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Characterization of Cultural and Morphological Variability in *Rhizoctonia solani* Isolates Associated with Black Scurf of Potato

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A B S T R A C T

Rhizoctonia solani Kühn [teleomorph :*Thanatephorus cucumeris* (Frank) Donk] is the most destructive and widely distributed soil borne pathogenic fungus causing black scurf of Potato. Cultural and morphological variability was studied in eleven potato isolates of *R. solani* collected from different potato growing areas including Haryana, Punjab, Uttarakhand, Rajasthan and Maharashtra etc. Based on the radial colony growth rate, all the isolates were categorised into 3 groups as slow, medium and fast growing. Majority of isolates showed slow colony growth rate. Hyphal width in all the isolates ranged between 6 to 10.5 µm. Most of the isolates produced appressed or raised and pale yellow to very pale brown, mycelium with varied patterns of sclerotial formation. Majority of isolates had sclerotia that aggregated at the centre of the colony. Isolates produced brown to dark brown sclerotia which were either present in the form of concentric ring at the centre or periphery or scattered throughout the colony. Studies on cultural and morphological characterization of *R. solani* isolates showed that isolates were highly variable both in mycelial and sclerotial parameters, with no consistent characters related geographic origin.

Introduction

Rhizoctonia solani Kühn [teleomorph *Thanatephorus cucumeris* (Frank) Donk] is the most destructive and widely distributed soil borne pathogen and most studied fungal species causing diseases in many plant species world-wide. It was originally described by Julius Kühn from potato in 1858 (Ogoshi, 1996). *R. solani* (AG- 3), is the principal cause of the black scurf disease of potato (Carling and Leiner, 1986; Truter and Wehner, 2004; Yanar *et al.*, 2005; Mahmoud, 2010). It is one of the oldest diseases of potato affecting tuber, stem and stolons. The most easily observable symptom is the formation of black, irregular lumpy encrustations on the surface of potato tubers commonly called black scurf which reduce their quality and market value (Arora 2008). This disease is ubiquitous in India and it is serious in fields where potato is grown year after year (Khurana *et al.*, 1998 and Arora, 2012). Potato black scurf is an economically important disease in potato-growing areas all over the world, leading to marketable yield losses up to 30 % (Banville, 1989).

Existence of variability is well known phenomenon in all the organisms including plant pathogens. Variations may affect the success of breeding programme and chemical control strategies. Variation in the progeny of plant or pathogens is introduced primarily through segregation and recombination of genes during sexual reproduction. However, *Rhizoctonia solani* is a basidiomycete fungus that does not produce any asexual spores and only occasionally produces sexual spores. Therefore, the main source of development of variation in *R. solani* is the anastomosis. Hyphal anastomosis criteria have been used extensively to place isolates of *Rhizoctonia* into taxonomically distinct groups called anastomosis groups (AG Group).

R. solani has been reported to show a variation in the cultural and morphological characters that can affect management of the disease (Basu *et al*., 2004; Guleria *et al.,* 2007; Hussain *et al*., 2014). Thus, the studies on variability in *R. solani isolates* are essential to devise economic and effective control measure for disease management. Therefore we studied the cultural and morphological variability among the *R. solani* population in several potato growing areas in India especially from Haryana and Punjab.

Materials and Methods

Collection and maintenance of *Rhizoctonia solani* isolates

Potato tubers showing black scurf symptoms at harvest were collected from different potato growing areas of Haryana as well as from other parts of the country (Table 1) during February and March 2015. These samples were brought to the laboratory in clean paper bags. These tubers were washed thoroughly with tap water and dried between folds of the filter papers. The tubers were then kept in paper bags in the laboratory for further studies. Bark of potato tubers showing black scurf were removed and cut in to small pieces, washed thoroughly in sterilized water to remove the dirt etc. The washed pieces were surface sterilized with 0.1% mercuric chloride ($HgC1_2$) solution for 40-60 seconds and subsequently rinsed in sterile distilled water 3-4 times to remove the traces of mercuric chloride. Surface sterilized bits (3 bits/ plate) were then aseptically placed in sterilized Petri dishes containing pre-sterilized potato dextrose agar (PDA) medium. Inoculated Petri dishes were incubated at 27±1°C in BOD incubator. The cultures of the pathogen were purified through single hyphal tip method (Rangaswami and Mahadevan, 2004), maintained on PDA slants and stored in refrigerator at 4^0 C for further studies. The cultures were designated as P1, P2, P3 and so on. Numbers 1, 2, 3 and others represent different locations from which disease samples had been collected (Table 1).

Colony texture

Colony texture recorded in all isolates revealed that 5 potato isolates (P2, P3, P6, P7 and P10) were occurred appressed, four isolate showed raised (P1, P5, P8 and P11), while colony texture fluffy was found in isolates P4and P9.

Colony colour

Colony colour in all the 11 isolates from potato was observed while culturing on PDA. It was observed that colony color was varied from pale yellow to very pale brown. The isolates viz., P1, P3, P6, P7 and P8 had pale yellow whereas isolates such as P2, P4, P5, P9, P10 and P11 had very pale brown color.

Cultural and morphological variability

The basic cultural characteristics such as colony diameter, colour and growth pattern

were studied. Colony growth rate was recorded by measuring colony diameter after 24, 48 and 72 h at 27±1°C. The observations on colony colour and texture were recorded after incubation for 5 days. The colour of colony was determined with the help of Munsell's Soil Colour Chart (Munsell, 1954). The culture and key colour card was placed side by side and colour of the colony was observed. Based on the mycelial pigmentation, the cultures were assigned to different groups. Measurement of the hyphal width was done with the help of ocular micrometer. Ten measurements were taken for each isolate to calculate the average minimum and maximum width of hyphae. A sclerotial character of each isolate was observed after incubation for 15 days. The sclerotial intensity, pattern of production (central, peripheral and scattered), presence or absence of honey dew secretion and location of sclerotia formed were recorded while colour of sclerotia was observed using a Munsell's Soil Colour Chart.

Results and Discussion

Cultural variability

Growth rate

The considerable variations in cultural characters were observed among the 11 *R. solani* isolates of potato (Table 2). Colony diameter in isolates was recorded at 24, 48 and 72 hr after inoculation. The colony growth rate less than 30 mm per day was observed in seven isolates (P3, P4, P5, P7, P9, P10 and P11) whereas isolate P1, P2 and P8 showed growth rate 30.0, 30.2 and 31.3 mm/day respectively. The maximum (37.6 mm/day) growth rate was found in isolate P6 (Jalandhar 1 isolate) while minimum (27.7 mm/day) recorded in P3, Pantnagar isolate.

Hyphal width

Hyphal width from potato isolates was measured as per standard methods and it was observed that hyphal width ranged from 6.1 µm to 10.1 µm. Majority of the potato isolates (P2, P3, P4, P7, P8 and P10) had hyphal width ranged between 8 to 10 µm. Hyphal width less than 8 µm were found in isolates P5, P6, P9 and P11. Maximum hyphal width (10.1µm) was observed in isolate P1 (Hisar1) while least (6.1 µm) was observed in isolate P9, Hoshiarpur isolate.

Based on cultural variations, potato isolates were categorised into different groups and isolates falling in each group are shown in Table 3. Based on colony growth rate, the potato isolates were grouped as slow, medium and fast growing having a colony growth rate < 30 as slow, medium having the growth rate ranged from 30 to 35 and fast having growth rate >35 mm/ day, respectively

The data revealed that out of 11 rice isolates, seven isolates exhibited medium growth rate, three isolates showed slow growth rate whereas only one isolate was found slow growing. On the basis of colony texture, all the isolates were categorized into three groups *i.e.*, appressed, raised and fluffy growth. Five potato isolates had appressed colony, four isolates showed raised colony texture, while only two isolates (P4 and P9) having fluffy growth. The hyphal width of six of isolates was appeared ranged between 8 to 10 µm. Four isolates showed growth rate les than 8 µm whereas growth rate >10 µm was found only in one isolate.

Morphological variability in potato isolates

The significant differences in morphological characters among the potato isolates of *R. solani* were observed. Sclerotial characters of each isolate were observed after 10 days of incubation. The characters such as sclerotial arrangement, colour, intensity location of sclerotia formation and presence/absence of honey dew like secretion were recorded. Because of aggregated nature of sclerotia in

most of the rice and potato isolates, the sclerotial count and diameter was could not be carried out individually. The observations observed were categorized colony into different group as discussed below.

Sclerotial arrangement

Most of the potato isolates (P1, P3, P4, P5 and P6) had sclerotia which showed aggregation at the centre of the colony. Isolates P2 and P7 showed sclerotial ring in the middle of the colony, while in two isolates (P9 and P11), there was a broad sclerotial ring appeared at both middle and the periphery. Sclerotial ring at periphery was observed in isolate P8 and P10.

Colour of sclerotia

Sclerotial colour of potato isolates varied from light brown to dark brown in color. Sclerotial light brown color was observed in isolates P2, P4, P6, P7, P10 and P11, whereas isolates P3, P5 and P8 showed brown in color. Dark brown color was appeared in isolates P1 and P9.

Location of sclerotia formation

In all potato isolates sclerotia were produced on at the surface of mycelium.

Sclerotial intensity

Among 11 potato isolates, five isolates *viz.*, P2, P4, P6, P8 and P10 showed moderate sclerotial intensity. Scanty sclerotial intensity was occurred in four isolates such as P3, P5, P7 and P11 whereas profuse sclerotial intensity was observed in isolates P1 and P9. Honey dew secretion was absent in all isolates except isolate P3, Pantnagar.

Based on the pattern of sclerotial formation all potato isolates were categorized into different groups (Table 5**)..** Five isolates (P1, P3, P4, P5 and P6) showed sclerotia that aggregated at the centre of the colony, two isolates (P2 and P7) produced sclerotial ring at middle, two isolates (P9 and P11) produced sclerotial ring at both middle & periphery whereas sclerotial ring at periphery was observed in isolates P8 and P10. On the basis of sclerotial intensity, all isolates were categorized in to three group's *viz*., scanty, moderate and profuse, five isolates showed moderate and four showed scanty sclerotial intensity whereas profuse sclerotial intensity was observed in two isolates in P1 and P9.

Table.1 List of potato isolates collected from different locations

Isolate No.	District	Code
P1	Hisar (HR)	HSR1
P2	Hisar	HSR2
P4	Yamuna Nagar	YN
P5	Ambala	AMB
P6	Jalandhar (PB)	JAL 1
P7	Jalandhar	JAL2
P8	Ludhiana	LDN
P9	Hoshiarpur	HSP
P3	Pantnagar(UK)	PNT
P10	Kota (RJ)	KOT
P11	Satara (MH)	STR

Table.2 Colony characters of *R. solani* isolates from different potato-growing region

Isolate No.	Code	Colony diameter[a] (mm) after			Colony growth rate (mm/day)	Hyphal[b] width	Colony texture	Colony colour[c]
		24h	**48h**	**72h**				
P1	HSR1	20.0	42.3	72.3	30.0	10.1	Raised	Pale yellow
P2	HSR2	25.0	49.6	79.8	30.2	9.6	Appressed	Very pale brown
P3	PNT	21.3	43.6	73.3	27.7	9.2	Appressed	Pale yellow
P4	YN	21.6	45.6	75.3	29.7	9.0	Fluffy	Very pale brown
P5	AMB	16.6	37.6	66.3	28.7	6.9	Raised	Very pale brown
P6	JAL 1	15.6	28.0	65.6	37.6	7.8	Appressed	Pale yellow
P7	JAL2	22.0	45.0	74.6	29.6	8.4	Appressed	Pale yellow
P8	LDN	22.3	47.3	78.6	31.3	8.2	Raised	Pale yellow
P9	HSP	15.6	40.6	68.8	28.2	6.1	Fluffy	Very pale brown
P10	KOT	18.0	40.8	69.6	28.8	9.2	Appressed	Very pale brown
P11	STR	19.5	42.6	72.0	29.4	7.2	Raised	Very pale brown
CD (P=0.05)		3.4	6.0	6.1	3.73	0.9		

[a]Mean of three replications , [b]Mean of five replications , [c] Based on Munsell's Soil Color Chart

Table.3 Grouping of *Rhizoctonia solani* isolates from potato based on colony characters

Sr. No.	Group description			Isolates	Isolate Code	Number of isolates in each group out of 18
1	Growth rate (mm/day)	Slow	<30	P3, P4 , P5, P7, P9, P10, P11	PNT ,YN, AMB, JAL2, HSP KOT, STR	7
		Medium	30 - 35	P1, P2, P8	HSR1, HSR2, LDN	3
		Fast	>35	P6	JAL1	1
2	Colony texture	Appressed		P2, P3, P6, P7, P10	HSR2,PNT, JAL1 ,JAL2 ,KOT	5
		Raised		P1, P5, P8, P11	HSR1,AMB, STR ,LDN	4
		Fluffy		P4, P9	YN, HSP	2
3	Hyphal width	<8 µm		P5, P6, P9, P11	AMB ,JAL1,HSP ,STR	4
		8 to 10 µm		P2, P3, P4, P7, P8, P10	HSR2, PNT, YN, JAL2, KOT, LDN	6
		>10 µm		P1	HSR1	1

Table.4 Sclerotial characters of *R. solani* isolates from different potato-growing regions

Isolate No.	Sclerotial arrangement	Sclerotial colour [a]	Location of sclerotia	Sclerotial intensity	Honey dew secretion
P1	Aggregated at centre	Dark brown	Surface	Profuse	-
P2	Sclerotial ring at middle	Light brown	Surface	Moderate	-
P3	Aggregated at centre	Brown	Surface	Scanty	+
P4	Aggregated at centre	Light brown	Surface	Moderate	-
P5	Aggregated at centre	Brown	Surface	Scanty	-
P6	Aggregated at centre	Light brown	Surface	Moderate	-
P7	Sclerotial ring at middle	Light brown	Surface	Scanty	-
P8	Sclerotial ring at periphery	Brown	Surface	Moderate	-
P9	Sclerotial ring both at middle & periphery	Dark brown	Surface	Profuse	-
P10	Sclerotial ring at periphery	Light brown	Surface	Moderate	-
P11	Sclerotial ring both at middle & periphery	Light brown	Surface	Scanty	-

[a] Based on Munsell's Soil Color Chart , + = Honey dew secretion , - = No honey secretion

Table.5 Grouping of *R. solani* isolates from potato based on pattern of sclerotial formation

Sr. No.	Group description		Isolates	Isolate Code	No. of isolates
1	Sclerotial arrangement	Central	P1, P3, P4, P5, P6	HSR1, PNT , YN, AMB, JAL1	5
		Scattered	NIL	NIL	NIL
		Sclerotial ring at middle	P2, P7	HSR2, JAL2	2
		Sclerotial ring at both middle & Periph.	P9, P11	HSP, STR	2
		Scle. ring at Periphery	P8, P10	LDN, KOT	2
2	Sclerotial intensity	Scanty	P3, P5, P7, P11	PNT, AMB, JAL2, STR	4
		Moderate	P2, P4, P6, P8, P10	HSR2 ,YN, JAL1, LDN KOT	5
		Profuse	P1, P9	HSR1, HSP	2

Variation is a common phenomenon in organism may affect the success of disease management strategies. Thus, the studies on variability of pathogen are essential to devise

economic and effective control measures for disease management. In present study, isolates collected from eleven different locations of potato growing states of Northern India and it revealed considerable variations in their cultural and morphological characters.

Among 11 potato isolates, the maximum growth rate was found in isolate P6 (Jalandhar 2) while minimum was recorded in isolate P3 (Pantnagar isolate). Based on the radial colony growth rate, all the isolates were assigned in to 3 groups as slow, medium and fast growing. Seven potato isolates out of 11 were slower growing having growth rate of < 30.0 mm⁄ 24 h.

Similarly, Hussain *et al.*, (2014) categorized *R. solani* isolates from potato in to three groups as fast, medium and slow growing on the basis of radial colony growth rate and observed that majority of isolates showed medium growth. Studies on hyphal width revealed that all isolates ranged between 6 to 10.5 µm. The, maximum hyphal width was recorded in P1 (Hisar-1 isolate) and minimum in isolate P9 (Hoshiarpur). Similar , variation in hyphal width of isolates has been reported by many researches (Lal and Kandhari, 2009; Vijayan and Nair 1985; Upmanyu and Paul, 2013).

Three types of colony texture *i.e.* appressed, raised and fluffy were recorded in potato isolates. The observations showed that most of the potato isolates had colony texture that was either appressed or raised. Thind and Agarwal (2008) also reported that colony growth of most potato isolates were appressed. Colony colour was found varied from pale yellow to very pale brown.

On the basis of differences in sclerotial arrangement, all potato isolates were assigned into five different categories. Majority of isolates from potato had sclerotia that aggregated at the centre of the colony. The sclerotial formation in the same manner *i.e.* central, peripheral or scattered has already been reported by many workers (Singh *et al.*, 1990; Singh *et al*., 2002). The sclerotial colour of the potato isolates varied from light brown to dark brown. Hoa (1994) also reported that sclerotial colour ranged from brown, light/dark brown and black brown. Among eleven isolates, five isolates showed moderate and four showed scanty sclerotial intensity whereas profuse sclerotial intensity was observed in P1 (Hisar 1 isolate) and P9 (Hoshiarpur isolate). Similar, morphological characterization of *R. solani* isolates has been done on the basis of mycelial colour, size and position of sclerotia by several workers (Banniza *et al.*, 1996; Sherwood, 1969; Vijayan, *et al.*, 1985; Vilgalys and Cubeta, 1994).

In conclusion, the present study indicated presence of great diversity in the potato isolates of *R. solani* with respect to cultural and morphological features. The finding of variability in morphological and cultural characteristics among the isolates may provide knowledge about the presence of different races within the geographic regions. In the present study, no specific correlation was observed between cultural, morphological characteristics and their geographical origin.

References

Arora, R. K. (2008). Eco-friendly management of soil and tuber born diseases of potato. *Indian Phytopath.* 65:116-121.

Banniza, S., Rutherford, M. A., Bridge, P. D., Holderness, M. And Mordue, J. E. (1996). Biological characterization of *Rhizoctonia solani* in rice-based cropping systems. Proceedings of Brighton Crop Protection Conference. *Pests & Dis*. 1: 399-404.

Banville, G. J. (1989). Yield losses and damage to potato plants caused by *Rhizoctonia solani* Kühn. *American Potato J.* 66:821-834.

Basu, A., Podder, M. and Sengupta, P. K. (2004). Variability and anastomosis among the rice isolates of Rhizoctonia solani. Indian Phytopath. 57 (1): 70-72.

Guleria, S., Aggarwal, R., Thind, T.S. and Sharma, T. R. (2007). Morphological and pathological variability in rice isolates of *Rhizoctonia solani* and molecular analysis of their genetic variability. *J. Phytopath.* 155: 654-661.

Hoa, T. T. C. (1994). *Characterization and pathogenicity of Rhizoctonia solani Kuhn isolates from different rice zone and management of sheath blight of rice*. Ph. D Thesis, Indian Agricultural Research Institute, New Delhi-12. 122p.

Hussain, A., Saeed, M. A., Muhammad, I., Muhammad, A. and Sartaj A. (2014).Variability among the potato sclerotial isolates of *Rhizoctonia solani* of mountainous region, Gilgit - Baltistan, Pakistan. *African J. Microbio. Res.* 8(30): 2849-2860

Khurana, S. M. P., Pandey, S. K., Bhale, R. L., Patel, B. K. and Lakra, B. S. (1998). Surveillance for potato diseases in India over last five years. *J. Indian Potato Asso.* 25: 16-20.

Lal, M. and J. Kandhari (2009). Cultural and morphological variability in *Rhizoctonia solani* isolates causing sheath blight of rice. *J. Mycol. Pl. Pathol.* 39 : 77- 81.

Munsell, (1954). Munsell's Soil Colour Chart. Munsell Colour Co. Inc. Baltimore, Maryaland, U.S.A.

Rangaswami, G. and Mahadevan, A. (Eds) (2004). *Disease of crop plants in India*. Prentice-Hall of India Private Limited Publisher, New Delhi, India. 507 p.

Sherwood, R. T. (1969) Morphology and pathology of four anastomosis group of *Thanatephorus cucumeris*. *Phytopathology*. 59: 1924-29.

Singh, S. K., Satyanarayan, K. and Reddy, A. P. K. (1990). Studies on morphology growth habit, hyphal anastomosis and virulence pattern of five isolates of sheath blight pathogen of rice. *Indian Phytopth.* 43: 368-371.

Singh, V., Singh, U. S., Singh, K. P., Singh, M. and Kumar, A. (2002). Genetic diversity of *R. solani* isolates from rice: Differentiation by morphological characteristics, pathogencity, anastomosis behavior and RAPD finger printing. *J. Mycol. Pl. Pathol.* 32: 332-344.

Thind, T. S. and Aggarwal, R. (2008). Characterization and Pathogenic Relationships of *Rhizoctonia solani* Isolates in a Potato–Rice System and their Sensitivity to Fungicides. *J. Phytopath*.156: 615–621.

Upmanyu, S. and Paul, Y.S. (2013). Cultural, morphological and physiological variations among different anastomosis groups of *Rhizoctonia solani* Kuhn in Himachal Pradesh. *Pl. Dis. Res.* 28 *(2):* 113-120.

Vijayan, M. and Nair, M. C. (1985). Anastomosis grouping of isolates of *Rhizoctonia solani* Kuhn (*Thanatephorus cucumeris* (Frank) Donk causing sheath blight of rice. *Curr. Sci.* 54: 289-291.

Vilgalys, R. and Cubeta, M. A. (1994). Molecular systemic and population biology of *Rhizoctonia*, *Ann. Rev. Phytopathology*. 32: 135-155.

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