Development of a Detection Technique for Identification of Rhizome Rot Diseases of Ginger.

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ABSTRACT

Ginger rhizome rot is the major problem faced by farmers and it significantly contributes to the yield loss. Hence, development of detection technique to identify the causal organism of rhizome rot disease is a must. Collected samples were cultured on PDA media then; KOH test, Carrot test, *Ralstonia solanacearum* identification test, and its biovar identification test were carried out to distinguish the presence of rhizome rot disease. Majority of cultures were identified as *Erwinia spp.*, in carrot test. *Ralstonia solanacearum* also could be identified as 2nd major causal organism. It appears that biovar 3 is the most commonly distributed biovar type in ginger cultivation of Sri Lanka. The developed technique for the identification of rhizome rot is very useful for the planting material production.

KEY WORDS: Bacteria wilt, *Erwinia spp*, Ginger, *Ralstonia solanacearum*, Rhizome rot, Soft rot

INTRODUCTION

Ginger (*Zingiber officinale*) is a one of the most economical important crop in Sri Lanka. It is mainly cultivated Mid, Western and North-Western provinces (Gunesekara, 1974). According to year 2003/2004 statistics, ginger was grown in 1234ha and the yield was 5693t (Anon, 2005). Local, Chinese and Rangon are the major ginger cultivars grown in Sri Lanka.

The rhizome rot disease causes significant yield loss of ginger. It may be due to the infection of soft rot disease and bacterial wilt disease.

Soft rot is the most destructive disease, which results in total loss of the affected clumps (Anon, 2004). All ginger cultivars available today are equally susceptible to soft-rot disease (Kavitha and Thomas, 2006). Bacterial soft rot is the principal post harvest bacterial disease of ginger (Anon, 2003). Depending on the ecological conditions, *Erwinia carotovora ssp. carotovora*, *Erwinia carotovora ssp. atroseptica* and *Erwinia chrysanthemi* caused soft rot disease (Farra *et al.*, 2000; De Boer, 2002, Stirling, 2005).

Bacterial wilt (*Ralstonia solanacearum*) is another major disease in ginger. *Ralstonia solanacearum* is an aerobic, non spore –forming, rod shaped gram negative bacterium (Mehan, *et al.*, 1994). *Ralstonia solanacearum* in ginger shows variation in their serological and physiological properties (Tsuchiya *et al.*, 2005).

In the absence of resistant cultivars, disease control focus on screening and certification of pathogen free rhizome (Alvarez *et al.*, 2005). Farmers use their own planting materials. It enhances the incidence of rhizome rot disease. According to Alvarez *et al.*, (2005), there is no test available for growers to test ginger propagative material and also for testing soil in production field. Therefore objective of this study is to identify the causal organisms of rhizome rot diseases and the biovar type of *Ralstonia solanacearum*.

MATERIALS AND METHODS

This study was conducted in the faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Markandura during the period of January 2006 to August 2006.

Sample Collection

Survey was conducted in Puttalam, Kurunegala, and Gampaha districts. Twenty-two farmers were contacted from the three districts and ten ginger rhizome samples were collected from each farmer.

Culturing on PDA Media

The samples were cultured on the potato dextrose agar media (Lelliott and Stead, 1987). The culture plates were incubated at 25°c.

KOH Test

Bacteria grown in the cultures were streaked on a drop of 5% KOH solution (Lelliott and Stead, 1987).

Carrot Test

Healthy carrot slices were placed on sterile filter paper in petri dishes. Sterile distilled water was added it. Bacteria culture showing sticky nature to KOH test was inoculated on to these carrot slices. As a control some slices were inoculated with sterile water. They were inoculated at 25°C for 72hrs (Kelaniyangoda *et al.*, 2004).

Identification of Ralstonia solanacearum

Bacteria suspected as *Ralstonia solanacearum* were cultured on tetra zolium chloride (TZC) media (Kelman, 1954) and the plates were incubated for 48hrs at 30°C to confirm identification of *Ralstonia solanacearum.*

Biovar Identification Test

The basal medium was prepared with by dissolving of 10g $NH_4H_2PO_4$, 0.2g Kcl, 1.0g Peptone, 0.2g $MgSO_4.7H_2O$, 0.3ml 1% Bromothymole blue, and 18.0g Agar, in one liter of distilled water. The

carbon compounds (Manitol, Sorbitol, Dulcitol, Lactose, Moltose, Cellobiose) were added to the basal medium to obtain final concentration of 1% (W.V) (Heyward, 1964 and He *et al.*, 1983). Agar slopes were inoculated with one day old culture of the different isolates and incubated at 34°C for 24hrs and then maintained at room temperature for the remaining period of the observation. Observations were made on the presence or absence of yellow colouration, 14 days after inoculation (Kelaniyangoda, 1994 and 1995).

Biochemical reaction and observation of five biovars of *Ralstonia solanacearum* was indicated in table 1.

Table 1 - Biochemical Reaction of Five Biovars of *Ralstonia solanacearum* :

Test	Biovars				
	1	2	3	4	5
Cellobiose	-	+	+	-	+
Lactose	-	+	+	-	+
Maltose	-	+	+	-	+
Mannitol	-	-	+	+	+
Sorbitol	-	-	+	+	-
Dulcitol	-	-	+	+	-

+ = Positive reaction or yellow coloration
− = Negative reaction or no yellow coloration
Source: Hayward (1964), He *et al.*, (1983)

RESULTS AND DISCUSSION

Culturing on PDA Media

After 48 hours of inoculation on PDA, bacteria growth was observed around some pieces of cultured samples. These bacteria colonies were smooth, cream in colour and slightly raised on PDA media. These bacteria can be either soft rot causing *Erwinia spp.* or bacterial wilt causing *Ralstonia solanacearum.*

KOH Test

Some cultures of bacteria were sticky. They were recognized as gram negative (Lelliott and Stead, 1987). Non-sticky ones were rejected.

Carrot Test

Some carrot tissues exhibited water soak appearance and harsh odour. It was due to *Erwinia spp.* (Kelaniyangoda *et al.*, 2004).

Identification of Ralstonia solanacearum

On the TZC media, some bacteria showed the pink colour with white margin. It was due to *Ralstonia Solanacearum* (Kelman, 1954). Others were rejected.

Biovar Identification Test

In biochemical text, the development of yellow colour in the medium indicates the production of acid from the oxidation of the carbon source.

All the isolates from the Kurunagele, puttalam and Gampaha district confirmed the presence of biovar 3 (Table 2). Ginger cultivation in Indonesia, Japan and Thailand have proved to be *Ralstonia solanacearum* biovar type 3 and 4 (Tsuchiya, *et al.*, 2005). However in this study, the biovar type 3 was found in ginger cultivation area. It appears that biovar 3 is the most commonly distributed biovar type in Sri Lanka. According to Bandara (1983), biovar 2 was isolated from potato grown in the central highlands and it was found to be delimited to isotherm 16°c. Biovar 3 which was pathogenic to all solanaceous crop was isolated from almost all the sampling sites throughout the island.

Table 2 - Relationship between the Biovar Type and the District:

District	Number of isolation	Biovar type
Kurunagala	3	3
Puttalam	4	3
Gamphaha	3	3

Rapid detection method developed in this study could be used as identification of rhizome rot disease in ginger cultivation (Figure 1).

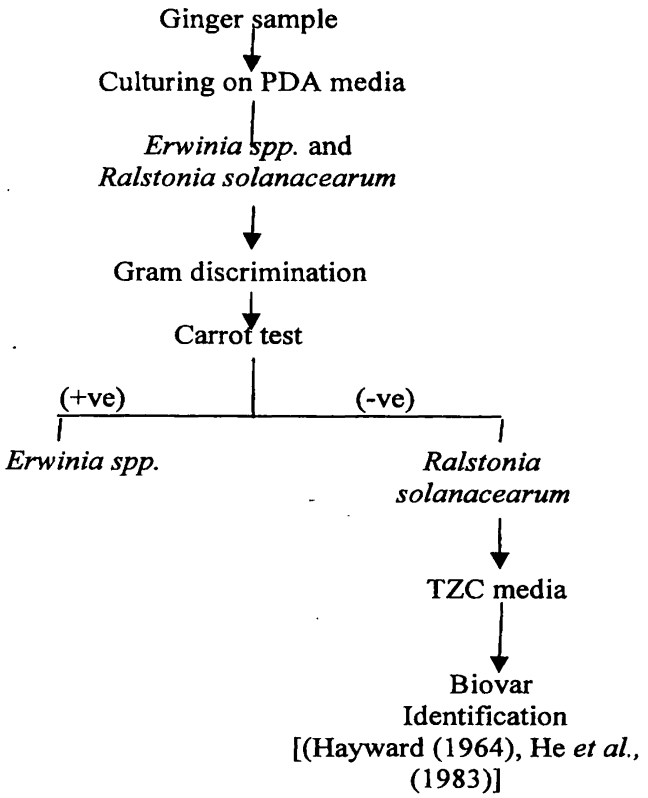

Figure 1 - Procedures Developed for Rapid Detection of Ginger Rhizome Rot Disease:

The distribution of the disease incidence in different districts indicated in table 3.

Most of samples were infected as *Erwinia spp.* while the small samples were infected as *Ralstonia solanacearum.* In Australia *Erwinia chrysanthemum* have been found in rhizome rot in ginger (Stirling, 2005). Therefore, it proves that *Erwinia spp.* significantly contribute to the ginger rhizome rot disease.

Table 3-Incidence of Rhizome Rot in Ginger:

District	Number of samples			
	Number of samples infected by *Erwinia spp*	Number of samples infected by *Ralstonia solanacearum*	Rotted due to others	Total number of infected samples
Kurunagala	37	3	5	45
Puttalam	31	4	2	37
Gamphaha	7	3	0	10
Total	75	10	7	92

The biochemical methods, take more than two weeks to provide conclusive results. Serological test are relatively costly and may give inaccurate results. The currently practice method of detection (Lelliott and stead, 1987) take three weeks to identify *Erwinia spp.*. Therefore, it can be conclude that the technique used in this study could be adopted in the laboratories to detect latent infection of *Erwinia spp.* and *Ralstonia solanacearum* in five days. The develop technique reduced the cost by 75% compared to the serological studies presently used.

CONCLUSIONS

Ginger rhizome rot disease was caused mainly by *Erwinia spp.* *Ralstonia solanacearum* identified as 2nd major causal organism. It appears that biovar 3 of *Ralstonia solanacearum* is the most commonly distributed biovar type in Ginger cultivation of Sri Lanka. The developed technique for the identification of rhizome rot is very useful for the planting material production.

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