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MORPHOLOGICAL AND PATHOGENIC VARIATIONS IN THE ISOLATES OF *RHIZOCTONIA SOLANI* IN BANGLADESH

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Abstract

Rhizoctonia solani isolates were collected from soil of different agro-ecological zones of Bangladesh and also from infected plant parts of different crops and grasses. Collected isolates were classified into five different cluster groups on the basis of morphological and cultural characters. Five isolates taking one from each of the five different cluster groups were selected to study their pathogenicity and host range on 35 different crops. Pathogenicity and host range of the isolates were determined by planting the seeds in water agar plate infested with *R. solani* isolates and incubated at 25 ^{0}C temperatures. After analyzing the morphological and cultural characters of the isolates, it was found that there was no relations between the isolates with respect to their origin from where they were collected. It indicated that the diversity among the isolates was not correlated with their origin. In case of host range and pathogenicity among the five selected isolates of different cluster groups, the isolate JES-16 was an avirulent isolate. The isolate SYL-30 had narrow host range and a low virulent isolate. The isolates DIN-8 and GAZ-18 possessed wide host range and might be considered as virulent isolates. The isolate GAZ-9 was a highly virulent isolate with a wide host range.

Keywords: Rhizoctonia solani, morphological and pathogenic variations, isolates.

Introduction

Rhizoctonia solani Kuhn is a plant pathogen that attacks a large number of plant species around the world with diverse symptoms (Ploetz *et al.,* 1985). *Thanatophorus cucumeris* (Frank) Donk is the sexual stage of this pathogen. Single basidiospore progeny are considered to have low genetic variability. In contrast, the asexual stage *(R. solani)* is generally considered to have wide genetic variability between pathogenicity groups. Several attempts have been made to group the isolates of this pathogen taxonomically. So far, anastomosis of mycelia is the criterion most widely accepted and used to group the isolates of this fungus (Anguiz, 1989). But grouping by anastomosis does not always correspond to grouping by colony morphology, pathogenicity or other physiological features (Carling, 1996). In Bangladesh, research on classification of *Rhizoctonia solani* isolates on the basis of pathogenicity and their ecological distribution is scarce. This paper reported the variability of 50 isolates of *R. solani* on the basis of morphological characters and pathogenicity on 35 crop species.

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Materials and Method

Isolates of *Rhizoctonia solani* were obtained mainly from soil collected from different areas representing major agro ecological zones of Bangladesh. Isolates were also collected from potato tuber and stem, mungbean, seedlings of cauliflower and cabbage, amaranth and grass like *Imperata cylindrical* L (ulu.). To isolate the fungus from soil, sterilized dry buckwheat stems were used as baits. The stems were cut into small pieces and put it into the soil at 10-15 cm depth for 3-5 days. Then the pieces of bait were recovered from the soil and washed in a jet stream of tap water for one hour. After washing, bait materials were kept into 200ppm streptomycin sulphate solution for 10 minutes and blotted with sterilized blotting paper. The baits were then plated (two pieces per plate) on Petri dishes containing 1.5% water agar (WA) amended with 200 ppm streptomycin sulphate and 100 ppm metalaxyl. In case of plant samples infected tissues were cut into small pieces (1-2 cm) along with healthy tissues. The pieces were washed in tap water and surface sterilized by dipping in 70% ethanol for 30 sec. The surface sterilized specimens were rinsed three times with sterilized water. The excess water on the surface of the cut pieces were removed by blotting with sterile blotting paper and plated on 1.5% water agar medium containing 200 ppm streptomycin sulphate and 100 ppm metalaxyl. For both the cases, the plates were incubated at 25 ± 1^0C. After appearance of the characteristic growth of the fungus, hyphae were transferred aseptically to Petri dishes containing solidified potato dextrose agar (PDA) amended with 200 ppm streptomycin sulphate. Contamination free plates were selected and young mycelium was transferred to PDA slants in test tubes to obtain pure culture of individual isolate. The test tube having sufficient growth was preserved in refrigerator at 10 ^{0}C. All the collected isolates were studied for cultural and morphological characters by growing them separately on PDA plates. Data were collected on mycelial growth rate after 24 hours of incubation, structure, zonation no., colour of the colony, number of sclerotia/cm^2, colour of sclerotia, shape and size of sclerotia, days to sclerotial initiation and the formation patterns of sclerotia. Variations among the isolates based on cultural and morphological characters were analyzed following cluster analysis (CLSA). Among the 50 isolates of *R. solani,* five homogeneous groups were identified. Five isolates, namely DIN-8, GAZ-9, JES-16, GAZ-18, and SYL-30 taking one isolate from each cluster group were selected to study the host range and pathogenicity. Thirty five crop species, namely wheat, rice, maize, prosomillet, foxtail millet, barley, sorgham, groundnut, soybean, sunflower, mustard, sesame, niger, safflower, chickpea. blackgram, lentil, grasspea, mungbean, cabbage, cauliflower, radish, stringbean, spinach, Indian spinach, Lady's finger, country bean, carrot, cucumber, white gourd, ribgourd, tomato, brinjal, potato and kangkon were tested against the isolates under field and laboratory conditions.

For laboratory test, direct inoculation technique was conducted by petriplate test method (Yang *et al.,* 1996). Selected five isolates were grown on PDA plates at 25 ^{0}C separately. After two days of incubation, a PDA plug (3mm in diameter) bearing mycelium of an isolate was transferred to 2% water agar plates. All inoculated plates were incubated at room temperature for 3 to 5 days until the mycelium covered most of the plates. Twenty five surface sterilized seed (10 minutes in 1% NaOCl solution) of each crop species were placed on each inoculated plate. Four replicated plates of each isolate were arranged in a completely randomized design and incubated at room temperature for seven days. Seed germination, seedling infection and post emergence mortality were recorded.

Field tests were done by soil inoculation method. Inocula were developed on sterilized barley grains (Yang *et al.,* 1996). Seedbed with 3.0m × l.5m size was used for the test. Soil of the seedbed was treated with 1% formalin solution. After two weeks, the sterilized seedbed was inoculated with the inocula 94 g/m^2 (Yuen *et al.,* 1994). After two weeks of inoculation, 25 surface sterilized seeds of each crop were sown in the inoculated bed. Bed without inoculum was also maintained as a control. Randomized complete block design with 3 replications was used in the experiment. Emergence of seedlings was recorded at 7 days after planting and post emergence mortality was recorded upto 21 days of planting. Comparing the two methods, founding the better performance, direct inoculation method was used to find out the variation of host range.

Results and Discussion

The isolates of *R. solani* were grouped into five cluster groups on the basis of their morphological characters. Five cluster groups showed that three isolates were distributed in cluster-1, one in cluster-II, ten in cluster-III, sixteen in cluster -IV and twenty isolates were in cluster-V (Table 1). The isolates were randomly distributed in different cluster groups disregarding their origin from where they were collected. The results indicated that the diversity among the isolates was not correlated with their origin of collection. The results of the present study are in agreement with that of Barid *et al.* (1996). On the basis of morphological characters they did not find any difference between Japanese and United States isolates.

Contribution of different components cluster means of five morphological components of 50 isolates are presented in Table 2. The lowest growth rate and the highest number of sclerotia were observed in cluster-I. The lowest zonation was observed in cluster-III., which was followed by cluster-I, V, and IV. The maximum zonation was found in cluster-II. The lowest duration of Sclerotial initiation was observed in cluster-III, which was followed by cluster-I, V, and II. The highest sclerotia initiation was found in cluster-IV. Width of hyphae was the lowest in cluster-II, which was followed by-III, IV & I and the highest in cluster-V.

Table 1. Distribution of isolates of *Rhizoctonia solani* under different clusters based on five morphological characters.

Cluster number	Number of isolates	Isolates
I	3	JAM-l, JES-16, KHA-36
II	1	GAZ-18
III	10	SER-7, NOA-17, BAR-20, CHI-25, SYL-30, RAG-37, CHI-44, MOU-46, GAZ-47, TAN-50
IV	16	TAN-2, GAZ-9, COM-10, KUS-14, KHU-21, GAZ-22, POT-23, CHI-24, RAJ-27, MYM-28, SYL-31, JAM-32, KHA-34, CHI-41, CHI-45, TAN-49
V	20	RAN-3, GAB-4, DIN-5, RAN-6, DIN-8, GAZ-11, DIN-12, KUS-13, JES-15, GAZ-19, DIN-26, MUN-29, RAN-33, KHA-35, RAG-38, RAG-39, CHI-40, CHI-42, CHI43, GAZ-48

Table 2. Mean values of five different morphological characters of *Rhizoctonia solani* isolates under five different clusters.

Component	Cluster				
	I	II	III	IV	V
Growth rate (cm/24 hrs.)	2.844	3.5000	3.203	3.362	3.270
Zonation of colonies (No.)	1.222	3.667	0.891	3.104	2.883
Width of hyphae (µ.m)	6.978	5.83	6.460	6.844	7.3 85
Duration for sclerotia initiation (days)	6.111	8.000	2.963	14.000	7.567
No. of sclerotia /cm^2	79.000	23.670	3.533	16.708	45.067

Pathogenic variations in five selected isolates of *R. solani* are shown in Table 3. Out of 35 crops, only mungbean and lady's finger were infected by the isolate JES-16, however, severity of mortality was 2% in mungbean and 3% in lady's finger. JES-16 did not cause any symptom of pre or post emergence mortality in other 33 crops. It indicated that the host range of the isolate is very narrow. The isolate SYL-30 infected 14 crops. The mortality was 1-5% in soybean, groundnut, sesame, safflower, chickpea, blackgram, grasspea, and mungbean, 6-8% in sunflower, niger, lentil, brinjal, and tomato. Maximum of 11% mortality was found in potato. Other 21 crops were free from mortality. The isolate DIN-8 infected all crops except proso millet and foxtail millet. The mortality of the infected crops varied considerably. The lowest mortality of 2% was found in rib gourd. The mortality was 10 to 20% in rice, wheat, maize, spinach, cucumber, and white gourd. Percent mortality ranged from 20 to 30 in chickpea, grasspea, indian spinach, lady's finger, carrot and kangkon. Upto 77% mortality was recorded in rest of the crops due to DIN-8. All the 35 crops were infected by the isolate GAZ-18. The lowest mortality of the isolate GAZ-18 was 10% in foxtail

millet. The mortality was 20% in proso millet, spinach, rib gourd, indian spinach, and carrot. The mortality recorded in maize, cucumber, and white gourd was 30%. The mortality was 35 to 80% in other 26 crops. All the crops were also infected by the isolate GAZ-9.The pathogen caused 20 to 30% mortality in proso millet, foxtail millet, soybean, niger, indian spinach, rib gourd, tomato, brinjal, and kangkon. Mortality of other 26 crops was 31 to 90%.

Table 3. Reaction of different crops against five different isolates of *Rhizoctonia solani.*

Crop	% Mortality				
	DIN-8	GAZ-9	JES-16	GAZ-18	SYL-30
Wheat	15	80	0	50	0
Rice	10	38	0	35	0
Barley	30	58	0	40	0
Maize	20	31	0	30	0
Prosomillet	0	25	0	20	0
Foxtail-millet	0	28	0	10	0
Sorgham	40	50	0	40	0
Groundnut	50	36	0	40	5
Soybean	55	30	0	50	1
Sunflower	70	85	0	70	7
Mustard	60	85	0	50	0
Sesame	50	28	0	60	5
Niger	75	30	0	50	6
Safflower	77	65	0	70	4
Chickpea	30	50	0	70	5
Blackgram	32	60	0	70	3
Lentil	40	85	0	50	7
Grasspea	28	72	0	60	3
Mungbean	34	45	2	80	5
Cabbage	55	85	0	40	0
Cauliflower	60	90	0	45	0
Radish	60	60	0	80	0
Stringbean	40	38	0	60	0
Spinach	20	50	0	20	0
Indian spinach	26	20	0	20	0
Lady’s finger	28	50	3	80	0
Country bean	40	60	0	60	0
Carrot	28	45	0	20	0
Cucumber	15	40	0	30	0
Whitegourd	16	38	0	30	0
Ribgourd	2	25	0	20	0
Tomato	45	30	0	50	7
Brinjal	37	28	0	60	8
Potato	40	32	0	80	11
Kangkon	26	30	0	60	0

The result indicated that the isolate JES-16 was an avirulent isolate of R. *solani*. The isolate SYL-30 possessed narrow host range and seemed to be a low virulent isolate. The isolates DIN-8 and GAZ-18 had wide host range and might be considered as virulent isolates. The isolate GAZ-9 was a highly virulent isolate with a wide host range. Similar results were recorded by Muyolo *et al.* (1993) with *R. solani* AG-4 on soybean. Leach and Clapham (1992) reported difference in virulence among isolates of AG-5 on sugar beat and white lupine, respectively. Carling *et al.* (1987) reported that the isolates of AG-9 were avirulent on cereal crops, forages, field pea, and tomato, but highly virulent on cauliflower and moderately virulent on flax. Monga and Sheo (1994) studied with 13 isolates of *R. solani.* They categorized them into four groups on the basis of cultural character. They selected one isolate from each group for pathogenicity test and found that isolates of groups 2, 3, and 4 showed lesser pathogenicity, whereas isolate of group 1 was highly pathogenic. The findings of the present study confirmed the existence of distinct variation among the isolates of *R. solani* based on host range and pathogenic variability.

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