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Application of seed coating with endophytic bacteria for Fusarium wilt disease reduction and growth promotion in tomato

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Abstract Tomato seed coating with endophytic bacteria (SuRW02) was used to control Fusarium wilt disease of tomato and stimulate**d** plant growth in field conditions. Results showed that seed coating with isolate SuRW02 reduced disease incidence and severity which similar to seed coating with chemical fungicide (Captan). Seed coating with endophytic bacteria and Captan showed disease incidences of 38 and 25% and disease severity indices of 0.37 and 0.25, respectively. Uncoated seed**s** and seed coating without endophytic bacteria had a disease incidences of 70 and 50% and disease severity indices of 2.00 and 1.25, respectively. In addition, seed coating with endophytic bacteria promoted tomato plant growth and quality of tomato production.

Keywords: seed coating, tomato, biological control, endophytic bacteria

Introduction

Seed production now employs seed coating technology to increase seed quality. In the past, seed coating with chemicals was mostly used to control pathogens; however, while proven to be efficient, these chemical coatings were not allowed for organic farming. Therefore, a technique is required which will improve seed quality, offer pathogen protection and stimulate plant growth that could be widely used in both conventional and organic farming. Seed coating with beneficial microorganisms has recently gained attention as a promising method. Endophytic bacteria are microbes that live inside plants without causing detrimental effects. They can also accelerate seedling emergence, promote plant growth and prevent disease development (Ryan *et al*., 2008). Tomato plants inoculated with endophytic bacteria showed significantly increased growth attributes (Khan *et al*., 2014). Abdallah *et al*. (2016) found that endophytic bacteria promoted growth of tomato plants by creating stimulatory mechanisms.

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Several biologically controlled studies of seed coating with antagonistic microorganisms have shown that they can effectively reduce diseases. Groundnut seeds coated with *Pseudomonas fluorescens* reduced charcoal rot disease caused by *Macrophomina phaseolina* at 61-70% (Shweta *et al*., 2008) while cucumber seeds coated with *Bacillus subtilis* controlled gummy stem blight disease at 20% (Kaewkham *et al*., 2016) and sweetcorn seed coated with *Bacillus amyloliquefaciens* KPS46 reduced bacterial leaf streak disease caused by *Acidovorax avenae* subsp. *avenae* at 68.31% (Kasem *et al*., 2011). Thus, seed coating with endophytic bacteria has a synergistic effect on seed improvement and biological control activities. In tomatoes, Ahmed *et al.* (2016) found that tomato seeds coated with nickel nanoparticles could be used to control Fusarium wilt, while Zohora *et al.* (2016) reported that tomato seed coated with *Basillus subtilis* RB14 could suppress *Rhizoctonia solani*. Kipngeno *et al*. (2015) determined that coating tomato seeds with *Bacillus subtilis* and *Trichoderma asperellum* was effective for the management of damping-off disease. In our previous studies, we found that tomato seeds coated with endophytic bacteria SuRW02 reduced disease incidence in the laboratory and under greenhouse conditions at about 35 and 34% respectively (Prasom *et al*., 2017). Therefore, here, tomato seeds coated with endophytic bacteria SuRW02 was tested to control Fusarium wilt disease in the field condition. Results will act as a guideline for food production safety in the future.

Materials and methods

Effect of seed coating with endophytic bacteria to control Fusarium wilt disease

In this experiment, endophytic bacteria were used as active ingredients for seed coating by mixing with a binder substance at a concentration of 70%. Endophytic bacterial isolate SuRW02 was used as a bacterial cell suspension at $1x10^{8}$cfu/65g seed. For the seed coating process, tomato seeds were cleaned with 0.6 percent sodium hypochlorite for 5 minutes, then washed with distilled water, sterilized 3 times and dried in laminar airflow. The sterilized tomato seeds were coated with a binder substance mixed with bacterial cell suspension using rotary plate type seed-coating machine. Coated seeds were stored at cool temperatures in a refrigerator.

This experiment was laid out in a completely randomized design (CRD) with 4 treatments and 8 replications as follows:-Treatment1 non-coated seed (NCS), Treatment2 coated seed (CS), Treatment3 coated seed with endophytic bacteria (CS+B) and Treatment4 coated seed with Captan (CS+C). Tomato seeds in each treatment were planted in peat moss. After 21 days, the tomato seedlings in each treatment were transplanted in soil mixed with *Fusarium oxysporum* (pathogen) at a concentration of 10^4 spores/ml at a volume of 100 ml per 1kg soil. Disease severity was evaluated at 7, 14 and 28 days after inoculation by using a 0-3 scoring method modified from Marlatt *et al.* (1996); wherein: 0 = healthy, 1 = temporary wilt, 2 = permanent wilt and 3= plant death.

Effect of seed coating with endophytic bacteria on the growth promotion

The experimental design was described previously. It was divided into 2 groups of non-inoculated and inoculated. Tomato seeds in each treatment were planted in peat moss. After 21 days, the tomato seedlings in each treatment were transplanted into soil inoculated with *Fusarium oxysporum* and soil without pathogen inoculation. Growth promotion of the tomato plants was evaluated at 25 days after transplantation by measuring shoot height and stem diameter. Data were collected every week for 100 days, with harvesting after 60 days of fruiting. Measurements included shoot height, stem weight, yield, weight per plant and total soluble solids.

Detection of endophytic bacteria in tomato plants

Endophytic bacteria in tomato seedlings were grown from bacterial-coated seeds isolated from the root, stem and leaf tissue which followed the process of Prasom *et al.* (2017). Colony morphology was compared with the morphology of SuRW02 by counting only similar colonies.

Results

Pathogenicity test

A pathogenicity test found that tomato seedlings showed wilt symptoms at 14 days after inoculation (Figure 1).

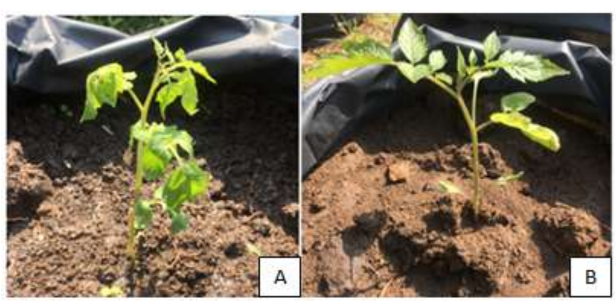

Figure 1. Symptom of Fusarium wilt disease on tomato seedling at 14 days after inoculation (A) and normal tomato seedling (B)

Effect of seed coating with endophytic bacteria to control Fusarium wilt disease

Results showed that tomato plants grown from seeds coated with endophytic bacteria reduced disease incidence and disease severity similar to seed coated with Captan. Plants grown from seed coated with endophytic bacteria and seed coated with the chemical (Captan) had disease incidences of 37.5 and 25.0 % and disease severity indices of 0.37 and 0.25, respectively. Coated seeds gave significantly different when compared with the uncoated seeds and seeds coated without endophytic bacteria with disease incidencse of 70.0 and 50.0 % and disease severity indices of 2.00 and 1.25, respectively (Table 1).

Table 1. Effect of seed coating for each method on disease incidence and severity of Fusarium wilt disease

Treatment	Disease incidence (%)	Disease severity index
NCS[1]	75.00	2.00a[2]
CS	50.00	1.25ab
CS+B	37.50	0.37b
CS+C	25.00	0.25b
F-test	-	*[2]

[1]NCS = non-coated seed, CS = coated seed, CS+B =coated seed with endophytic bacteria and CS+C = coated seed with Captan

[2]ns =non-significant,* = significant difference at P=0.05 according to Duncan's multiple range test.

Table 2. Effect of seed coating for each method on growth, yield and quality of tomato

Treatment		Height of shoot (cm)	Fresh weight of shoot (g)	Stem diameter (mm)	Yield weight per plant (g)	Total soluble solids(°brix)
Non-inoculated	NCS[1]	119.375b	134.25	4.10	366.98	5.67b
	CS	114.875b	146.50	3.70	399.11	5.45b
	CS+B	153.00a	182.75	4.33	357.72	6.95a
	CS+C	117.75b	138.00	4.51	403.16	5.57b
F-test		*	ns	ns	ns	*
Inoculated	NCS[1]	57.75c	116.50	1.79c	42.96c	5.23b
	CS	75.87b	124.00	2.84bc	163.17b	5.61b
	CS+B	128.12a	139.75	4.45a	407.85a	5.95a
	CS+C	115.00ab	128.75	4.10ab	355.78a	5.85ab
F-test		*[2]	ns	*	*	*

[1] NCS = non-coated seed, CS = coated seed, CS+B =coated seed with endophytic bacteria and CS+C = coated seed with Captan

[2] ns =non-significant,* = significant difference at P=0.05 according to Duncan's multiple range test.

Effect of seed coating with endophytic bacteria on growth promotion

Seed coating with SuRW02 improved growth and yield of plants that affected by the pathogen. Shoot height and total soluble solids were significantly different from the other treatments. Shoot weight, stem diameter and yield weight per plant in all treatments showed no significant differed. In addition, the inoculated group containing tomato plants grown from seeds coated with SuRW02 (CS+B) showed the best growth and yield compared with other treatments but with no significant difference from seeds coated with Captan (CS+C). Both the growth and yield of coated treatments were higher than uncoated seed and seed without endophytic bacteria (Table 2).

Detection of endophytic bacteria in tomato plants

The amount of endophytic bacteria in the roots, leaves and shoots of tomato plants grown from seeds coated with SuRW02 were detected at 4.36, 3.75 and 3.71 log cfu/g, respectively (Figure 2), while endophytic bacteria were not detected in tomato tissue grown from uncoated-seed (control) as the typical colony.

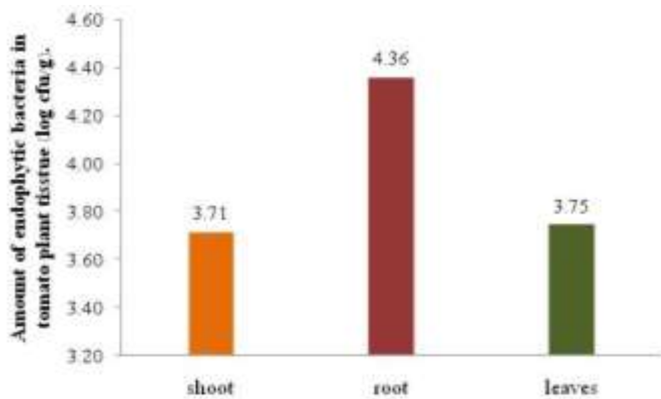

Figure 2. Amount of endophytic bacteria detected in tomato plants

Discussion

This experiment revealed that tomato plants grown from bacterial-coated seeds showed increased tolerance to Fusarium wilt disease, similar to chemical treatment (seeds coating with Captan), while disease incidence and disease severity observed in the field condition were not significantly different. Similarly, to our previous experiment, we found that tomato seed coating with endophytic bacteria SuRW02 reduced disease incidence and disease severity both under laboratory and greenhouse conditions at 34.75 and 34.09%, respectively (Prasom *et al.,* 2018). Abdallah *et al.* (2016) reported that endophytic bacteria reduced wilt disease caused by *Fusarium* sp. in tomato at 75%. The endophytic bacteria gave indirect protection by promoting growth, and reduced disease development in the plant. Kaewkham (2017) also reported that seed coating with beneficial bacteria reduced disease through preventing the destruction of pathogens before and after seed germination by various mechanisms including antagonistic, antibiotics, metabolites, antifungal enzymes, competition with pathogens and induction of resistance in plants. Furthermore, Abdallah *et al*. (2016) and Nandhini *et al.* (2012) reported that endophytic bacteria from tomato plants could control *F. oxysporum* by synthesizing various allelochemicals that affect fungal growth, such as chitinase and β-1,3-glucanase, related to fungal cell wall digestion. In addition, several authors reported the use of seed coating with antagonistic bacteria for controlling many pathogens such as groundnut seed coating with *Pseudomonas fluorescens* to reduce charcoal rot disease caused by *Macrophomina phaseolina* at 61-70 percent. This technique also increased the yield of peanuts. Kaewkham *et al*. (2016) reported that cucumber seed coating with *Bacillus subtilis* effectively controlled gummy stem blight disease caused by *Didymella bryoniae* at 22-24 percent, while seed coating with *Bacillus subtilis* Tu-100 reduced disease *et al* caused by *Sclerotium rolfsii* on oilseed rape (Pate *et al*., 2012).

Plant growth promotion by endophytic bacteria was reported in tomato plants by creating stimulants such as indole-3-acetic acid (IAA), resulting in stimulated cell elongation, cell division and cell transformation (Hu., 2011; Shahab *et al.*, 2009). In addition, bacteria can produce other hormones such as cytokinin, ethylene, gibberellin, and 1-aminocyclopropane-1-carboxylate deaminase (Noel *et al*., 1996). Glick (2012) and Kaewkham (2017) reported that beneficial bacteria have other mechanisms to promote growth in plants including nitrogen fixation, plant nutrient dissolution, and by creating siderophore and plant growth regulators. Thus, application of endophytic bacteria as seed coating has been proven effective for plant disease control and growth promotion.

At the end of the experiment, the amount of endophytic bacteria in plant tissues were detected by a cultural technique, with only similar colonies counted according to the morphological characteristics of SuRW02. Results indicated that detectable amounts of endophytic bacteria remained in shoots, root and leaves of tomato plants grown from coated seed, while endophytic bacteria were not detected in tomato tissue grown from uncoated seeds (control). This indicated that the beneficial effect obtained from the experiment originated from endophytic bacteria. Therefore, we can conclude that endophytic bacteria SuRw02 isolated from tomato tissue are capable of reducing Fusarium wilt disease and promoting plant growth under laboratory conditions. In addition, when used as a seed coating to reduce Fusarium wilt disease in the field condition, results were similar to using a chemical coating. Thus, seeds coated with endophytic bacteria SuRW02 show promising applications for controlling Fusarium wilt disease and promoting the growth of tomato.

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