RESEARCH ARTICLE Legume Research- An International Journal, Volume 47 Issue 10: 1809-1814 (October 2024)

Diversity Studies of *Sclerotium rolfsii* Sacc. Isolates Associated with Collar Rot of Soybean Through Molecular and Biochemical Approaches

Ishwari G. Hiremath[1], Shamarao Jahagirdar[1], P.U. Krishnaraj[1], S.A. Ashtaputre[1], D.N. Kambrekar[1], K. Priyanka[1]

10.18805/LR-5221

ABSTRACT

Background: *Sclerotium rolfsii* Sacc. is one of the potential soil-borne plant pathogens due to its significant loss in terms of yield and quality in several crop plants. In soybean the pathogen is associated with collar rot disease.

Methods: Sixteen samples which were showing collar rot symptoms were collected from different soybean growing regions of the country and were subjected for isolation and purification. Molecular detection was carried out using ITS1/4 primers followed by phylogenetic analysis was performed using the MEGA 11 bioinformatics tool. Further, the biochemical test was made for oxalic acid production to determine the variability across the isolate.

Result: DNA was amplified for ITS rDNA region at 650-700 bp in all the sixteen isolates. BLAST detection of nucleotide sequence of sixteen isolates framed mainly into two distinct phylogenetic clusters. Cluster B consisted of two isolates (MPSe 1 and MHSa 3) whereas, Cluster A contains the remaining fourteen isolates. There was a variation with respect to oxalic acid production among the isolates which was in the rage of 1.15 to 2.23 mg/ml.

Key words: Collar rot, Oxalic acid, Phylogeny, *Sclerotium rolfsii*, Soybean, Variability.

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] has become an important oil yielding crop for steady increase in worldwide population which accounts for approximately 50 per cent of the total oil seed production in the world (Fehr, 1989). Over the past decade, productivity trend of soybean shows that yields achieved are not attained due to several abiotic and biotic factors. The average soybean yield in the world is about 2880 kg/ha while it is only 991 kg/ha in India (Anonymous 2022). More than 100 pathogens are known to affect the crop of which 35 are economically important and soil-borne fungal diseases play a vital role to hamper the yield production at field soil (Sinclair, 1978).

Sclerotium rolfsii (teleomorph: *Athelia rolfsii* (Curzi) Tu and Kimbrought) is one of the devastating soil borne plant pathogenic fungus which attacks the crop at all crop growth stages (Punja, 1985). The pathogen has a wide host range of over 500 plant species throughout the world, mostly infecting dicotyledonous plants and few monocotyledonous species. The disease causes 25-50 per cent loss through infection of seedlings and the annual yield losses by disease in soybean have been reported 10- 30 percent (Hartman *et al*., 1999). It was first observed from Madhya Pradesh and later on from different soybean growing areas of India (Agrawal and Kotasthane, 1971). Ansari and Agnihotri (2000) reported upto 65 per cent loss in soybean due to collar rot under severe condition at Indore.

The analysis of genetic variation in pathogen population is an important pre-requisite for understanding the evolution occurring in plant pathogen system. The present study aims to identify the variability present among the isolates which were collected from different geographical locations as there is a limited information on variability of *S. rolfsii* infecting soybean in India.

Oxalic acid, a metabolite of *S. rolfsii*, is known to play a role in pathogenesis of this fungus (Kirtzman *et al*., 1977) and there is a positive correlation between oxalic acid production and virulence of the isolates of *S. rolfsii* (Ansari and Agnihotri, 2000). The oxalic acid sequesters the calcium in the host cell wall thereby favouring the pectic enzymes secreted by the pathogen to rapidly hydrolyse the pectate in the middle lamella to cause the collar rot/ root rot symptoms.

[1]Department of Plant Pathology, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India.

Corresponding Author: Shamarao Jahagirdar, Department of Plant Pathology, University of Agricultural Sciences, Dharwad-580 005, Karnataka, India. Email: shamaraoj@gmail.com

How to cite this article: Hiremath, I.G., Jahagirdar, S., Krishnaraj, P.U., Ashtaputre, S.A., Kambrekar, D.N. and Priyanka, K. (2024). Diversity Studies of *Sclerotium rolfsii* Sacc. Isolates Associated with Collar Rot of Soybean Through Molecular and Biochemical Approaches. Legume Research. 47(10): 1809-1814. doi: 10.18805/LR-5221.

Submitted: 03-08-2023 **Accepted:** 30-01-2024 **Online:** 01-03-2024

MATERIALS AND METHODS

Collection of infected samples and isolation of the fungus

The symptoms showing collar rot portion on host were collected from sixteen different soybean growing areas of the country and infected plant parts were subjected for isolation using the standard tissue isolation method (Brunda, 2018) on sterilized Potato Dextrose Agar (PDA) medium and incubated at 28°C. Subsequently sub-culturing was carried out to purify the cultures and were maintained on Potato Dextrose Agar (PDA) slants for further use. The present investigations were carried out at the Microbial Genetics Laboratory, Department of Agricultural Microbiology, University of Agricultural Sciences Dharwad during 2021-22.

Genomic DNA extraction

The genomic DNA of all the sixteen isolates was carried as per the procedure given by Murray and Thompson (1980) with slight modifications. Mycelium from 2 to 3 days old pure broth culture was harvested using sterile Whatman filter paper and transferred to pre-chilled pestle and mortar. The mycelium was homogenized to fine powder using liquid nitrogen and about 100 mg of the fine powder was transferred to pre-sterilized micro-centrifuge tubes containing freshly pre heated CTAB buffer. Ten microlitre of beta-mercaptoethanol was added to each tube and mixed well by inversion. The tubes were incubated in hot water bath at 65°C for 45 minutes with intermittent mixing of the tubes. After incubation, equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and mixed well followed by centrifugation at 10000 rpm for 10 minutes. The upper aqueous phase was collected in the fresh tubes to which equal volume of chloroform: iso-amyl alcohol was added and mixed. Later, the tubes were centrifuged at 10000 rpm for 10 minutes in order to obtain the three phases in the tube from which, the upper aqueous phase was collected and transferred into fresh micro-centrifuge tubes. Equal volume of pre-chilled iso-propanol followed by mixing and incubation at -20°C overnight. The tubes were centrifuged at 10000 rpm for 20 minutes and decanted to retain the pellets. The pellets were washed with 70 percent ethanol, air-dried a room temperature until the traces of ethanol was removed and dissolved in TE buffer and stored at -20°C for further use.

PCR amplification

Molecular detection was carried out using primers complementary to 5.8S RNA gene with the flanking ITS, namely ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) (White *et al*., 1990). The PCR cocktail was prepared, which consists of 2 mM $MgCl_2$, 200 µM of each dNTP's and 2.5 U of Taq polymerase (Takara, Japan) in a 0.2 ml micro-centrifuge tube and to each tube, 50 ng of DNA was dispensed to make a final volume of 20 µl then the tubes were vortexed gently. PCR was performed in a thermocycler with the following conditions: Initial denaturation of 94°C for 5 minutes, 32 cycles each of denaturation at 94°C for 50 seconds, 52°C for 45 seconds and extension of 72°C for one minute followed by one cycle of final extension of 72°C for 5 minutes. A buffer control (no template control) was maintained along with the sixteen samples. The PCR product was sequenced by using forward and reverse primers at Eurofins Genomics India Pvt. Ltd., Bangalore and the nucleotide sequences of the isolates were subjected to BLAST analysis for homology search in the NCBI bioinformatics tool and the sequences were deposited in GenBank of NCBI. Multiple alignments for homology search were performed using the Clustal W algorithm software and the phylogenetic tree was constructed using ITS sequence of sixteen isolates of the pathogen (Tamura *et al*., 2013).

Oxalic acid estimation

All the sixteen isolates of *Sclerotium rolfsii* were tested for oxalic acid production in the culture filtrates. For this purpose each isolate was grown separately in 250 ml flasks containing 50 ml Potato Dextrose Broth (PDB) which was sterilized earlier at 121°C for 20 min. Each flask was inoculated with individual isolate of *S. rolfsii* (by putting 5mm mycelial disc of five days old culture) and incubated at 28°C for 10 days. The broth containing mycelial growth was filtered through Whatman filter paper No.1 to remove the mycelial mat. The obtained filtrate was centrifuged at 5000 rpm for 10 min. to remove the mycelial fragment, if any and to this 10 ml cell free culture filtrate, 8 ml of calcium chloride-acetate buffer (pH 4.5) was added and mixed well. The mixture was allowed to stand overnight at room temperature and then centrifuged at 5000 rpm for 10 min. The supernatant was discarded and residue was washed with 10 ml of 50 percent acetic acid (saturated with calcium oxalate) and then centrifuged. Then the residue obtained was dissolved in 10 ml of 4 N H_2SO_4 and transferred to 100 ml flask and heated at 80°C on a water bath. While hot, titration was carried out with 0.02 N potassium permanganate until a faint pink colour persisted. For each isolate three replications were maintained and mean value was calculated. For oxalic acid quantification, the amount of oxalic acid present in the culture filtrate was calculated as 1ml of 0.02 N potassium permanganate reacted with 1.265 mg of oxalic acid (Mahadevan and Sridhar, 1986).

RESULTS AND DISCUSSION

The pathogen was isolated from the infected samples where it initially produced dense, white radiating mycelium on potato dextrose agar plates followed by production of sclerotial bodies from fifth day of inoculation (Plate 1). Similar culture morphology was observed by many researchers (Prabhu, 2003; Sangeeta, 2011; Brunda, 2018 and Vivekanand, 2020).

Amplification of DNA at ITS1/4 was carried out for which all the sixteen isolates amplified at 650 to 700 bp (Plate 2).

However, no amplification was noticed in the buffer control. During the homology search in the NCBI database, the isolates showed up to 97 percent similarity with already deposited sequences of *Athelia rolfsii* strains (Table 1). Further, the results of phylogenetic analysis which was carried out for ITS sequence of sixteen isolates in MEGA 11.0 bioinformatics tool indicated the formation of two distinct phylogenetic clusters. Cluster B consisted of two isolates (MPSe 1 and MHSa 3) which were collected from Madhya Pradesh and Maharashtra and the remaining fourteen isolates grouped together to form Cluster A. Again this Cluster A comprised of two sub clusters A1 and A2. Cluster A1 consists of only one isolate (MPSe 2) and all the remaining isolates grouped in the cluster A2 which revealed the variation among the isolates of *Sclerotium rolfsii* (Fig 1). This is the first report of understanding molecular variability of *Sclerotium rolfsii* infecting soybean in India. The results were in conformity with the results of Jebaraj *et al*. (2017) who reported the DNA amplification with specific ITS 1 and ITS 4 universal primers at the amplicon size of approximately 650 to 700 bp for all the 22 isolates of *S. rolfsii* of groundnut. Similar amplification was reported from the isolates collected from different hosts *viz*., soybean, chickpea, groundnut, sunflower and from different geographical locations (Nagamma *et al*., 2015).

Oxalic acid produced by *Sclerotium rolfsii* during the invasion of host tissue during pathogenesis acts synergistically with endo-polygalacturonase, lowering the pH of the infected tissues to a level optimal for the activity of this enzyme. Oxalic acid was the principal toxic agent produced in the culture filtrates of *S. rolfsii* and it was responsible for the death of host tissues. The calcium present in structural pectates can be strongly chelated by oxalic acid. As a consequence, plant tissues are rendered more susceptible to invasion by the pathogen (Paramasivan *et al*., 2013). With respect to oxalic acid production, notable variation was detected across the isolates. Maximum oxalic acid production of 2.23 mg/ml was observed in KADh 6 isolate followed by 2.20 mg/ml in KADh 7 isolate, 2.18 mg/ml in KABe 11 isolate, 2.13 mg/ml in KADh 5 isolate and 2.11 mg/ml in KAHa 13 isolate which were on par with each other. Significantly least oxalic acid production (1.15 mg/ml) was observed in AMJo 4 isolate and all the remaining isolates were intermediate to the oxalic acid production.

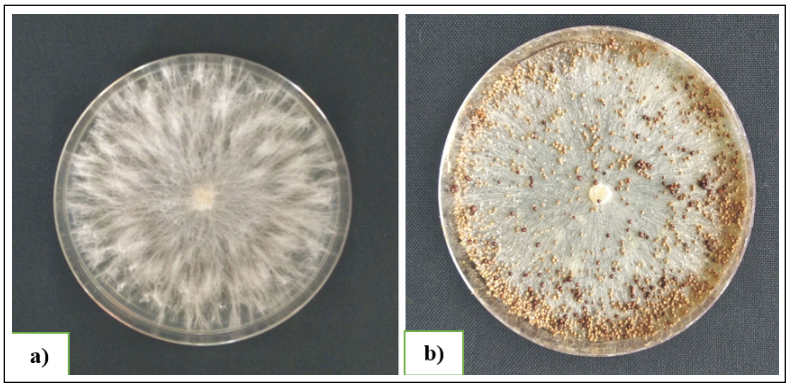
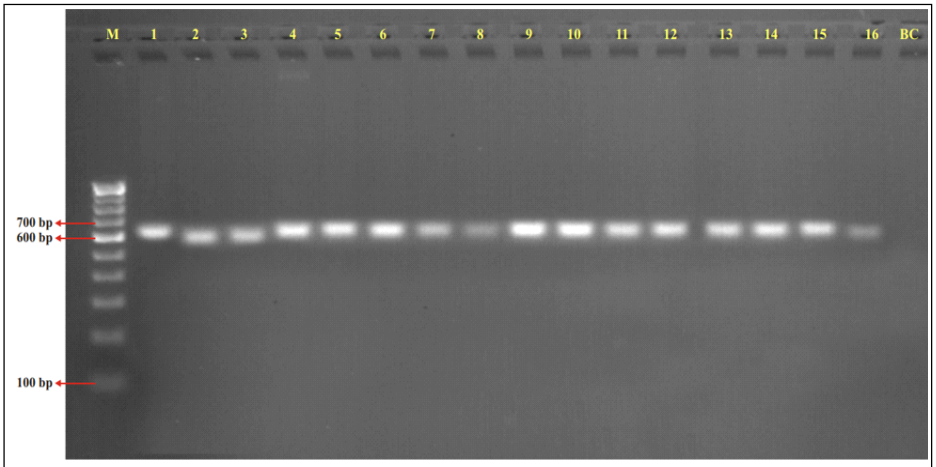


Plate 1: Mycelial (a) and sclerotial stage (b) of *Sclerotium rolfsii* on potato dextrose agar.



Lane M is the 1kb DNA marker, BC is the buffer control (no template control), 1-16 = MPSe 1, MPSe 2, MHSa 3, AMJo 4, KADh 5, KADh 6, KADh 7, KADh 8, KABe 9, KABe 10, KABe 11, KABa 12, KAHa 13, KADh 14, KABe 15 and KABi 16 respectively.

Plate 2: Gel electrophoresis for PCR product of ITS1/4 regions.

The maximum oxalic acid production was observed in the isolates which were producing small sized and dark brown sclerotial bodies and *vice versa*. This oxalic acid is considered as one of the key pathogenicity factors in *Sclerotium rolfsii* which have resulted in suppression of host and increased virulence (Table 2).

Similar findings were reported by Gawande *et al.* (2013) where in oxalic acid production in culture filtrate of *Sclerotium rolfsii* infecting different crops varied from 1.04 to 2.87 mg/ml. Ferrar and Walker (1993) reported that oxalic acid production enhances the success of the pathogen by decreasing the defense mechanisms of host. Ansari and Agnihotri (2000) observed the positive correlation between oxalic acid production and virulence of the isolates. Biochemical variability of 12 isolates (Six isolates which were collected from different parts of Karnataka and six from All India Co-ordinated Research on soybean centres of India) of *S. rolfsii* infecting soybean was studied where the highest oxalic acid production was in Dharwad isolate followed by Bagalkot isolate (Sr BGT) whereas the least production of oxalic acid was in Kota isolate (Sr KTA) (Prabhu and Patil, 2005). Among the four isolates of *Sclerotium delphinii* isolated from jackfruit, Chafa, Khirni and plants of western Maharashtra region, the highest oxalic acid production was observed in Chafa isolate (8.86 mg/g of mycelial mat) whereas the least production was from jackfruit isolate (4.94 mg/g of mycelial mat) (Gawande *et al*., 2014). The genetic diversity studies of 20 *S. rolfsii* isolates with 29 RAPD markers revealed the amplification size of the products between 200-2000 bp. The similarity coefficients values ranged from 0.15 to 0.72 and the UPGMA dendrogram grouped the isolates into two major clusters where the maximum number of isolates (18) were grouped in cluster I and cluster II has two isolates (Srividya *et al*., 2022). The molecular analysis of ten isolates from the current investigation shared more than 97 per cent nucleotide identity with A. rolfsii-HS-1(MN244450, MN243786), which infects the *Cynanchum stauntonii* crop. phylogenetic analysis the current isolates were shown to belong to two separate clades of *Athelia rolfsii*. SrD1 was assigned to clade 1, while the remaining nine isolates (SrB1, SrB2, SrC1, SrC2, SrD2, SrP1, SrP2, SrY1 and SrY2) were assigned to clade 2 (Hawaldar *et al*., 2021). Ten isolates of *S. rolfsii* collected from groundnut grown areas were investigated for their genetic variability study through ITS amplification that produced rDNA amplicon size of 650-700 bp which confirmed that all isolates belong to *Sclerotium rolfsii*. RAPD primers confirmed genetic variation among the isolates. Dendrogram and cluster analysis cleared that Group I was more genetically diverse among the isolates (Meena *et al*., 2023). Paramasivan *et al*. (2009) reported that a wide diversity among fungal groups can occur within a limited area, within a host or in geographically isolated regions. Hence, studying the morphological and genomic background of isolates promotes clear understanding of the ecology and pathogenicity aspects of *S. rolfsii*.

Table 1: Isolates collected from collar rot infected samples from different soybean growing areas of India and their sequence information.

State	Location	Isolate name	Accession no.	Hit results	Host	% identity
Madhya Pradesh	Sehore	MPSe 1	ON972520	*Athelia rolfsii* Clone FJSR1	*Stephania tetrandra*	94.00
Madhya Pradesh	Sehore	MPSe 2	ON629754	*Athelia rolfsii* isolate SR1	Brinjal	94.00
Maharashthra	Sangli	MHSa 3	KU885934	*Athelia rolfsii* isolate BLH-1	*Macleaya cordata*	84.00
Assam	Jorhat	AMJo 4	MN191516	*Athelia rolfsii* isolate kanpur	Chickpea	92.00
Karnataka	MARS, Dharwad (Dharwad)	KADh 5	MK926449	*Athelia rolfsii* isolate MGB17	*Vigna unguiculata*	93.09
Karnataka	Naredra (Dharwad)	KADh 6	MN191518	*Athelia rolfsii* isolate Gadag	Chickpea	87.32
Karnataka	Garag (Dharwad)	KADh 7	MT129499	*Athelia rolfsii* isolate Mudhol	Wheat	96.20
Karnataka	Kalaghatagi (Dharwad)	KADh 8	KT750883	*Athelia rolfsii* strain FP15	*Canavalia ensiformis*	93.72
Karnataka	Kittur (Belagavi)	KABe 9	KY678444	*Athelia rolfsii* isolate BZBJ 1	*Atractylodes macrocephala*	97.00
Karnataka	Chikkodi (Belagavi)	KABe 10	MW288292	*Athelia rolfsii* isolate Kb	*Sarcandra glabra*	82.00
Karnataka	Ugar Khurd (Belagavi)	KABe 11	OK017870	*Athelia rolfsii* strain LH2	Groundnut	82.39
Karnataka	Bagalkote	KABa 12	MN191517	*Athelia rolfsii* isolate Bagalkot	Chickpea	92.21
Karnataka	Haveri	KAHa 13	OK017873	*Athelia rolfsii* strain LY1	*Macleaya cordata*	88.00
Karnataka	MARS, Dharwad (Dharwad)	KADh 14	MT023434	*Athelia rolfsii* isolate Ar2019	*Valeriana officinalis*	93.00
Karnataka	Hirebagewadi (Belagavi)	KABe 15	OK017893	*Athelia rolfsii* strain RH4	Groundnut	87.00
Karnataka	Bidar	KABi 16	MN191518	*Athelia rolfsii* isolate Bijapur	Chickpea	91.00

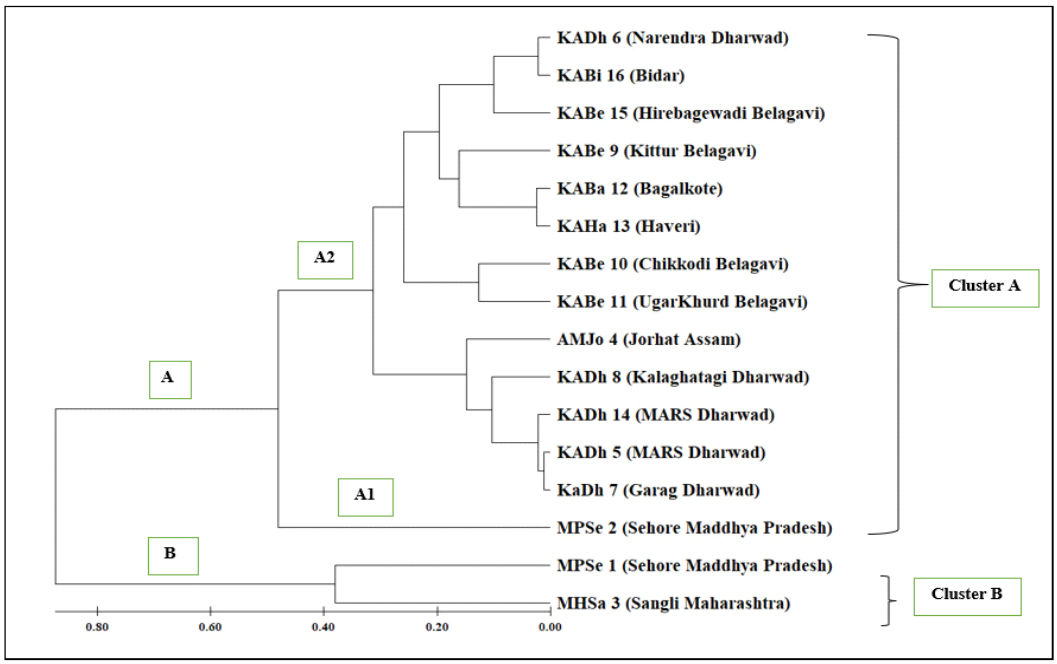


Fig 1: UPGMA phylogenetic tree based on ITS rDNA sequences of different isolates of *Sclerotium rolfsii* Sacc.

Table 2: Oxalic acid production by isolates of *Sclerotium rolfsii* Sacc.

State	Location	Isolate name	Oxalic acid (mg/ml)
Madhya Pradesh	Sehore	MPSe 1	1.59
Madhya Pradesh	Sehore	MPSe 2	1.38
Maharashthra	Sangli	MHSa 3	1.42
Assam	Jorhat	AMJo 4	1.15
Karnataka	MARS, Dharwad (Dharwad)	KADh 5	2.13
Karnataka	Naredra (Dharwad)	KADh 6	2.23
Karnataka	Garag (Dharwad)	KADh 7	2.20
Karnataka	Kalaghatagi (Dharwad)	KADh 8	1.76
Karnataka	Kittur (Belagavi)	KABe 9	2.01
Karnataka	Chikkodi (Belagavi)	KABe 10	1.92
Karnataka	Ugar Khurd (Belagavi)	KABe 11	2.18
Karnataka	Bagalkote	KABa 12	1.98
Karnataka	Haveri	KAHa 13	2.11
Karnataka	MARS, Dharwad (Dharwad)	KADh 14	1.82
Karnataka	Hirebagewadi (Belagavi)	KABe 15	1.78
Karnataka	Bidar	KABi 16	1.90
	S.Em. ±		0.08
	C.D. @ 1%		0.28

CONCLUSION

Soybean [*Glycine max* (L.) Merrill] is an important pulse and oil seed crop grown in India which is affected by many soil-borne fungi of which *Sclerotium rolfsii* Sacc. is one of the destructive pathogens. In this study, we could report the variability with respect to molecular and biochemical level among the isolates where the isolates formed two different clusters in the phylogenetic analysis. To date this is the first report of understanding molecular variability of *Sclerotium rolfsii* infecting soybean in India.

Conflict of interest

All authors declare that they have no conflict of interest.

REFERENCES

Agrawal, S.C. and Kotasthane, S.R. (1971). Resistance in some of the soybean varieties against *Sclerotium rolfsii* Sacc. Indian Phytopathology. 24: 401-403.

Anonymous, (2022). Annual Report, 2021-22, All India Coordinated Research Project on Soybean. ICAR - IISR, Indore. pp. 2-3.

Ansari, M.M. and Agnihotri, S.K. (2000). Morphological, physiological and pathological variations among *Sclerotium rolfsii* isolates of soybean, Indian Phytopathology. 53: 65-67.

Brunda, K.S. (2018). Studies on Fungal and Bacterial Endophytes of Soybean [*Glycine max* (L.) Merrill] Against the Soilborne Pathogens M. Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, Karnataka, India.

Fehr, W.R. (1989). Soybean. Oil crops of the world: Their breeding and utilization. Mc Graw Hill Publishing Co., New York, 283p.

Ferrar, P.H. and Walker, J.R.L. (1993). O-Diphenol oxidase inhibition an additional role of oxalic acid in the phytopathogenic arsenals of *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. Physiological and Molecular Plant Pathology. 43: 415-422.

Gawande, S.P., Borkar, S.G. and Chimote, V.P. (2013). Variation in growth and oxalic acid production by different crop isolates of *Sclerotium rolfsii* Sacc. Journal of Mycopathological Research. 51(1): 95-100.

Gawande, S.P., Borkar, S.G., Chimote, V.P., Nagrale, D.T. and Sharma, A.K. (2014). Determination of morphometric, biochemical and genetic variation in *Sclerotium delphinii* isolates. African Journal of Microbiological Research. 8(16): 1696-1703.

Hartman, G.L., Sinclair, J.B. and Rupe, J.C. (1999). Compendium of soybean diseases. American Phytopathological Society, St. Paul.

Hawaldar, S., Nandan, M., Vinaykumar, H.D., Hadimani H.D., Hiremath, S., Venkataravanappa, V., Jahir Basha, C.R., Lakshminarayana Reddy, C.N. (2021). Morphological and molecular characterization of *Sclerotium rolfsii* associated with stem rot disease of groundnut (*Arachis hypogaea* L.). Indian Phytopathology. https://doi.org/10.1007/s42360-021-00419-y.

Jebaraj, D.M., Aiyanathan, K.E.A. and Nakkeeran, S. (2017). Virulence and genetic diversity of *Sclerotium rolfsii* Sacc., infecting groundnut using nuclear (RAPD and ISSR) markers. Journal of Environmental Biology. 38: 147-159.

Kirtzman, G., Chet, I. and Henis, Y. (1977). The role of oxalic acid in the pathogenic behaviour of *Sclerotium rolfsii* Sacc. Experimental Mycology. 1: 280-285.

Mahadevan, A. and Sridhar, R. (1986). Methods in Physiological Plant Pathology, 3rd Ed. Sivakami Publications, Madras, pp. 182.

Meena, P.N., Meena, A.K. and Ram, C. (2023). Morphological and molecular diversity in *Sclerotium rolfsii* Sacc., infecting groundnut (*Arachis hypogaea* L.). Discover Agriculture. 1:3. https://doi.org/10.1007/s44279-023-00003-0.

Murray, M.G. and Thompson, W.F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research. 84: 321-325.

Nagamma, G., Nagaraja, A., Syeda, S.A. and Patil, S. (2015). Molecular variability of *Sclerotium rolfsii* causing foot rot diseases of major field crops of Karnataka. Journal of Pure and Applied Microbiology. 9(4): 3225-3230.

Paramsivan, M., Mohan, S., Muthukrishnan, N. and Chandrasekaran, A. (2013). Degradation of oxalic acid (OA) producing *Sclerotium rolfsii* (Sacc.) by organic biocides. Archives of Phytopathology and Plant Protection. 46(3): 357-363.

Paramasivan, M., Mathiyazhagan, S., Mohan, S., Ali, G.S. and Karthikeyan, M. (2009). Molecular variability of *S. rolfsii* in tropical sugar beet based on restriction fragment length polymorphism (RFLP) of ITS region of ribosomal DNA. Archives in Phytopathology and Plant Protection. 42: 327-333.

Prabhu, H.V. (2003). Studies on collar rot of soybean caused by *Sclerotium rolfsii* Sacc. M. Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, Karnataka, India.

Prabhu, H.V. and Patil, P.V. (2005). Morphological, biochemical and pathogenic variation among *Sclerotium rolfsii* isolates of soybean. Karnataka Journal of Agricultural Sciences. 18(4): 990-994.

Punja, Z.K. (1985). The biology, ecology and control of *Sclerotium rolfsii*. Annual Review of Phytopathology. 23: 97:127.

Sangeeta, N. (2011). Variability and Management of Collar Rot Chickpea Caused by *Sclerotium rolfsii* Sacc. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, Karnataka, India.

Sinclair, J.B. (1978). The seed borne nature of some soybean pathogens. The effect of phomopests and Bacillus subtilis on germination and their occurrence in soybean produced in Illinois. Seed Science and Technology. 6: 957-964.

Srividya, P.V., Ahamed, M.L., Ramana, J.V. and Ahammed, S.K. (2022). Studies on diversity of *Sclerotium rolfsii* causing collar rot in chickpea using morphological and molecular markers. Legume Research: An International Journal. 45(1): 82-89. doi: 10.18805/LR-4199.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 30: 2725-2729.

Vivekanand, B.K. (2020). Studies on Mechanisms of Endophytes in Suppressing Seed and Soil Borne Diseases of Soybean. M. Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, Karnataka, India.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In: PCR Protocols: A Guide to Methods and Applications. [Innis M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. (eds.)], Academic Press, San Diego. pp. 315-322.