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Morpho-pathogenic and Molecular Variability of Chickpea Collar Rot in Bundelkhand Region of Uttar Pradesh, India

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ABSTRACT

Background: Collar rot of chickpea infected by *Sclerotium rolfsii* is considered one of the major biotic factors for low productivity in chickpea. The present study was conducted to determine twenty nine isolates of collar rot were characterized for their Morphological, Pathogenic and Molecular variability.

Methods: Twenty nine collar rot infected chickpea plant were collected from different places of Bundelkhand region of Uttar Pradesh. Pathogen was isolated from collar rot infected sample using PDA media followed by morphological, pathogenic and molecular variability studies from all isolates. Type of growth rate, mycelia colour, sclerotia size, sclerotial pattern, test weight of isolates was recorded by observing cultural plate after complete growth of mycelium. Pathogenicity test were done to check the aggressiveness of isolates in three varieties of chickpea. The isolated fungal DNA was amplified with specific primer pair, the quality and quantity of DNA was checked using gel electrophoresis and UV trasnliminator.

Result: A morphological evaluation of isolates showed notable differences in identification characteristics, including colony size and sclerotial properties like count, test weight, dimensions and form on PDA media. Among the all isolates SRC 25 and SRC 28 considered as most virulent causes highest disease incidence in all selected chickpea variety under greenhouse experiments. RAPD analysis revealed distinct banding patterns, with PIC values between 0.59 and 0.89. The highest genetic similarity was observed between SRC-11 and SRC-29.

Key words: Chickpea, Collar rot, Molecular variability, Morphology, Pathogenicity.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the primary pulse crop cultivated in India. India is about 75 per cent of the world's chickpea production. In Bundelkhand region of Uttar Pradesh chickpea is the predominant pulse crop. (Kumar *et al*., 2025). The crop production in this region limited due to various biotic restrictions. Collar rot is one of the most severe disease of chickpea. It is a well known polyphagous pathogen. The fungus was first reported by Rolfs (1892) as a cause of tomato blight in Florida. Fungi in the genus Sclerotium form sclerotia and sterile mycelia but no spores (Saccardo, 1899) and include more than 40 plant pathogenic species (Farr, 2008). Although there are several geographical variability among *S. rolfsii* populations was demonstrated by earlier workers (Punja, 1985; Sarma *et al*., 2002; Remesal *et al*., 2013; Le *et al*., 2012) and contemporary studies that there is shift of paradigm in the pathogen's prevalence due to climatic changes therefore major yield loss (Rajalaxmi, 2020; Sood *et al*., 2020). Collar rot is a fast-spreading and severe chickpea disease. *S. rolfsii* have become more important in recent years due to drastic climate change which makes the pathogen more aggressive and increased with adaptability to the environment (Ghatak and Ansar, 2017; Kumar *et al*., 2017; Savary *et al*., 2011). Traditional methods on only morphological characters based that is not sufficient for the differentiation of the specimens.

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Consequently, the current research was conducted to evaluate the diversity of *S. rolfsii* isolates through morphological and molecular.

MATERIALS AND METHODS

Study area

The present investigation entitled" Morpho-Pathogenic and molecular Variability of Chickpea collar rot in Buldelkhand region of Uttar Pradesh, India" was carried out in the Department of Plant Pathology's laboratory and performed in the greenhouse, College of Agriculture, BUAT, Banda (U.P.) during 2020-23.

Isolation and purification of pathogen

Twenty Nine isolates of *S. rolfsii* addressing chickpea growing districts of Bundelkhand Region of U.P. was collected, isolated by using PDA in BUAT, Banda, Department of Pathology Laboratory. The fungal cultures were purified by a single hyphal tip from a pathogen culture, which are then transferred on PDA slants or PDA plates. The PDA plates were incubated at 27±1°C three days for growth of test fungus.

Morphological variability

Twenty-nine isolates of *S. rolfsii* from different regions of Bundelkhand, Uttar Pradesh, were examined for morphological variation. Five-mm culture discs were positioned centrally on petri dishes with three repetitions for each isolate and incubated at 27±1°C for 15 days. Colony diameter was measured daily for three days, taken at right angles. Mycelial and sclerotial characteristics were noted on the 7th and 15th day, respectively. Replicate data were gathered and statistically examined to evaluate morphological differences between isolates.

Pathogenic variations in *Sclerotium rolfsii*

Pathogenic variability of 29 isolates of *S. rolfsii* was evaluated under net house conditions at BUAT, Banda through a pot experiment complete randomized design(CRD). Sterilized soil was combined with sorghum grain-based inoculum @ 25 g/kg soil. Chickpea seeds of three varieties that were surface-sterilized were planted in inoculated pots, while non-inoculated pots served as controls. Every treatment was replicated three times and the pots were arranged in a random manner. Sterilized water ensured sufficient hydration. Data on seedling emergence and incidence per cent were receorded 10-15 days after sowing and final plant stand was counted after 60 days of sowing.

$$\text{Per cent collar rot incidence} = \frac{\text{Infected plants}}{\text{Total plant}} \times 100$$

Molecular diversity

Fungal DNA isolation and quantification

Isolates of Sclerotium rolfsii were grown in 2% potato dextrose broth for 12 days at 26±1°C. A modified CTAB method was employed to extract DNA from the fungal mycelial mats. Mycelia were crushed using liquid nitrogen and combined with prewarmed 2X CTAB buffer (containing CTAB, NaCl, EDTA, Tris-HCl and β-mercaptoethanol). The extract was cleaned using chloroform-isoamyl alcohol and spun at 10,000 rpm for 5 minutes. DNA was precipitated with cold isopropanol, kept on ice and then centrifuged once more. The pellet underwent a wash with 70% ethanol, was air-dried and then dissolved in TE buffer. DNA quality and quantity were evaluated using gel electrophoresis and a UV transilluminator. The quality and quantity of DNA was checked using gel electrophoresis and UV trasnliminator. In Table 1 show the oligonucleotide primer sequence of RAPD primers.

RESULTS AND DISCUSSION

A roving survey was conducted during *Rabi* season 2021-22 and 2022-23 for occurrence and distribution of collar rot of chickpea in seven chickpea growing district of Bundelkhand region of U.P. The severe form of incidence is mainly due to monocropping and sowing time when high moisture conditions prevail because of the rainy days. Singh *et al.* (2019) also reported the incidence of chickpea diseases in all seven districts of Bundelkhand region.

Morphological variability

Mycelial characters

Data in Table 2 show Morphological and cultural characters of 29 isolates significant differences among the isolates for total growth and growth rate on PDA. The average colony diameter varied from 40.00 mm (SRC29) to 90.00 mm (SRC5, SRC25, SRC28), indicating substantial differences among the isolates. Among the isolates, 10 showed rapid growth, 3 demonstrated slow growths and 16 displayed medium growth. Mycelial color ranged from very white to dull white, with very white being frequent among rapidly growing isolates. The mycelial growth distribution also differed: 10 isolates exhibited fluffy growth, 13 displayed dense growth and 6 were sparse. Interestingly, fluffy growth

Table 1: Sequence of oligonucleotide primers used in RAPD analysis to check molecular variability among the *S. rolfsii* isolates.

Primer name	Sequence (5'-3')
OPA-11	CAATCGCCGT
OPA-19	CAAACGTCGG
OPB-18	CCACAGCAGT
OPB-19	ACCCCCGAAG
OPB-20	GGACCCTTAC
OPS-12	CTGGGTGAGT
OPY-01	GTGGCATCTC
OPY-13	GGGTCTCGGT
OPR-14	CAGGATTCCC
OPS-12	CTGGGTGAGT

was frequently linked to rapidly growing isolates. The identified morphological variability, with a coefficient of variation (C.V.) of 2.17% and a critical difference (C.D.) of 2.42 at p=0.05, emphasizes the varied phenotypic characteristics of the fungal isolates among regions. Similar, reports were given by Srividya *et al.* (2018); Manu *et al.* (2018). Okereke and Wokocha (2007) observed the variation in colony diameter.

Sclerotial characters

Out of twenty-nine isolates of *S. rolfsii* obtained from various chickpea cultivation areas showed considerable sclerotial diversity when grown on potato dextrose agar (PDA) medium. Sclerotia yield differed considerably among isolates, spanning from 108.33 (SRC22) to 1005.33 (SRC2) per plate. The weight of sclerotia varied from 66.33 mg (SRC10, SRC20) to 538.33 mg (SRC2) and the diameter of sclerotia ranged from 0.93 mm (SRC5) to 1.59 mm (SRC2). The majority of isolates formed brown to light brown Sclerotia. The distribution was generally uniform, with only a few isolates exhibiting peripheral (SRC1, SRC14) and concentric patterns (SRC15). C.D. values at p=0.05 and low coefficients of variation confirmed notable differences (Table 3). The variability in number of sclerotia produced among the isolates of *S. rolfsii* collected from different location and hosts was also recorded in earlier reports (Sekhar *et al*., 2017; Manu *et al*., 2018). Palaiah and Adiver (2004) also recorded the similar variation in sclerotial test weight of various isolates of *S. rolfsii.*

Pathogenic variability

Data in Table 4 show a notable difference in disease occurrence was noted among the 29 *S. rolfsii* isolates

Table 2: Mycelial characters of *Sclerotium rolfsii* on PDA medium.

Isolates	Location	Mean colony diameter 72 hrs after incubationat 25+_1°C	Type of growth	Mycelium colour	Distribution of mycelial growth
SRC1	Barokharkhurd	81.33	Fast	Extrawhite	Fluffy
SRC2	Barokharkhurd	40.33	Slow	Extrawhite	Dense
SRC3	Barokharkhurd	50.00	Medium	Extrawhite	Fluffy
SRC4	Tindwari	60.33	Medium	Extrawhite	Dense
SRC5	Tindwari	90.00	Fast	White	Fluffy
SRC6	Naraini	86.33	Fast	White	Fluffy
SRC7	Muskara	89.33	Fast	Extrawhite	Fluffy
SRC8	Muskara	59.00	Medium	Dull white	Dense
SRC9	Kurara	85.00	Fast	White	Dense
SRC10	Kurara	60.00	Medium	Dull white	Dense
SRC11	Maudaha	63.33	Medium	Dull white	Sparse
SRC12	Maudaha	68.33	Medium	Extrawhite	Fluffy
SRC13	Balgaon	53.00	Medium	White	Dense
SRC14	Balgaon	62.00	Medium	Dull white	Dense
SRC15	Balgaon	66.00	Medium	Dull white	Dense
SRC16	Bamur	67.00	Medium	White	Dense
SRC17	Chirgaon	46.33	Slow	White	Dense
SRC18	Karwi	80.33	Fast	Dull white	Dense
SRC19	Karwi	53.33	Medium	Dull white	Dense
SRC20	Ramangar	89.33	Fast	White	Dense
SRC21	Mau	61.00	Medium	Dull white	Sparse
SRC22	Kabrai	84.00	Fast	White	Dense
SRC23	Kabrai	62.33	Medium	White	Dense
SRC24	Charkhari	50.66	Medium	Dull white	Sparse
SRC25	Jaitpur	90.00	Fast	White	Sparse
SRC26	Baar	60.00	Medium	Dull white	Sparse
SRC27	Baar	82.33	Fast	Extrawhite	Fluffy
SRC28	Jalaun	90.00	Fast	Dull white	Dense
SRC29	Kadora	40.00	Slow	Dull white	Sparse
C.D. (p=0.05)		2.42			
C.V. (%)		2.17			

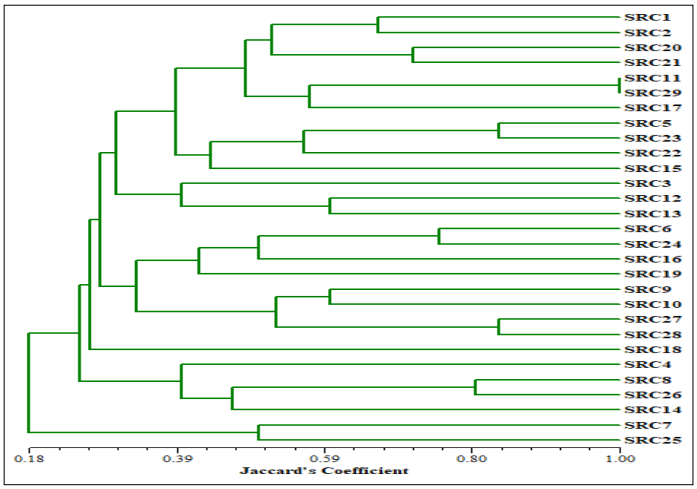

Fig 1: Cluster analysis of *S. rolfsii* isolates generated by UPGMA analysis of RAPD bands.

Table 3: Sclerotial characters of *Sclerotium rolfsii* on PDA medium.

Isolates	Location	No.fosclerotia per plate	Test wt (100 sclerotia)	Diameter (mm)	Colour	Shape	Arrangement
SRC1	Barokharkhurd	204.66	74.33	1.04	Brown	Oval	Peripheral
SRC2	Barokharkhurd	1005.33	538.33	1.59	Light brown	Oval	Peripheral
SRC3	Barokharkhurd	255.00	166.66	1.31	Brown	Spherical	Peripheral
SRC4	Tindwari	352.33	78.66	1.10	Light brown	Spherical	Scattered
SRC5	Tindwari	348.00	70.00	0.93	Brown	Irregular	Scattered
SRC6	Naraini	172.66	102.00	1.21	Light brown	Spherical	Scattered
SRC7	Muskara	263.33	84.33	0.98	Dark brown	Spherical	Scattered
SRC8	Muskara	256.66	145.00	1.23	Brown	Spherical	Scattered
SRC9	Kurara	316.66	145.00	1.40	Light brown	Irregular	Scattered
SRC10	Kurara	414.33	66.33	1.12	Light brown	Spherical	Upper petriplate
SRC11	Maudaha	374.33	113.00	1.40	Brown	Spherical	Scattered
SRC12	Maudaha	106.33	70.66	1.12	Brown	Spherical	Scattered
SRC13	Balgaon	372.00	105.33	1.23	Brown	Spherical	Scattered
SRC14	Balgaon	201.66	91.66	1.01	Light brown	Spherical	Peripheral
SRC15	Balgaon	187.33	85.00	1.16	Light brown	Spherical	Concentric ring
SRC16	Bamur	356.00	102.00	1.21	Dark brown	Oval	Scattered
SRC17	Chirgaon	198.33	94.00	1.17	Brown	Oval	Upper Petriplate
SRC18	Karwi	373.33	76.00	1.33	Dark brown	Spherical	Scattered
SRC19	Karwi	326.66	95.00	1.14	Brown	Irregular	Scattered
SRC20	Ramangar	394.00	66.33	1.01	Brown	Spherical	Scattered
SRC21	Mau	462.00	100.00	1.21	Brown	Spherical	Peripheral
SRC22	Kabrai	108.33	92.33	1.13	Dark brown	Spherical	Scattered
SRC23	Kabrai	275.00	90.66	1.16	Brown	Oval	Scattered
SRC24	Charkhari	404.00	81.66	1.06	Light brown	Spherical	Peripheral
SRC25	Jaitpur	131.33	128.33	1.41	Light brown	Spherical	Scattered
SRC26	Baar	371.33	101.00	1.54	Light brown	Spherical	Scattered
SRC27	Baar	141.00	82.66	1.25	Light brown	Spherical	Scattered
SRC28	Jalaun	425.00	73.66	1.06	Light brown	Spherical	Peripheral
SRC29	Kadora	347.33	77.66	1.33	Light brown	Spherical	Peripheral
C.D (p=0.05)		9.06	4.76	0.03			
C.V. (%)		1.75	2.63	1.99			

Table 4: Pathogenic variability of twenty nine isolates of collar rot on three chickpea variety.

Treatment	2022 incidence (%)			2023 incidence (%)			Pooled		
	JG14	JG16	L550	JG14	JG16	L550	JG14	JG16	L550
SRC1	74.33 (59.59)	88.89 (73.98)	66.66 (54.76)	71.00 (57.45)	83.33 (65.94)	61.11 (51.47)	72.67 (58.52)	86.11 (69.96)	63.89 (53.11)
SRC 2	44.44 (41.82)	44.44 (41.82)	41.66 (40.17)	41.66 (40.17)	41.66 (40.17)	44.44 (41.82)	43.05 (41.00)	43.05 (41.00)	43.05 (41.00)
SRC 3	61.22 (51.69)	52.77 (46.62)	44.44 (41.82)	66.66 (54.76)	50.00 (45.02)	44.44 (41.82)	63.94 (53.22)	51.39 (45.82)	44.44 (41.82)
SRC 4	63.88 (53.11)	63.88 (53.11)	50.00 (45.02)	61.11 (51.51)	66.66 (54.76)	49.00 (44.45)	62.50 (52.31)	65.27 (53.94)	49.50 (44.74)
SRC 5	83.33 (65.94)	83.33 (65.94)	100.00 (90.05)	80.55 (63.97)	80.55 (63.97)	100.00 (90.05)	81.94 (64.95)	81.94 (64.95)	100.00 (90.05)
SRC 6	75.00 (60.24)	83.33 (65.94)	80.55 (63.97)	77.77 (62.21)	86.11 (72.01)	83.33 (75.04)	76.39 (61.23)	84.72 (68.97)	81.95 (69.50)
SRC 7	66.66 (55.40)	69.44 (56.52)	72.22 (58.49)	61.11 (51.62)	66.66 (54.76)	77.77 (66.52)	63.89 (53.51)	68.05 (55.64)	75.00 (62.50)
SRC 8	41.66 (40.17)	50.00 (45.02)	58.33 (50.02)	44.44 (41.82)	55.55 (48.27)	55.00 (47.89)	43.05 (41.00)	52.78 (46.64)	56.67 (48.96)
SRC 9	77.77 (62.21)	75.00 (60.03)	83.33 (65.94)	75.00 (60.24)	75.00 (60.03)	86.11 (72.01)	76.39 (61.23)	75.00 (60.03)	84.72 (68.97)
SRC 10	74.33 (59.59)	77.77 (62.21)	66.66 (54.76)	77.78 (62.00)	77.77 (62.21)	69.44 (56.84)	76.06 (60.80)	77.78 (62.21)	68.05 (55.80)
SRC 11	58.33 (50.02)	50.00 (45.02)	55.55 (48.27)	61.11 (51.51)	50.00 (45.02)	58.33 (50.02)	59.72 (50.77)	50.00 (45.02)	56.94 (49.14)
SRC 12	75.00 (60.03)	77.77 (62.21)	72.22 (58.49)	77.77 (66.52)	72.22 (58.49)	77.77 (66.52)	76.39 (63.27)	75.00 (60.35)	75.00 (62.50)
SRC 13	50.00 (45.02)	55.55 (48.27)	69.44 (56.52)	55.00 (47.89)	61.11 (51.51)	72.22 (58.49)	52.50 (46.46)	58.33 (49.89)	70.83 (57.50)
SRC 14	50.00 (45.02)	41.66 (40.17)	55.55 (48.27)	55.55 (48.27)	41.66 (40.17)	61.11 (51.51)	52.78 (46.64)	41.66 (40.17)	58.33 (49.89)
SRC 15	72.22 (58.49)	77.77 (62.21)	77.77 (66.52)	75.00 (60.24)	83.33 (65.94)	77.77 (66.52)	73.61 (59.36)	80.56 (66.23)	77.77 (66.52)
SRC 16	83.33 (65.94)	77.77 (62.21)	77.77 (62.21)	80.55 (63.97)	72.22 (58.49)	77.77 (66.21)	81.94 (64.95)	75.00 (60.35)	77.77 (62.21)
SRC 17	44.44 (41.82)	41.66 (40.17)	45.00 (42.15)	50.00 (45.02)	47.22 (43.42)	49.00 (44.45)	47.22 (43.42)	44.44 (41.80)	47.00 (43.30)
SRC 18	83.33 (65.94)	83.33 (65.94)	100.00 (90.05)	80.55 (63.97)	83.33 (65.94)	94.44 (82.01)	81.94 (64.95)	83.33 (65.94)	97.22 (86.03)
SRC 19	41.66 (40.17)	44.44 (41.82)	40.00 (39.25)	44.44 (41.82)	50.00 (45.02)	44.44 (41.82)	43.05 (41.00)	47.22 (43.42)	42.22 (40.54)
SRC 20	88.89 (73.98)	83.33 (65.94)	100.00 (90.05)	94.44 (82.01)	88.89 (73.98)	100.00 (90.05)	91.67 (77.99)	86.11 (69.96)	100.00 (90.05)
SRC 21	75.00 (60.24)	75.00 (60.03)	66.66 (54.76)	77.77 (66.52)	77.77 (62.21)	63.88 (53.11)	76.39 (63.38)	76.39 (61.12)	65.27 (53.94)
SRC 22	100.00 (90.05)	94.44 (82.01)	100.00 (90.05)	100.00 (90.05)	88.89 (73.98)	94.44 (82.01)	100.00 (90.05)	91.67 (77.99)	97.22 (86.03)
SRC 23	77.77 (62.21)	75.00 (60.24)	72.22 (58.49)	80.55 (63.97)	72.22 (58.49)	72.22 (58.49)	79.17 (63.09)	73.61 (59.36)	72.22 (58.49)
SRC 24	41.66 (40.17)	45.00 (42.15)	33.33 (35.28)	44.44 (41.82)	44.44 (41.82)	33.33 (35.28)	43.05 (41.00)	44.72 (41.99)	33.33 (35.28)
SRC 25	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	94.44 (82.01)	100.00 (90.05)	94.44 (82.01)	97.22 (86.03)	100.00 (90.05)	97.22 (86.03)
SRC 26	41.66 (40.17)	33.33 (35.28)	33.33 (35.28)	40.00 (39.25)	40.00 (39.25)	33.33 (35.28)	40.83 (39.72)	36.67 (37.27)	33.33 (35.28)
SRC 27	100.00 (90.05)	94.44 (82.01)	100.00 (90.05)	100.00 (90.05)	88.89 (73.98)	100.00 (90.05)	100.00 (90.05)	91.67(77.99)	100.00 (90.05)
SRC 28	100.00 (90.05)	94.44 (82.01)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	100.00 (90.05)	97.22 (86.03)	100.00 (90.05)
SRC 29	33.33 (35.28)	41.66 (40.17)	33.33 (35.28)	40.00 (39.25)	33.33 (35.28)	33.33 (35.28)	36.67 (37.27)	37.50 (37.73)	33.33 (35.28)
Control	7.41 (12.99)	9.26 (14.53)	5.55 (8.03)	9.26 (14.53)	9.26 (14.53)	5.55 (8.03)	8.34 (14.76)	9.26 (14.53)	5.55 (8.03)
Mean	66.22 (56.92)	66.16 (56.60)	66.72 (58.47)	67.27 (57.82)	66.27 (56.49)	67.13 (59.04)	66.75 (57.37)	66.22 (56.54)	66.93 (58.75)
C.D. at 5%	6.25 (4.58)	6.05 (6.10)	5.65 (4.69)	6.66 (6.27)	5.65 (5.51)	8.61 (8.55)	4.71 (3.73)	3.61 (3.63)	5.50 (5.51)
C.V. (%)	11.55 (9.85)	11.20 (13.21)	10.37 (9.82)	12.13 (13.28)	10.45 (11.95)	15.70 (17.73)	8.65 (7.97)	6.68 (7.86)	10.06 (11.49)
SE(m)	12.53	12.13	11.33	13.36	11.34	17.26	9.45	7.24	11.03
SE(d)	4.42	4.28	3.99	4.71	4.00	6.09	3.33	2.55	3.89

*Value given in arcsin A transformation.

Table 5: Results of RAPD primers used for *S. rolfsii* isolates.

Primer name	Sequence (5'-3')	Total no. of bands amplified	Number of monomorphic bands	Number of polymorphic bands	Polymorphic bands (%)	Polymorphic index content (PIC)
OPA-11	CAATCGCCGT	7	5	3	35.98	0.59
OPA-19	CAAACGTCGG	8	4	5	54.04	0.71
OPB-18	CCACAGCAGT	5	3	4	55.62	0.77
OPB-19	ACCCCCGAAG	8	5	4	42.92	0.89
OPB-20	GGACCCTTAC	7	3	5	60.98	0.74
OPS-12	CTGGGTGAGT	8	3	6	65.15	0.78
OPY-01	GTGGCATCTC	7	3	5	60.98	0.61
OPY-13	GGGTCTCGGT	6	2	5	69.91	0.75
OPR-14	CAGGATTCCC	7	2	6	63.48	0.83
OPS-12	CTGGGTGAGT	9	3	6	65.15	0.78

across three chickpea varieties in the 2022 and 2023 growing seasons. Data collected over two years showed considerable differences in the occurrence of *S. rolfsii* across 29 isolates on chickpea varieties JG14, JG16 and L550. Disease incidence varied between 66.22% (JG14) and 66.93% (L550), with L550 exhibiting marginally greater vulnerability. Mostvirulent isolates such as SRC25, SRC27 and SRC28 resulted in 97.22-100% incidence, whereas those with lower virulence exhibited 33.33-44.72%. Control treatments exhibited low levels of infection (5.55-9.26%). Notable differences (CD at p=0.05) and low CV values (6.68-15.70%) validated the dependability of findings. Similar pathogenic variability has also been reported by Kumari and Ghatak (2018). Sennoi *et al*. (2010) evaluated pathogenecity test of ten *S. rolfsii* and found most of isolates were aggressive nature.

Molecular variability of *S. rolfsii* Isolates by RAPD

Data in Table 5 show ten random RAPD primers were selected to study the genetic diversity among the 29 isolates of *S. rolfsii*. RAPD primers employed for detecting genetic diversity produced clear and reproducible banding patterns. These primers generated 357 amplified bands which ranged from 100 to 3,600 bp. The total number of polymorphic bands was 49. The per cent polymorphism ranged from 69.91 (OPY-13) to 35.98 (OPA-11) percent. The minimum size of 300 base pairs was generated from OPA-19 and OPY-01 while maximum size 3600 base pairs were generated with primer OPS-12. The primers OPB-18, OPB-20, OPS-12, OPY-01, OPY-13, OPY-14 and OPS-12 and OPB-19 were found to be most informative based on the level of polymorphism detected by them.

Data in Table 6 show Genetic similarities were analyzed through the data obtained on the basis of 10 RAPD primers from the 29 isolates of the *S. rolfsii*. The genetic similarity between *S. rolfsii* isolates exhibited different levels, with Jaccard's similarity coefficients varying from 0.05 to 1.0. The greatest similarity was noted between genotypes SRC-11 and SRC-29, suggesting a close genetic relationship. Conversely, the minimum similarity was noted between SRC-5 and SRC-25, SRC-13 and SRC-7, as well as SRC-18 with SRC-8 and SRC-25. A dendrogram showed (Fig 1) that all isolates, apart from SRC-18, grouped into a larger cluster, while SRC-11 and SRC-29 created a separate pair. Prasad *et al*. (2010) also reported similar findings in their studies involving *S. rolfsii*. 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 6: Similarity index of different isolates of *S. rolfsii*.

	SRC 1	SRC 2	SRC 3	SRC 4	SRC 5	SRC 6	SRC 7	SRC 8	SRC 9	SRC 10	SRC 11	SRC 12	SRC 13	SRC 14	SRC 15	SRC 16	SRC 17	SRC 18	SRC 19	SRC 20	SRC 21	SRC 22	SRC 23	SRC 24	SRC 25	SRC 26	SRC 27	SRC 28	SRC 29
SRC1	1.00																												
SRC2	0.67	1.00																											
SRC3	0.29	0.38	1.00																										
SRC4	0.43	0.50	0.22	1.00																									
SRC5	0.50	0.57	0.11	0.22	1.00																								
SRC6	0.38	0.30	0.20	0.18	0.33	1.00																							
SRC7	0.14	0.11	0.13	0.11	0.13	0.22	1.00																						
SRC8	0.33	0.43	0.29	0.43	0.13	0.22	0.14	1.00																					
SRC9	0.33	0.43	0.29	0.43	0.13	0.38	0.14	0.33	1.00																				
SRC10	0.33	0.25	0.13	0.25	0.13	0.38	0.14	0.14	0.60	1.00																			
SRC11	0.50	0.57	0.43	0.38	0.25	0.33	0.13	0.29	0.50	0.29	1.00																		
SRC12	0.33	0.43	0.50	0.25	0.13	0.22	0.14	0.33	0.33	0.14	0.50	1.00																	
SRC13	0.33	0.43	0.29	0.25	0.29	0.22	0.00	0.14	0.33	0.14	0.50	0.60	1.00																
SRC14	0.29	0.38	0.25	0.38	0.11	0.20	0.13	0.50	0.50	0.29	0.43	0.29	0.13	1.00															
SRC15	0.50	0.38	0.25	0.22	0.43	0.33	0.29	0.29	0.13	0.13	0.43	0.29	0.13	0.43	1.00														
SRC16	0.50	0.38	0.25	0.38	0.25	0.50	0.13	0.29	0.29	0.29	0.43	0.29	0.29	0.11	0.25	1.00													
SRC17	0.43	0.33	0.22	0.33	0.22	0.30	0.11	0.11	0.25	0.25	0.57	0.25	0.25	0.22	0.38	0.38	1.00												
SRC18	0.29	0.22	0.11	0.22	0.43	0.33	0.13	0.00	0.13	0.29	0.25	0.13	0.29	0.00	0.25	0.25	0.38	1.00											
SRC19	0.33	0.25	0.29	0.11	0.13	0.38	0.14	0.33	0.14	0.33	0.29	0.33	0.14	0.13	0.29	0.50	0.25	0.29	1.00										
SRC20	0.50	0.38	0.25	0.22	0.25	0.33	0.29	0.13	0.29	0.29	0.43	0.29	0.29	0.11	0.25	0.43	0.57	0.25	0.29	1.00									
SRC21	0.57	0.63	0.33	0.44	0.33	0.27	0.22	0.38	0.38	0.22	0.50	0.38	0.38	0.33	0.33	0.33	0.44	0.20	0.22	0.71	1.00								
SRC22	0.57	0.44	0.20	0.44	0.50	0.40	0.22	0.22	0.22	0.22	0.33	0.22	0.22	0.20	0.50	0.50	0.44	0.33	0.22	0.50	0.56	1.00							
SRC23	0.43	0.50	0.10	0.20	0.83	0.30	0.11	0.11	0.11	0.11	0.22	0.11	0.25	0.10	0.38	0.22	0.33	0.38	0.11	0.38	0.44	0.63	1.00						
SRC24	0.38	0.30	0.20	0.18	0.20	0.75	0.10	0.22	0.38	0.38	0.33	0.22	0.22	0.20	0.20	0.50	0.44	0.20	0.38	0.50	0.40	0.40	0.30	1.00					
SRC25	0.13	0.22	0.25	0.22	0.00	0.09	0.50	0.29	0.29	0.13	0.25	0.29	0.13	0.25	0.11	0.11	0.22	0.00	0.13	0.43	0.50	0.20	0.10	0.20	1.00				
SRC26	0.29	0.38	0.25	0.38	0.11	0.20	0.13	0.80	0.29	0.13	0.25	0.29	0.13	0.43	0.25	0.25	0.22	0.00	0.29	0.25	0.50	0.33	0.22	0.33	0.43	1.00			
SRC27	0.29	0.22	0.11	0.22	0.25	0.50	0.29	0.13	0.50	0.50	0.25	0.13	0.13	0.25	0.25	0.25	0.38	0.25	0.13	0.43	0.33	0.50	0.38	0.50	0.25	0.25	1.00		
SRC28	0.25	0.20	0.10	0.20	0.22	0.44	0.25	0.11	0.43	0.67	0.22	0.11	0.11	0.22	0.22	0.22	0.33	0.38	0.25	0.38	0.30	0.44	0.33	0.44	0.22	0.22	0.83	1.00	
SRC29	0.50	0.57	0.43	0.38	0.25	0.33	0.13	0.29	0.50	0.29	1.00	0.50	0.50	0.43	0.43	0.43	0.57	0.25	0.29	0.43	0.50	0.33	0.22	0.33	0.25	0.25	0.25	0.22	1.00

CONCLUSION

The study revel wider heterogeneity within a small area in Bundelkhand region of U.P. at the pathogenic, molecular and morphological level in *S. rolfsii*, which causes chickpea collar rot. The study of aggressiveness and the genetic basis of variability can be based on the identification of isolates based on differences in physical and cultural characteristics. Further, in the isolate, the highest similarity was observed between genotypes SRC-11 and SRC-29. The lowest similarities were reviled between the lines SRC-5 and SRC-25, SRC-13 and SRC7, SRC-18 and SRC8, SRC-25 and SRC-18 registered the minimum similarity value indicating the maximum genetic distance between them.

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Conflict of interest

In relation to publishing this work, the author declare that they have no conflicts of interest.

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