Identification of anastomosis group of *Rhizoctonia solani* associated with tobacco in Karnataka

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ABSTRACT

Sore shin of tobacco, *Nicotiana tabacum*, L., caused by the fungus *Rhizoctonia solani* Kuhn is present in all FCV tobacco growing areas of Karnataka Light Soil (KLS). Occurrence of *R. solani* anastomosis group and the vegetative compatibility groups among the isolates were studied. 27 isolates of *R. solani* were recovered from sore shin infected tobacco seedlings collected during disease survey during 2006-2008. Based on the cultural characteristics the isolates were grouped into seven groups. All of them were found to be multinucleate. The cultures were tested for anastomosis reaction by water-agar cellophane culture method and vegetative compatibility group test. All the isolates showed cytoplasmic fusions with cell death or killing reaction. A C2 type of anastomosis reaction was observed. All seven isolates from KLS made cytoplasmic fusions at 55.0-65.0% with those from Japan. A barrage line is formed during confrontation of related but vegetatively incompatible strains of *R. solani*. AG determination revealed that all isolates belong to AG-4. This is the first report on AG determination of *R. solani* isolates in Karnataka light soil, India.

Keywords: Tobacco, sore shin disease, *Rhizoctonia solani*, mycelial compatibility, anastomosis group

INTRODUCTION

Rhizoctonia solani Kuhn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) is a soilborne pathogen responsible for severe damage on many crop species [1]. Sore shin disease caused by *R. solani* is an important disease in tobacco nursery worldwide [2]. This hitherto unrecorded disease was noticed for the first time in Karnataka Light Soil (KLS) nurseries during 2005 season [3]. *R. solani* is genetically diverse fungus and separated into subgroups known as Anastomosis Groups (AGs). AGs and their subgroups have been used for identification and classification of *R. solani* [4]. The anastomosis subgroups have been divided on the basis of host range, cultural morphology and biochemical or molecular characteristics [5]. It is found that anastomosis occurs between isolates of the same groups but not between isolates of different groups [6]. Currently 14 AGs (designated AG-1 through AG-13, AG- BI) have been identified and characterized based on morphology, host range and ability to infect plants [7,8]. Each anastomosis group therefore, seems to be genetically independent from all others [6]. Among 14 AGs, AG-4 has been divided into AG-4 HG-I, HG-II and HG-III subgroups. Anastomosis subgroups are partially based on differences in one or more biochemical, genetic or pathogenic characteristics [9]. Anastomosis groups appear to be fairly host plant specific [10]. For instance AG-4 is regularly associated with Pinaceae, Chenopodiaceae, Cruciferae, Leguminosae, Malvaceae and Solanaceae [11]. Mycelial compatibility groups (MCGs)

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have been used to evaluate genetic variability in fungal pathogens within the species such as *Amylostereum areolatum* and *A. chailletti* [12], *Sclerotium rolfsi* and *S.delphinii* [13], *Sclerotinia sclerotiorum* [14], *Fusarium oxysporium* f. sp. *cubens* [15], *Rhizoctonia solani* [12]. In many species mycelia incompatibility results in the formation of macroscopic reaction lines (barrages) between fungal colonies, which indicates their failure to anastomose [17].

Despite the reports of *R. solani* AG groups causing the sore shin disease on tobacco from different parts of the world, information is not available on this disease in India with reference to tobacco. The objective of this study was to identify the anastomosis group (AG) and to determine the mycelial compatibility of these isolates of *R. solani* associated with sore shin disease of tobacco which is commercially grown in KLS.

MATERIALS AND METHODS

Tobacco seedlings showing the sore shin disease symptoms were collected from the nursery fields of KLS, viz., Hunsur, Periyapatna and H.D. Kote to record the variation in the disease incidence. The diseased plants were collected in polythene bags (Sterile) taken in plastic cover, brought to the laboratory for isolating the disease-causing fungus. Isolation was done using diseased root pieces collected from different nurseries. Root pieces were washed under tap water for about 5 minutes to remove soil particles. The root pieces were dipped in 70% ethyl alcohol for 2 minutes and then transferred to sterile distilled water for 2-3 minutes twice. The treated root pieces were blot dried and then transferred to petriplate containing sterilized potato dextrose agar medium with five pieces per plate. All plates were kept at 25 ± 2°C for 7 days. For confirmation of identification, the isolates were sent to IARI, New Delhi, India. The fungal mycelium was transferred to fresh Potato Dextrose Agar medium. The fungus was then sub cultured whenever needed during the present study.

Seven isolates of *R. solani* were tested for their ability to anastomose with known Japanese standard cultures (AG-1,IA; AG-1,IB; AG-1,IC; AG-2-1; AG-2-2,IIIB; AG-2-2,IV; AG-2-2,LP; AG-4,HGI and AG4,HGII) of *R. solani*. The isolates from Japan were provided by Dr. Shiro Kuninaga, Health Sciences University of Hokkaido, Ishikari-tobetsu, Hokkaido, Japan. Hyphal anastomosis was tested by WA-cellophane culture method [18]. The mycelial plugs of 5mm diameter from the margins of actively expanding young cultures on CDA of unknown isolates and known tester isolates were opposed 2-3 cm apart on the cellophane strip resting on 2% WA (Water agar) in 9cm petriplate, the strip was 6×3 cm in size, disinfected with 70% ethanol for 5 min, washed in sterile distilled water and dried in sterile filter paper before using. The cultures were incubated at 25°C for 24-28 hours until the advancing hyphae from the opposite side made contact and properly overlapped. Each unknown isolate was paired in combination with the tester isolates obtained from Japan experiment was performed in three replicates.

The overlapped area of hyphae by Water-agar cellophane culture was stained with cotton blue in lactophenol and scanned for hyphal anastomosis. Observations were made at the point of contact with one isolate with the opposite tester isolate by using light microscope. Fusion frequency of hyphal anastomosis was determined using the formula: % F = A (100)/B, where A=Sum of fusion points in 15 microscopic fields, B=Sum of contact points in 15 such fields [19].

Representative seven isolates of *R. solani* which are distinctly different in their cultural characteristics were selected. These isolates were then paired in all combinations on PDA to identify the compatible strains. Mycelial compatibility was determined [20]. To determine the macroscopic vegetative reaction between two isolates, the cultures were paired on PDA. Inoculum plugs (5mm in diameter) were placed 4 cm apart on Potato Dextrose Agar in 9cm petridish and incubated at 28°C in pairs of isolates, and were assessed 7 days later for compatibility and incompatibility reactions. Compatibility reaction (+) was recorded if the two colonies merged. Incompatibility (-) reaction was noted as barrage line (a clear area at the junction of paired cultures).

RESULTS AND DISCUSSION

The disease incidence recorded at Hunsur, Periyapatna and H.D. Kote were 30%, 10% and 6% respectively. It might be due to conducive weather conditions prevailing in these areas, and hence caused seedling losses every year which resulted in transplant shortage in these areas. The fungi isolated were identified as *Rhizoctonia solani* and 7 groups were formed based on their typical colony characteristics. Seven isolates obtained from infected tobacco seedlings in KLS made cytoplasmic fusions followed by killing reaction with AG-4, HGI Japanese standard culture of *R. solani* by anastomosis test. All the isolates anastomosed with the known tester isolate AG-4, HGI but did not anastomose with other tester isolates used in this study. Microscopic examination showed that in all isolates cell walls join but membrane connection is uncertain, death of anastomosing and adjacent cells was observed (Figure 1). This predicts a C2 type of anastomosis reaction [4]. The frequency of cytoplasmic fusion was 55.0-65.0%. Incompatible hyphal fusion was observed among all the seven isolates (Table 2). A barrage line was formed during confrontation of related but vegetatively incompatible *R. solani* isolates (Figure 2). This incompatible reaction was clearly visible after 7 days (Table 1).

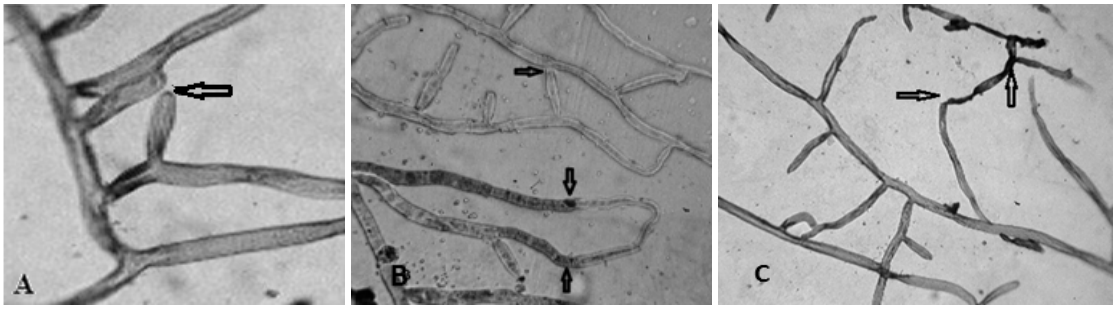

Figure 1. A: Hyphal tips attracting each other; B, C: Death of anastomosing and their adjacent cells (arrow mark indicates the region of anastomosis).

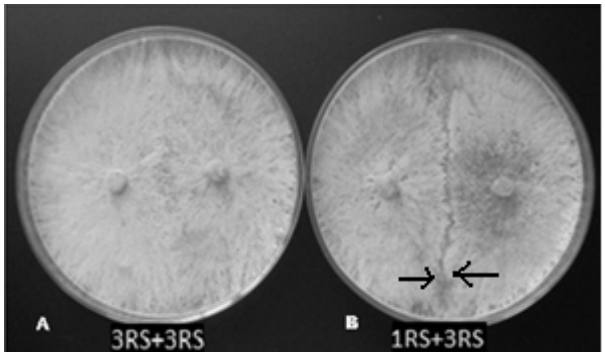

Figure 2. Mycelial interactions of *R. solani*. A: Compatible reaction, B: Incompatible reaction showing barrage line.

Table 1. Frequency of hyphal anastomosis among the isolates of *Rhizoctonia solani* from KLS and those of *R. solani* AG-4, HG1 from Japan.

KLS Isolates	% of cytoplasmic fusion with AG-4, HG1 isolate from Japan
1RS	55.0
2RS	65.0
3RS	58.8
4RS	60.2
5RS	59.0
6RS	62.0
7RS	55.2

Table 2. Mycelial compatibility groups (MCGs) of different isolates of *R. solani*.

Rhizoctonia solani isolates	1RS	2RS	3RS	4RS	5RS	6RS	7RS
1RS	+ve	-ve	-ve	-ve	-ve	-ve	-ve
2RS		+ve	-ve	-ve	-ve	-ve	-ve
3RS			+ve	-ve	-ve	-ve	-ve
4RS				+ve	-ve	-ve	-ve
5RS					+ve	-ve	-ve
6RS						+ve	-ve
7RS							+ve

+ve: Mycelia were compatible; -ve: No mycelia compatibility occurred.

Anastomosis group determined by using 9 tester isolates of *R. solani* showed that all the isolates of KLS belonged to same anastomosis group AG-4, HGI. It is the first report on AG identification on *R. solani* occurring on FCV tobacco in KLS, India. However, reports of AG-4 in tobacco are available from other countries [21]. *R. solani* AG-4 is also reported on many economically important plants like potato [18,22,23] and cotton [8]. More than one AG group of *R. solani* are identified in tobacco in other countries [24], the present work has revealed that in the KLS region all the isolates belonged to the same AG group and exhibited C2 reaction with the AG-4, HGI tester isolate. AG-4 is reported to be dominating throughout the world infecting tobacco seedlings [21, 25].

The isolates obtained from different geographical zones of KLS tested for mycelial compatibility showed that the isolates were compatible within the group but showed a barrage reaction indicating that the isolates were vegetatively incompatible with those of the other groups and with the tester isolates. The results are in congruent with the previous reports of AG-4 isolates infecting flax [26]. This may indicate the presence of significant diversity of the pathogen and thus mycelial compatibility groups (MCGs) are valuable tools to evaluate genetic variability in fungal plant pathogens and to understand the epidemiology of diseases caused by them [12]. The present study indicates AG-4 consists of an assemblage of genetically diverse individuals in KLS. Previous reports on such variations are available across distant geographical areas. However, the current investigation gives an evidence for the existence of variations within a much smaller geographical region. This knowledge on the diversity of the pathogen may help in formulating the better disease control strategies. In addition, this will also be valuable in plant breeding programs.

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