

BACTERIAL WILT (*Ralstonia solanacearum* E.F. SMITH) MANAGEMENT IN POTATO ROOTED STEM CUTTINGS IN THE NET-HOUSE

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ABSTRACT

Bacterial wilt (*Ralstonia solanacearum*) is a detrimental disease that impairs nucleus seed potato production through the non-conventional clonal seed system based on the rapid multiplication technique (RMT). In this approach, rooted stem cuttings (RSC) produced in the aphid-proof nethouse are used to generate nucleus seed material. In order to develop management strategies to control the disease, investigations were carried out to study the influence of rooting medium, rooting medium steaming duration and irrigation water treatment on the incidence of bacterial wilt (*R. solanacearum*) in rooted stem cuttings in the nethouse at the Regional Agricultural Research and Development Centre, Bandarawela during February 1994 to May 1996. The investigations revealed that bacterial wilt in potato rooted stem cuttings in the nethouse could be managed effectively by integrating component technologies such as ordinary mixture of cowdung and sub soil (1:1), steaming of the rooting medium for 3 h and treating irrigation water with 2 ppm of calcium hypochlorite for 6 h or 2.75 ppm for 1 h. It was also noticed that the 2 ppm calcium hypochlorite concentration was not phytotoxic to potato plants.

KEY WORDS: Bacterial wilt, Potato rooted stem cuttings, Nucleus seed, Rooting medium, Steaming, Chlorination.

INTRODUCTION

Potato is a high-value crop in Sri Lanka. The major cultivation constraints are high seed cost and poor seed quality. The seed cost alone accounts for about 50-60% of the total production cost. These constraints can be minimized by the production of quality seed tubers locally through a non-conventional seed system based on the rapid multiplication technique (RMT). Several modifications in the conventional seed potato production system have been introduced recently in different countries according to their national needs (Uyen and Vander Zaag, 1993; Potts,

1986; Kim *et al.*, 1986; Pact and Zamora, 1991). In this approach, rooted stem cuttings (RSC) generated from tissue-cultured plants in the nethouse are used for the production of pre-basic seed (G_0) potatoes. These seeds are used as nucleus planting material in certified seed potato production. Bacterial wilt (*Ralstonia solanacearum* E.F. Smith) is one of the major diseases affecting potato RSC generated from tissue-cultured material in the nethouse. The incidence of this disease has been consistently reported after transferring tissue-cultured materials to an "aseptic" nethouse for further multiplication. Rooting media and irrigation water from different

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sources are used to raise the RSC in propagating trays in the net house. It is suspected that the disease originates from the rooting medium and the irrigation water. Hence, formation management strategies to check the disease spread is necessary for a successful local seed production programme based on non-conventional clonal seed system. Therefore, experiments were carried out to study the effect of the type of rooting medium, duration of steaming of the rooting medium and irrigation water treatment on the incidence of bacterial wilt on potato RSC grown in the nethouse.

MATERIALS AND METHODS

Experiment-1: Effect of rooting medium on bacterial wilt (*R.solanacearum*) incidence on potato stem cuttings.

An experiment was carried out in the nethouse at the Regional Agricultural Research and Development Centre, Bandarawela during Maha 1994/1995, Yala 1995, Maha 1995/1996 and Yala 1996 seasons to study the influence of rooting medium on bacterial wilt incidence.

The different types of rooting media used were saw dust, river sand, metal grit, and paddy husk. These materials were mixed in the ratio as follows; saw dust: river sand: paddy husk (1:1:1); saw dust: river sand: paddy husk (2:1:1). The control treatment consisted of an ordinary mixture of cowdung and sub soil in the ratio of 1:1. All rooting media were subjected to steaming for a period of 3 h prior to potting.

A 5 cm deep layer of each medium was spread in 45 x 30 cm plastic trays. Each treatment had 24 plants replicated three times. Stem cuttings were planted in Maha 1994/1995 and the experiment was repeated in Yala 1996. Trays were placed inside the nethouse

for about a month. During this period, recommended cultural practices of the Department of Agriculture were carried out. The bacterial wilt incidence was recorded at seven day intervals commencing from 7 days after planting. The wilted plants were discarded after recording.

Experiment-2: Effect of the duration of steaming of rooting medium on bacterial wilt (*R.solanacearum*) incidence.

The experiment was conducted at the Regional Agricultural Research and Development Centre, Bandarawela during Yala 1994 to test the effectiveness of steaming treatments in controlling bacterial wilt incidence.

In order to test the effectiveness of steaming, ordinary mixture containing cowdung: subsoil = 1:1 or ordinary mixture with *R.solanacearum* inoculate (10^8 cfu/ml) were exposed to steaming for 3 and 6 h. Ordinary mixture with the inoculate (10^8 cfu/ml) without steaming was used as the control.

Each treatment consisted of 3 kg of ordinary mixture packed in bags made of nylon netting. Isolation of *R.solanacearum* from each treatment (with 10 petri-dishes) was carried out by seeding plates with triphenyl tetrazolium chloride (TZC) agar medium (Kelman, 1954) and was replicated 3 times. The number of colony formation units (cfu) was recorded in each treatment.

Experiment-3: Influence of chlorine treatments of irrigation water on bacterial wilt (*R.solanacearum*) incidence in rooted stem cuttings.

The influence of chlorine concentrations of CaOCl_2 2.0, 2.25, 2.5, 2.75 and 3.00 ppm was compared with water not

treated with CaOCl_2 as a control. The pathogen level after 1 and 6 h of treatment was investigated during February 1994, at the Regional Agricultural Research and Development Centre, Bandarawela.

Ten millilitres of *R. solanacearum* suspension (10^8 cfu/ml) were added to each treatment containing 90 ml of sterilized water. In the control treatment, ninety millilitres of sterilized water were mixed with *R. solanacearum* alone. Isolation of *R. solanacearum* from each treatment (with 10 petri-dishes) was carried out by seeding plates and was replicated three times. Number of cfu was observed in each treatment for the presence or absence of *R. solanacearum*. In a preliminary study, the potato plants were irrigated with chlorinated water (2 ppm) and non-chlorinated water (check). Phytotoxic effect on potato plants was also examined.

RESULTS AND DISCUSSION

Experiment-1: Effect of rooting medium on bacterial wilt (*R. solanacearum*) incidence in potato stem cuttings.

Results showed that bacterial wilt (*R. solanacearum*) incidence was low in cuttings planted in ordinary mixture survival of rooted potato stem cuttings.

(cowdung: sub soil=1:1 by volume) steamed for 3 h (Table 1). This treatment gave the highest plant survival of 96%, 92%, 100% and 100% in Maha 1994/1995, Yala 1995, Maha 1995/1996 and Yala 1996 plantings, respectively compared with the other treatments. In the other treatments, plant survival rate ranged from 50-79% in Maha 1994/1995, 32-83% in Yala 1995, 45-85% in Maha 1995/1996 and 45-85% in Yala 1996.

Experiment-2: Effect of the duration of steaming of rooting medium on bacterial wilt (*R. solanacearum*) incidence.

Steaming of the rooting medium for 3 or 6 h gave zero level of bacterial wilt incidence whereas no steaming resulted in high bacterial wilt incidence of 7.68×10^3 cfu/ml (Table 2).

Further, 3 h and 6 h steaming resulted in raising the rooting medium temperature to 72°C and 80°C, respectively. Though 6 h steaming resulted in a temperature of 80°C which was greater than that under 3 h steaming (72°C), no bacterial wilt incidence was observed under both temperature regimes. This indicated that 3 h steaming is adequate

Table 1. Influence of rooting medium on the

Rooting medium	Mean plant survival (%)				Arc. Sin $\sqrt{\%}$ transformation			
	Maha 94/95	Yala 95	Maha 95/96	Yala 96	Maha 94/95	Yala 95	Maha 95/96	Yala 96
Saw dust	79	58	53	57	62.7	49.6	46.7	49.0
River sand	79	79	85	85	62.7	62.7	67.2	67.2
Metal grit	51	80	73	73	45.6	63.4	58.7	58.7
Paddy husk	50	32	45	45	45.0	34.5	42.1	42.1
Saw dust:River sand :Paddy husk (1:1:1)	79	83	52	52	62.7	65.7	46.1	46.1
Saw dust: River sand: Paddy husk (2:1:1)	67	60	48	58	54.9	50.7	43.8	49.6
Ordinary mixture (cowdung:sub soil=1:1)	96	92	100	100	78.5	73.6	90.0	90.0
LSD (P=0.05)					10.0	12.0	15.0	11.0
CV(%)					11.0	12.0	10.5	11.0

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to prevent bacterial wilt incidence of plants in the rooting medium. According to Zamora *et al.* (1994), sterilization of the potting mixtures by steam sterilization chamber and by maintaining the temperature at least at 83°C for 3 to 5 h is required for thorough sterilization. This is in agreement with the results obtained from the study reported in this paper.

Experiment-3: Influence of chlorine treatments of irrigation water on bacterial wilt (*R.solanacearum*) incidence in rooted stem cuttings.

Complete sterilization of irrigation water using sophisticated techniques is very costly. Therefore, this study was undertaken to investigate the feasibility of using a cheap technique with chlorine (calcium

Table 2. Effect of the duration of steaming of the rooting medium on bacterial wilt incidence.

Rooting medium	Steaming (h)	cfu*/ml	Highest temp. (°C) recorded in medium
<i>R.solanacearum</i> inoculate + Ordinary mixture ¹	3	0	72
<i>R.solanacearum</i> inoculate + Ordinary mixture	6	0	80
Ordinary mixture	3	0	72
Ordinary mixture	6	0	80
<i>R.solanacearum</i> inoculate + Ordinary mixture (control)	None	7.68x10 ³	28

* cfu- colony formation unit

¹ - Ordinary mixture - Cowdung:sub soil= 1:1

Table 3. Population of *R. solanacearum* at different chlorine concentrations after 1 h and 6 h of treatment.

Chlorine concentration in irrigation water (ppm)	Population of <i>R. solanacearum</i> cfu/ ml in treated water.	
	1 h	6 h
0	1.47x10 ⁴	1.68x10 ⁴
2	15	0
2.25	6	0
2.50	6	0
2.75	0	0
3	0	0

hypochlorite-33% chlorine) to sterilize the irrigation water. Chlorine concentrations ranging from 0 to 3 ppm were tested and the number of colony formation units (cfu) of *R. solanacearum* were counted after 1 and 6 h of the treatment (Table 3).

The results showed that the irrigation water treated with 2 ppm of calcium hypochlorite for 6 h or 2.75 ppm for 1 h completely sterilized the water (Table 3). Also, chlorine concentration of 2 ppm had no phytotoxic effect on potato plants, whereas higher levels showed adverse effects on the plants.

Secondary spread of bacteria takes place from infected plants by root contact, irrigation water and farm implements. In areas where cut tubers are used for planting, cutting knives may contribute to transmission of the pathogen to healthy tubers (Khanna and Vishwadhar, 1974). In hilly areas of India, bacterium from infected plants is carried by the river water and the disease appeared wherever river water is used for irrigating the potato crop (Shekhawat *et al.*, 1992). Shekhawat *et al.* (1988) further observed that application of stable bleaching powder 12 kg/ha mixed with fertilizer in furrows at the time of planting or soil drenching before or after first earthing up reduced bacterial wilt incidence by 80 per cent and increased the yield.

The results of these investigations suggest that one approach will not be sufficient to manage the bacterial wilt in rooted potato stem cuttings in the nethouse. The three approaches need to be integrated appropriately for achieving effective bacterial wilt management in rooted stem cuttings in the nethouse.

CONCLUSIONS

Bacterial wilt in rooted potato stem cuttings in the nethouse can be managed effectively by the integration of component technologies such as use of ordinary mixture of cowdung and sub soil (1:1), steaming of the rooting medium for 3 h and treating irrigation water with 2 ppm of calcium hypochlorite for 6 h or with 2.75 ppm for 1 h.

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