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EFFECTIVENESS OF DIFFERENT SUBSTRATE MATERIALS TO PREPARE *Trichoderma harzianum* BASED BIO-FUNGICIDES TO CONTROL FOOT AND ROOT ROT *(Fusarium oxysporum)* OF TOMATO

M. I. FARUK[1], M. L. RAHMAN[2], M. M. RAHMAN[3]
R. ISLAM[4] AND M. A. RAHMAN[5]

Abstract

An investigation was undertaken to evaluate the effectiveness *Trichoderma harzianum* based bio-fungicides multiplied on different substrates. The substrates was rice bran, wheat bran, grass pea bran and their combinations with mustard oilcake (MOC) were used to mass culture *T. harzianum* for the management of foot and root rot disease of tomato seedling caused by *Fusarium oxysporum* in seedbed. All combinations of carrier materials were found effective for preparing *T. harzianum* based bio-fungicides to promote germination, seedling growth and reducing pre-emergence and post-emergence mortality of tomato seedling under *F. oxysporum* inoculated seedbed soils. The shoot length, shoot weight, root length and root weight of tomato seedling were enhanced significantly by the application of different substrate materials of *T. harzianum* based bio-fungicides under *F. oxysporum* inoculated seedbed conditions. The individual (rice bran, wheat bran, grass pea bran) and combination of substrates (rice bran + wheat bran, rice bran + mustard oilcake, rice bran + wheat bran + MOC and wheat bran + grass pea bran + MOC) were equally suitable for mass culturing of effective *T. harzianum* bio-fungicides for the management of foot and root rot disease of tomato seedling in seedbed condition.

Keyword: *Trichoderma harzianum*, bio-fungicide, *Fusarium oxysporum*, tomato seedling, seedbed

Introduction

Tomato *(Solanum lycopersicum* L.) is one of the most popular vegetables in Bangladesh. The crop is cultivated in 24,800 hectare of land and the production is 232459 mt of fruit annually (Anon., 2012). The crop suffers from many diseases and incurs 30-40% yield loss every year (Anon., 1992). Among the diseases, seedling mortality due to the soil borne fungus, *Fusarium oxysporum* is prevalent throughout the tomato growing areas of the country (Anon., 19992). Management of *F. oxysporum* is difficult using fungicides and cultural practices.

[1&3]Senior Scientific Officer, Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), [2]Chief Scientific Officer, Training and Communication Wing, [4]Scientific Officer, Plant Pathology Division, BARI, [5]Chief Scientific Officer, Plant Pathology Division, BARI, Bangladesh.

Therefore, an alternative method like biological control of disease management may be practice if available. Growth of pathogenic fungus can be reduced by means of antagonistic microorganisms, which kill or compete with the pathogenic fungus. The bio-control agent, *T. harzianum* is abundant in soil under all climates over different geographical regions. It is known as efficient decomposers of various substrates having rapid growth rates and antimicrobial properties. The bio- control agents are naturally present in soil usually in low population. Thus increasing of its population density through artificial inoculation is necessary to achieve successful control of target fungus in soil. Available reports reveal that rice bran, wheat bran, maize bran, sawdust (Das *et al.*, 1997); rice straw, chickpea bran, ,grasspea bran, rice course powder, blackgram bran (Shamsuzzaman *et al.* 2003) and cow dung, poultry manure, groundnut shell, black ash (Rettinassababady and Ramadoss, 2000) are good substrates materials for multiplication of *T harzianum.* Reports on the results of conclusive study on the use of substrates for multiplication of *T harzianum* for the preparation bio-fungicides are not available in Bangladesh. The present investigation was conducted to evaluate effectiveness of different organic substrates for the preparation of *T. harzianum* based bio-fungicides against *F. oxysporum* causing foot and root rot disease of tomato in seedbed.

Materials and Method

Effectiveness of ten *T. harzianum* based bio-fungicides multiplied on three substrate materials was evaluated in the present experiment to control foot and root rot of tomato in seedbed. The substrate materials were rice bran, wheat bran, grasspea bran and their combinations mixed with or without mustard oilcake (MOC) were used to prepare the bio-fungicides. The experiment was conducted in a seedbed used under nethouse conditions of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARl), Gazipur during three consecutive years from 2010 to 2014. In this experiment barley grains colonized with *F. oxysporum* were incorporated in the seedbed soils @ 100g/m^2 soil. The pathogen was allowed to colonize the soil in seedbed for 10 days. A pure culture of *T. harzianum* (TM7) was grown in potato dextrose agar (PDA) medium which was used as inocula for preparation bio-fungicides.

The experiment was conducted in seedbed under nethouse condition of Plant Pathology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur during three consecutive years from 2010 to 2014. In this experiment barley grains colonized with *F. oxysporum* were incorporated in the seedbed soils @100 g/m^2 soil for inoculation. The pathogen was allowed to multiply in seedbed soil for 10 days. A pure culture of *T. harzianum* (TM7) was grown in potato dextrose agar (PDA) medium which was used as inocula of bio-fungicide. The treatments in the experiment were T_1= Rice bran, T_2= Wheat bran, T_3= Grasspea bran, T_4=

Rice bran + Wheat bran (1:1), T_5=Rice bran + Grasspea bran (1:1), T_6= Rice bran + Mustard oilcake (1:1), T_7= Rice bran + Wheat bran + MOC (1:1:1), T_8= Rice bran + Grasspea bran + MOC (1:1:1), T_9= Wheat bran + Grasspea bran + MOC (1:1:1), T_{10}= Rice bran + Wheat bran + Grass pea bran+ MOC(1:1:1:1), T_{11}=Seed treatment with provax and T_{12}= Control. According to the treatment combinations 600 g of individual or combination of substrate materials were taken separately in 1000 ml Erlenmeyer flask. The flask with substrate materials were sterilized in an autoclave at 121^0C for 15 minutes and cooled down to make it ready for inoculation. The sterilized substrate was inoculated individually with 5 mm diameter mycelia disc of five-day old culture of *T. harzianum* grown on PDA and then incubated at room temperature (25±2 ^{0}C) for 15 days. After incubation the colonized substrates were removed from the flasks and air dried and finally preserved in refrigerator at 10 ^{0}C. The inoculum of *T. harzianum,* colonized on different substrates, were incorporated to the previously *F. oxysporum* inoculated seedbed soils @ 100 g/m^2 soil and kept for 7 days maintaining proper soil moisture to establish *T. harzianum* in the soils. The control bed did not receive any colonized substrate of *T. harzianum* except the inoculum of *F. oxysporum*. The seeds of BARI Tomato-2 (Raton) were sown in the seedbed @ 200 seeds per treatment. The initial germination of the seeds was 98% as per blotter test result. The percent emergence of the seedling was calculated on the basis of initial germination status of the seeds. The experiment was laid out in completely randomized design (CRD) with four replications. Proper weeding, irrigation and intercultural operations were done to raise tomato seedlings in the seedbed. Data were collected on seedling emergence after 15 days of seed sowing. Similarly seedling mortality was recorded at an interval of 7 days starting from seedling emergence and it was continued up to 35 days of seedling age. The height and weight of shoot and length and weight of tomato seedlings were recorded at 35 days of seedling age. The percent data were converted into arcsine transformation values before statistical analysis. Data were analyzed statistically by using the MSTATC program. The treatment effects were compared by applying the least significant different (LSD) test at P=0.05 level.

Results and Discussion

a) Seedling emergence and pre-emergence mortality

Every year, the seedling emergence of tomato was significantly increased over control due to treatment of *F. oxysporum* inoculated seedbed soil with *T harzianum* bio-fungicides. Among the treatments the seedling emergence varied from 65.67- 75.00, 75.67-78.33 and 70.67-76.67% where the emergence under control was 50.67, 61.00 and 49.67% in first, second and third year, respectively. Seedling emergence under various treatments with the bio-fungicides was not significantly different (Table 1).

Table 1. Effect of different carrier material based *T. harzianum* bio-fungicides on the emergence and pre-emergence mortality of tomato seedling in *F. oxysporum* inoculated seedbed soil.

Name of substrates	Seedling emergence of tomato (%)				Pre-emergence seedling mortality (%)			
	1st year	2nd year	3rd year	Mean	1st year	2nd year	3rd year	Mean
Rice bran	69.67 ab	75.67 a	73.00 a	72.78 a	30.33 b	24.33 b	27.00 c	27.22 c
	(56.62)	(60.47)	(58.74)	(58.60)	(33.30)	(29.53)	(31.26)	(31.36)
Wheat bran	69.67 ab	76.00 a	72.33 a	72.69 a	30.33 b	24.00 b	28.67 c	27.67 c
	(56.58)	(61.37)	(58.30)	(58.75)	(33.41)	(28.58)	(31.70)	(31.23)
Grasspea bran	65.67 ab	76.33 a	75.67 a	72.56 a	34.33 b	23.67 b	25.33 c	27.78 c
	(54.13)	(62.75)	(59.82)	(58.90)	(35.86)	(29.06)	(30.18)	(31.70)
Rice bran + Wheat bran	71.00 ab	77.33 a	76.67 a	75.00 a	29.00 b	22.33 b	23.33 c	24.89 c
	(57.42)	(60.95)	(61.21)	(59.86)	(32.25)	(28.13)	(28.79)	(29.72)
Rice bran + Grass pea bran	68.67 ab	78.33 a	76.67 a	74.56 a	31.33 b	21.67 b	23.33 c	25.44 c
	(55.96)	(62.31)	(61.15)	(59.81)	(33.94)	(27.69)	(28.85)	(30.16)
Rice bran + Mustard oilcake	75.00 a	77.33 a	75.33 a	75.89 a	25.00 b	22.67 b	23.67 c	23.78 c
	(60.00)	(61.66)	(60.29)	(60.65)	(31.12)	(28.40)	(29.72)	(29.75)
Rice bran + Wheat bran + MOC	71.67 ab	77.33 a	74.33 a	74.44 a	28.33 b	22.33 b	25.67 c	25.44 c
	(57.84)	(60.87)	(59.66)	(59.46)	(31.98)	(28.34)	(30.34)	(30.22)
Rice bran + Grasspea bran + MOC	66.67 ab	78.33 a	74.33 a	73.11 a	33.33 b	21.67 b	25.67 c	26.89 c
	(54.74)	(62.43)	(59.62)	(58.93)	(35.21)	(27.57)	(29.71)	(30.83)
Wheat bran + Grass pea bran + MOC	72.00 ab	78.00 a	70.67 a	73.56 a	28.00 b	22.00 b	29.33 c	26.44 c
	(58.05)	(62.37)	(57.27)	(59.23)	(31.74)	(27.63)	(32.74)	(30.70)
Wheat bran + Grass pea bran+ Rice bran + MOC	69.00 ab	77.33 a	71.33 a	72.55 a	31.00 b	22.67 b	28.67 c	27.45 c
	(56.17)	(61.60)	(57.67)	(58.48)	(33.76)	(28.40)	(32.33)	(31.50)
Seed treatment with Provax	59.67 bc	77.00 a	60.00 b	65.56 b	40.33ab	23.00 b	40.00 b	34.44 b
	(50.58)	(60.60)	(50.78)	(53.99)	(39.41)	(28.63)	(39.22)	(35.75)
Control	50.67 c	61.00 b	49.67c	53.78 c	49.33 a	39.00 a	50.33 a	46.22 a
	(45.38)	(52.58)	(44.81)	(47.59)	(46.61)	(38.63)	(45.19)	(43.48)

Values in a column having same letter did not differ significantly (p=0.05) by LSD; values within the parentheses were the Arcsine Transformed values.

On the contrary, treatment of seedbed soils with the bio-fungicides caused significant reduction in pre-emergence seedling mortality of tomato compared to control. The range of seedling mortality was 25.00-34.33% in first year, 21.67-24.33% in second year and 23.33-29.33% in third year. The corresponding mortality under control was 49.33, 39.00 and 50.33%, respectively. Efficacy of all treatments with the bio-fungicides to reduce the pre-emergence mortality was not significantly different (Table 1).

b) Post-emergence mortality

Post-emergence mortality of tomato in *F. oxysporum* inoculated seedbed soil was 23.67, 31.67 and 21.67% under control in 1st, 2nd and 3rd year of study, respectively. Treatment of seedbed soil with the bio-fungicides reduced the disease incidence to 60.58-74.65, 58.95-67.38 and 64.61-67.70%, respectively. The reduction was significant under every bio-fungicide. Efficacy of all treatments with the bio-fungicides to reduce the disease incidence was not significantly different (Table 2).

c) Shoot growth

Under control, shoot length was 23.53 cm in first year, 14.87 cm in second year and 16.13 cm in third year. Treatment of seedbed soils with *T harzianum* based bio- fungicides multiplied on rice bran, wheat bran, grasspea bran alone or in different combinations increased the shoot length to 27.07-30.13, 15.87-19.33 and 24.17-33.60 cm in 1st, 2nd and 3rd year, respectively. The shoot weight under control was 4.66, 4.10 and 4.23 $gplant^{-1}$ in first, second and third year, respectively. *T. harzianum* based bio-fungicidal treatments of seedbed soils increased the parameter to 7.34- 8.01, 5.51-6.71 and 6.97-10.03 $gplant^{-1}$, respectively. Every year, the increase in length and weight of shoot of tomato seedling due to bio-fungicidal seedbed soil treatment was significant compared to control. Effect of the treatments on shoot growth was more or less similar (Table 3).

d) Root growth

Every year, the root length of tomato seedling was significantly lower in non-treated seedbed (control) compared to bio-fungicide and Provax treated beds. In first, second and third year, the root length of tomato seedlings ranged 7.13-10.17, 6.93- 8.27 and 6.87-10.67 cm under different treatments and 5.50, 4.70 and 4.97 cm in control seedbeds, respectively (Table 4).

In first, second and third year, the ranges of root weight were 0.42-0.46, 0.50-0.58 and 0.71-0.95 $gplant^{-1}$, respectively in seedbed treated with bio-fungicides multiplied on various substrate materials and 0.33, 0.39 and 0.54 $gplant^{-1}$ in control seedbeds, respectively (Table 4). The root weight was significantly higher compared to seedbeds received no bio-fungicide or Provax. Effect of the treatments on root growth was more or less similar (Table 4).

Table 2. Effect of various carrier material based *T. harzianum* bio-fungicides on the post-emergence mortality of tomato seedling in *F. oxysporum* inoculated soils in seedbed.

Name of substrates	Post-emergence seedling mortality (%)				Seedling mortality reduced than control in consecutive three years (%)			
	1st year	2nd year	3rd year	Mean	1st year	2nd year	3rd year	Mean
Rice bran	9.00 b (17.46)	13.00 b (21.09)	7.33 b (15.66)	9.78 b (18.07)	61.98	58.95	66.17	61.90
Wheat bran	9.33 b (17.79)	11.00 b (19.36)	7.33 b (15.70)	9.22 b (17.62)	60.58	65.27	66.17	64.08
Grasspea bran	7.67 bc (16.08)	12.67 b (20.81)	7.33 b (15.60)	9.22 b (17.50)	67.58	59.99	66.17	64.08
Rice bran + Wheat bran	7.33 bc (15.71)	12.33 b (20.54)	7.33 b (15.70)	8.99 b (17.32)	69.03	61.07	66.17	64.98
Rice bran + Grasspea bran	8.00 bc (16.43)	10.33 b (18.72)	7.67 b (16.07)	8.67 b (17.07)	66.20	67.38	64.61	66.23
Rice bran + Mustard oilcake	7.67 bc (16.08)	12.33 b (20.51)	7.33 b (15.60)	9.11 b (17.40)	67.58	61.07	66.17	64.51
Rice bran + Wheat bran + MOC	8.33 bc (16.78)	12.00 b (20.26)	7.00 b (15.24)	9.11 b (17.43)	64.81	62.11	67.70	64.51
Rice bran +Grasspea bran +MOC	8.00 bc (16.43)	11.33 b (19.97)	7.33 b (15.68)	8.89 b (17.36)	66.20	64.22	66.17	65.37
Wheat bran + Grasspea bran + MOC	9.00 b (17.79)	12.67 b (21.07)	7.00 b (15.32)	9.56 b (18.06)	61.98	59.99	67.70	62.76
Wheat bran + Grasspea bran+ Rice bran + MOC	6.00 c (14.18)	12.33 b (20.54)	7.33 b (15.68)	8.55 b (16.80)	74.65	61.07	66.17	66.89
Seed treatment with Provax	8.67 bc (17.12)	13.00 b (21.09)	7.00 b (15.32)	9.56 b (17.84)	63.33	58.95	67.70	62.76
Control	23.67 a (28.66)	31.67 a (34.22)	21.67 a (27.70)	25.67 a (30.19)	-	-	-	

Values in a column having same letter did not differ significantly (p=0.05) by LSD; values within the parentheses were the Arcsine Transformed values.

Table 3. Role of different carrier material based *T. harzianum* bio-fungicides on the shoot growth of tomato seedling in *F. oxysporum* inoculated seedbed soil.

Name of substrates	Shoot length (cm)				Shoot weight (g/plant)			
	1st year	2nd year	3rd year	Mean	1st year	2nd year	3rd year	Mean
Rice bran	27.43 b	15.87 de	24.50 b	25.93 a	7.38 b	5.51 b	8.07 ab	6.99 ab
Wheat bran	27.20 b	16.93 c	24.43 b	22.85 ab	7.37 b	5.54 b	6.97 bc	6.63 a
Grasspea bran	27.10 b	17.23 c	24.17 b	22.83 ab	7.42 b	5.57 b	7.63 b	6.87 ab
Rice bran + Wheat bran	30.13 a	19.33 a	32.13 a	27.20 a	8.01 a	6.48 a	8.47 ab	7.65 a
Rice bran + Grasspea bran	27.80 b	18.63 ab	33.60 a	26.68 a	7.29 b	6.24 a	10.03 a	7.85 a
Rice bran + Mustard oilcake	27.07 b	18.47 ab	31.67 a	25.74 a	7.36 b	6.24 a	9.17 ab	7.59 a
Rice bran + Wheat bran + MOC	27.67 b	16.60 cd	31.33 a	25.20 a	7.34 b	5.63 b	8.30 ab	7.09 a
Rice bran +Grasspea bran +MOC	27.87 b	18.40 b	31.40 a	25.89 a	7.35 b	6.49 a	8.50 ab	7.45 a
Wheat bran + Grasspea bran + MOC	27.57 b	18.13 b	33.00 a	26.23 a	7.59 b	6.71 a	9.13 ab	7.81 a
Wheat bran + Grasspea bran+ Rice bran + MOC	27.80 b	16.73 cd	30.13 a	24.89 a	7.59 b	5.60 b	8.50 ab	7.23 a
Seed treatment with Provax	27.13 b	15.47 ef	22.83 b	21.81 ab	5.64 c	4.43 c	5.20 cd	5.09 c
Control	23.53 c	14.87 f	16.13 c	18.18 b	4.66 d	4.10 c	4.23 d	4.33 c

Values in a column having same letter did not differ significantly (p=0.05) by LSD.

Table 4. Role of various carrier material based *T. harzianum* bio-fungicides on the root growth of tomato seedling in *F. oxysporum* inoculated seedbed soil.

Name of substrates	Root length (cm)				Root weight (mg/plant)			
	1st year	2nd year	3rd year	Mean	1st year	2nd year	3rd year	Mean
Rice bran	7.20 b	6.93 b	8.47 b	7.53 abc	0.45 b	0.55 ab	0.71 ab	0.57 ab
Wheat bran	7.50 b	7.07 ab	6.87 cd	7.15 bc	0.42 e	0.50 b	0.72 ab	0.55 ab
Grasspea bran	7.97 b	7.47 ab	8.13 bc	7.86 ab	0.43 d	0.52 b	0.71 ab	0.55 ab
Rice bran + Wheat bran	7.77 b	7.20 ab	8.93 b	7.97 abc	0.43 d	0.58 a	0.95 a	0.65 a
Rice bran + Grasspea bran	7.43 b	7.87 ab	8.97 b	8.09 abc	0.43 d	0.54 ab	0.85 ab	0.61 a
Rice bran + Mustard oilcake	7.40 b	7.20 ab	9.73 ab	8.11 abc	0.42 e	0.57 a	0.93 a	0.64 a
Rice bran + Wheat bran + MOC	7.67 b	7.23 ab	9.23 ab	8.04 abc	0.43 d	0.52 b	0.87 a	0.61 a
Rice bran +Grasspea bran +MOC	8.50 b	8.27 a	9.53 ab	8.77 a	0.43 d	0.56 ab	0.90 a	0.63 a
Wheat bran + Grasspea bran + MOC	7.13 b	7.20 ab	10.67 a	8.33 ab	0.44 c	0.58 a	0.91 a	0.64 a
Wheat bran + Grasspea bran+ Rice bran + MOC	10.17 a	7.80 ab	8.53 b	8.83 a	0.46 a	0.58 a	0.81 ab	0.62 a
Seed treatment with Provax	7.73 b	5.80 c	6.27 de	6.60 c	0.37 f	0.42 c	0.67 ab	0.49 bc
Control	5.50 c	4.70 d	4.97e	5.06 d	0.33 g	0.39 d	0.54 b	0.42 c

Values in a column having same letter did not differ significantly (p=0.05) by LSD.

e) Effect of Provax

Treatment of seedbed soil infested with *F oxysporum* with Provax also reduced the incidence of foot and root rot and increased shoot and root growth of tomato seedlings over control. However, its efficacy was lower compared to bio-fungicides (Tables 1- 4).

Results of the present experiment reveal that efficacy of all *T harzianum* based bio- fungicides multiplied on rice bran, wheat bran, grasspea bran used alone or in different combinations mixed with or without MOC are effective to control foot and root disease of tomato seedling in seedbed and to achieve satisfactory increase in seed germination, pre- and post-emergence seedling mortality. Similar findings have been reported by other researchers (Bentez *et al.,* 2004; Mausam *et al.,* 2007; Pros ad and Anes, 2008; John *et al.,* 2010). The fungus *Trichoderma harzianum*, a well-known antagonistic fungus prevailing in the soil, was being used in many crops, like lettuce, tomato, onion, cotton, grapes, peas, apples, sweet corn and carrots to control various diseases caused by *Phytophthora, Pythium, Sclerotinia, Botrytis, Rhizoctonia* and *Fusarium* (Benítez *et al*., 2004; Mausam *et al.,* 2007). It was reported that *T. harzianum* remarkably proliferated the root system and accelerated biological nitrogen fixation in addition to the reduction of diseases caused by *F. oxysporum* and *Pythium* spp. in legume crops (John *et al*., 2010). This findings are in accordance with the observation of the present study where soil is treated with different carrier material based *T. harzianum* bio-fungicides that enhanced the growth of tomato seedling in *F. oxysporum* inoculated seedbed soils though the degree of shoot and root growth varied among the treatments. Harman, (2006) and Manju and Mall, (2008) also reported positive role of *Trichoderma* species in increasing plant growth and productivity. In present experiment there is significant increase in emergence, shoot and root length and also shoot and root weights of tomato seedling due to *T. harzianum* bio-fungicides which is supported by the findings of many investigators (Prasad and Anes, 2008; Mishra and Sinha, 2000; Chaur-Tsuen and Chien-Yih, 2002). Enhanced seed germination due to *Trichoderma* species has also been reported by Mukhtar (2008). It has been reported that *Trichoderma* isolates possesses the ability to compete for key exudates from seeds that stimulate germination of propagules of plant pathogenic fungi in the soil as they compete with microorganisms for nutrient and space. The three well known mechanisms associated with pathogen control by *Trichoderma* were competition for nutrients, antibiosis, and myco-parasitism (Chet, 1987). It has been noticed by Tjamos *et al*. (1992) that *T. harzianum* controls *F. oxysporum* by competing for both rhizosphere colonization and nutrients. They observe that bio-control of targeted pathogen became more effective with the decline of nutrient concentration of the soil. The study confirm the reports of other researchers regarding the role of *T. harzianum* to enhance seed germination and

root and shoot growth of seedlings (Dubey *et al*., 2007) as well as increasing the frequency of healthy plants (Rojo *et al*., 2007).

Rice bran based *Trichoderma* bio-fungicide gave maximum seed germination, reduced seedling mortality and increased growth of tomato seedling. Similar observation with wheat and rice bran for the formulation of *T harzianum* bio-fungicide was reported by Sangeetha *et at.* (1993). Disease incidence of tomato, water melon and cotton was reported to be reduced considerably by the application of *T harzianum* (Sivan and Chet, 1986). Shoresh *et al.* (2005) stated that *Trichoderma* spp. were effective bio-control agents for a number of soil borne plant pathogens and induced a potentate state in the plant enabling it to be more resistant to subsequent pathogen infection.

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