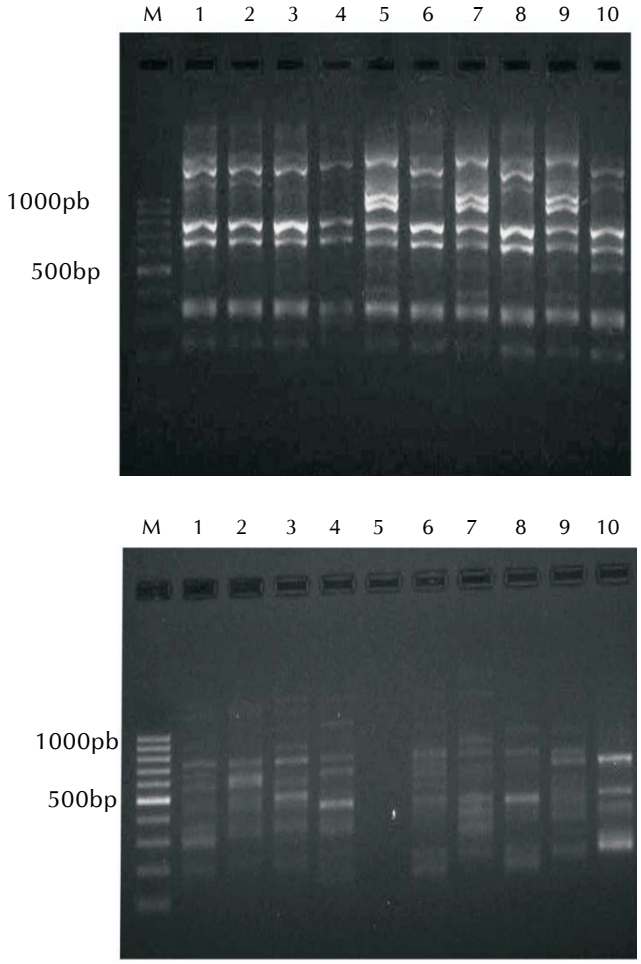


Figure 3a: RAPD profiles of *S. rolfsii* isolates obtained with random primers. A: C3 primer; B: OPA02 primer. Lane 1-10: SrSB1, SrSB2, SrSB3, SrTO1, SrST1, SrJ1, SrJ2, SrGL1, SrBA1, SrGN1; M: marker (100 bp)

produced by SrJ1, SrSB5, SrTO2 and SrGN2 (1.5 to 1.6 mm in dia). Mostly dark to light brown sclerotia with an exception in one of the sugarbeet isolates being a reddish brown (SrSB2) even after maturity (Table 2). The result indicated that the morphological variations existed among isolates of *S. rolfsii*.

Genetic variations

Considerable genetic variability among *S. rolfsii* isolates from different host species and geographic regions by RAPD-PCR analysis was confirmed by many researchers (Almeida *et al*., 2001; Kokub *et al*., 2007; Punja and Sun, 2001). In the present study, RAPD banding patterns were established for 10 isolates of *S. rolfsii* using five random primers. The amplicon patterns were consistent and the profiles were reproducible between two independent experiments. Size of randomly amplified DNA fragments with all five primers ranged from 100 bp to > 1 kb indicating polymorphism among *S. rolfsii* isolates. The primer C3 amplified two specific bands of approximately 900 bp and 1000 bp in the isolates SrST1, SrJ2, SrSB1 and such bands were absent in other isolates. Moreover, the same primer yielded three fragments with more than 1000 bp in the isolates SrSB1, SrSB2, SrJ1, SrGL1, two fragments (>1000 bp) in SrSB3, SrGN1 and single fragment (>1000 bp) in SrTO1, SrST1, SrJ2, SrSB1 isolates. A unique band of approximately 500 bp in SrSB1 and 300 bp in SrSTO1 amplified by the primer C3. The primer OPA02 amplified a unique fragment of approximately 200 bp in SrSB1, SrTO1, SrJ1, SrGL1 isolates, 300 bp in SrSB1, SrJ2 isolates, 850 bp in SrSB2, SrJ2 isolates, 1000 bp in SrTO1 isolate and fragments with more than 1000 bp in SrSB2, SrSB3, SrTO1, SrJ2 isolates (Fig. 3a). The primer OPX07 amplified two specific bands of approximately 100 bp and 200 bp in most of isolates, however such bands were absent in SrST1, SrJ2, SrBA1 isolates. Again these three isolates were observed with absence of approximately 225bp for the OPF01 primer. The same primer amplified a unique 1000 bp fragment in the isolate SrTO1 (Fig. 3b). The primer OPC20 yielded a specific band of approximately 700bp in SrT1, SrBA1 isolates, three bands with more than 1000 bp in the isolates SrT1, SrBA1,

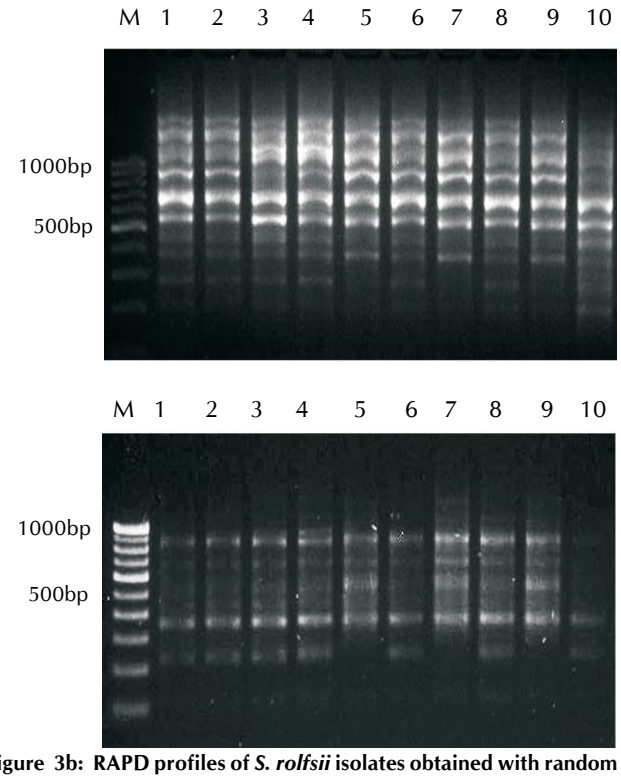


Figure 3b: RAPD profiles of *S. rolfsii* isolates obtained with random primers. C: OPX07 primer; D: OPF01 primer. Lane 1-10: SrSB1, SrSB2, SrSB3, SrTO1, SrST1, SrJ1, SrJ2, SrGL1, SrBA1, SrGN1; M: marker (100 bp)

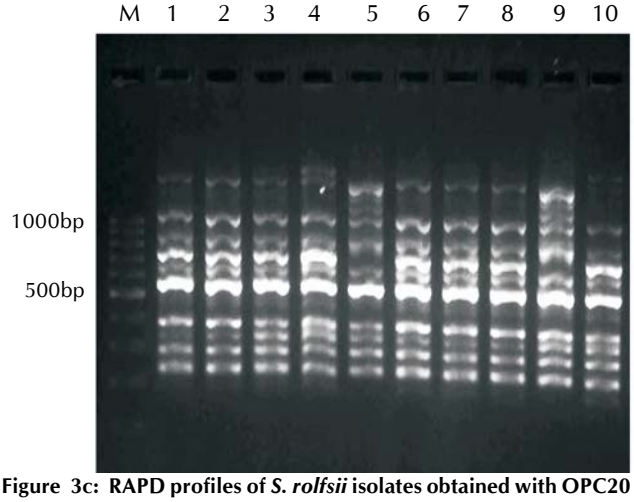


Figure 3c: RAPD profiles of *S. rolfsii* isolates obtained with OPC20 primer. Lane 1-10: SrSB1, SrSB2, SrSB3, SrTO1, SrST1, SrJ1, SrJ2, SrGL1, SrBA1, SrGN1; M: marker (100 bp)

two bands (> 1000bp) in SrTO1, SrGN1 and single fragment (> 1000bp) in other isolates (Fig. 3c). Similarly, Prasad *et al*. (2010) observed the polymorphic and distinguishable banding pattern for the DNA fragments amplified with five random primers signifying genetic diversity among all eight isolates of *S. rolfsii*. The findings of Perez-Moreno *et al*. (2002) demonstrated the genetic polymorphism among isolates of *Sclerotium cepivorum* from Mexico based on RAPD analysis. In the present study, relationships among the isolates were evaluated by cluster analysis. The result from UPGMA based

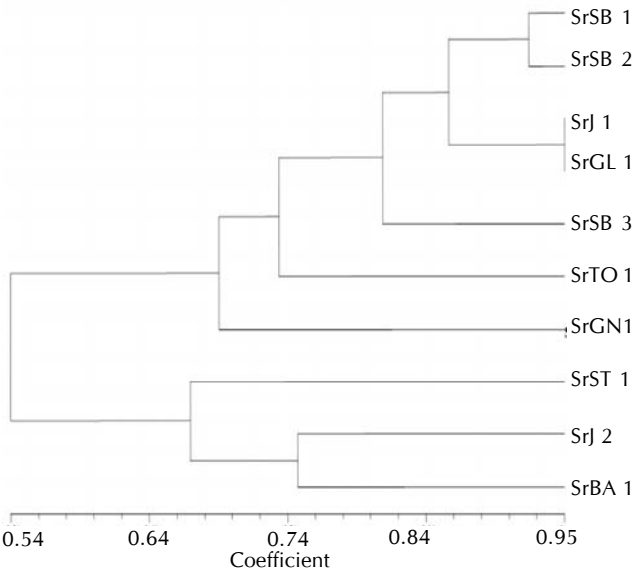


Figure 4: Dendrogram generated for the *S. rolfsii* isolates from different locations and host plants.

dendrogram generated for the *S. rolfsii* isolates revealed that they were divided into two main clusters, A and B which were further subdivided into subclusters. Among the isolates, SrST1, SrJ2 and SrBA1, fall in one cluster, which showed 66 per cent similarity coefficient between them. The isolates SrSB1, SrSB2, SrJ1, SrGL1, SrSB3, SrTO1 and SrGN1 isolates fall in another cluster, which showed 67 per cent similarity. SrSB1 and SrSB2 isolates showed 91 per cent similarity and SrJ1 and SrGL1 isolates showed 95 per cent between them, which fall in cluster B. Overall, all the isolates have about 54 per cent similarity coefficient (Fig. 4), indicating that they were genetically varied by their unique banding patterns however, they shared more number of common bands and clustered together. These results were in agreement with the findings of Prasad *et al*. (2010) and Punja and Sun (2001). According to Perez *et al*. (1998), a dendrogram based on pair-wise dissimilarities indicated that the isolates collected from a single field clustered into one group while they from different regions clustered into other group, hence they were most divergent. Kumar *et al*. (2010) investigated the isolates of rice stem rot fungus *Sclerotium oryzae* collected from major rice growing belt of Haryana were clustered into different groups based on DNA fingerprinting. Four distinct groups of *S. rolfsii* isolates based on DNA polymorphisms for 21 random primers were observed by Saude *et al*. (2004). Hence studying the morphological and genomic background of the groups should promote understanding the ecology and pathogenicity aspects of *S. rolfsii*.

ACKNOWLEDGEMENT

The authors thank the M/S Syngenta India limited for providing financial support to carry out this study.

REFERENCES

Akram, A., Iqbal, M. S. H., Ahmed, N., Iqbal, U. and Ghafoor, A. 2008. Morphological variability and mycelial compatibility among

the isolates of *Sclerotinia sclerotiorum* associated with stem rot of chickpea. *Pak. J. Bot*. **40(6):** 2663-2668.

Almeida, A. M. R., Abdelnoor, R. V., Calvo, E. S., Tessnman, D. and Yorinori, J. T. 2001. Genotypic diversity among Brazilian isolates of *Sclerotium rolfsii*. *J Phytopathol*. **149(9):** 493-502.

Gaitan, A., Valderrama, A. M., Saldarriaga, G., Velez, P. and Bustillo, A. 2002. Genetic variability of *Beauveria bassiana* associated with the coffee berry borer *Hypothenemus hampei* and other insects. *Res.*, **106(11):** 1307-1314.

Gomez, K. A. and Gomez, A. A. 1984. *Statistical Procedure for Agricultural Research. J. Wiley and Sons,* New York.

Harlton, C. E., Le´vesque, C. A. and Punja, Z. K. 1995. Genetic diversity in *Sclerotium* (*Athelia*) *rolfsii* and related species. *Phytopathology*. **85**: 1269-1281.

Jaccard, P. 1908. Nouvelles recherché sur la distribution florale. *Bull. Soc. V. Sci. Nat*. **44**: 223-270.

Kokub, D., Azam, F., Hassan, A., Ansar, M., Asad, M. J. and Khanum, A. 2007. Comparative growth, morphological and molecular characterization of indigenous *Sclerotium rolfsii* strains isolated from different locations of Pakistan. *Pak. J. Bot*. **39(5):** 1849-1866.

Kumar, A., Ram Singh, K., Boora, S. and Jalali, B. L. 2010. Genetic diversity in *Sclerotium oryzae* population of rice based on random amplified polymorphic DNA markers. *[abstract]*. **63**: 4.

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

Okabe, I., Morikawa, C. and Matsumoto, N. 2000. Variation in southern blight fungi detected by ITS-RFLP analysis. *http//:ss. Jirkas.Affrc.go.Jp/engpage/jarq/34-2/okabe/34-2(3). htm.*

Okereke, V. C. and Wokocha, R.C. 2007. *In vitro* growth of four isolates of*Sclerotium rolfsii* Sacc. in the humid tropics. *African J. Biotech*. **6(16):** 1879-1881.

Perez, L., Olalde, V., Vandemark, G. J., Martinez, O., Martinez, J., Entwistle, A., Lara, J. and Vasquez, G. 1998. Analysis of *Sclerotium cepivorum* by random amplified polymorphic DNA (RAPD) assay. Proceedings of the sixth international workshop on *Allium* white rot. **3**: 13.

Perez-Moreno, L., Olalde-Portugal, V., Vandemark, G. J., Martínez-de la Vega, O., Martínez-Soriano, J. P., VázquezMarrufo, G. and Lara-Reyna, J. 2002. Genetic relationships among isolates of *Sclerotium cepivorum* Berk. based on RAPD analysis. *Revista Mexicana de Fitopatologia*. **20**: 187192.

Prasad, S. D., Basha, S. T. and Eswara Reddy, N. P. G. 2010. Molecular variability among the isolates of *Sclerotium rolfsii* causing stem rot of groundnut by RAPD, ITS-PCR and RFLP. *Eur. Asia. J. Bio. Sci*. **4**: 80-87.

Punja, Z. K. and Damiani, A. 1996. Comparative growth, morphology and physiology of three *Sclerotium* species. *Mycologia*. **88**: 694-706.

Punja, Z. K. and Sun, L. J. 2001. Genetic diversity among mycelial compatibility groups of *Sclerotium rolfsii* (telemorph *Athelia rolfsii*) and *S. delphenii*. *Mycol. Res.* **105(5):** 537-546.

Rohlf, F. J. 1993. NTSYS-pc numerical taxanomy and multivariate analysis system. *Version 1.8. Exeter Publ., Setauket, New York.*

Sarma, B. K. and Singh, U. P. 2002. Variability of Indian isolates of *Sclerotium rolfsii*. *Mycologia*. **94(6):** 1051-1058.

Saude, C., Melouk, H. A. and Chenault, K. D. 2004. Genetic variability and mycelial compatibility groups of *Sclerotium rolfsii* [abstract]. *Phytopathology*. **94**: S92.

Singh, A., Mehta, S., Singh, H. B. and Nautiyal, C. S. 2003. Biocontrol of collar rot disease of betelvine (*Piper betle* L.) caused by *Sclerotium rolfsii* by using rhizosphere-competent *Pseudomonas fluorescens* NBRI-N6 and *P. fluorescens* NBRI-N. *Curr. Microbiol*. **47(2):** 153-158.

Welsh, J. and McClelland, M. 1990. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Research*. **18**: 7213-7218.

Zolan, M. E. and Pukkila, P. J. 1996. Inheritance of DNA methylation in *Coprinus cinereus*. *Mol. Cell Bio.***6**: 195-200.