



Research Article

Suppressing Bacterial Wilt (*Ralstonia solanacearum*) by Grafting Tomato Seedlings Grown on Six Comparable Media

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ABSTRACT

Bacterial wilt caused by *Ralstonia solanacearum* is an important disease of tomatoes. The grafting method with a resistant rootstock variety is a reliable control technique and has been carried out in many countries. Healthy tomato seedling are needed for grafting so this study was aimed to find a good seedling medium to grow the upper stem and rootstock of tomato for grafting and to know the effect of seed media on the intensity of bacterial wilt. Tomato seeds were grown in coir and rice husk charcoal with ratio A (10:0), B (9:1), C (8:2), D (7:3), E (6:4), F (5:5) (coir: rice husk charcoal, v/v). Seedlings were watered regularly and after seedlings aged 21 days, the grafting was conducted. Agronomic observations were justified by measuring germination style, plant height, leaf area, fresh mass and dry mass of plants at 21 days after planting. Tomato seedlings were grafted by using H-7996 variety as a rootstock and Servo variety as an upper stem. Tomato seedlings were transplanted into polybags and then inoculated with *R. solanacearum* (10^8 CFU/ml). The disease intensity and AUDPC (Area Under Disease Progress Curve) were recorded. The results revealed that the media with a ratio of 8:2 (coir : rice husk charcoal, v/v) is the best medium for growing tomatoes and the grafting treatment could reduce the disease intensity of bacterial wilt by 40% compared with non grafted treatment and reduce 16% disease intensity compared to the grafted treatment with seedling sown in other ratio media at the last observation.

Keywords: bacterial wilt; coir; grafting; *Ralstonia solanacearum*; tomato

INTRODUCTION

Bacterial wilt is an important disease of tomato in terms of area distribution and damage caused. In the field, 100% of Solanaceae plants were damaged by this disease (Goto, 1992). This disease commonly attacks during the rainy season because of high humidity and temperature, or poor drainage (Ashari, 1995). The pathogen attack the xylem tissue by inhibiting the water transportation of tomato plants caused the plants to become wilt, yellow, and stunted. Bacteria can infect the plants through the water flow, loose soil, and removal of plants. Bacteria can survive in the soil for several months to years (Arwiyanto, 2013).

Plant grafting is an effort to improve the growth and development of plants from unfavorable conditions, including to be resistance to disease. The tomato plants are grafted with eggplant rootstock to survive on the very humid soil conditions (because eggplant can survive on soils with high

water content) and to overcome soil-borne diseases (Arwiyanto *et al.*, 2015).

The good seedling media for tomato are needed to get healthy seedling for grafting. Growing media has an important role in the growth of seedlings after germinated will require sufficient nutrients and place to grow optimally. Organic material can be used as a growing medium is coconut waste (cocopeat). Cocopeat is a growing media produced from the process of coir by producing fiber and fine powder or cocopeat (Irawan & Hidayah, 2014). The use of organic materials such as coir and rice husk charcoal is very potential to be used as a composite of alternative planting media to reduce the use of topsoil. The advantage of using organic material as a planting medium is that it has a structure to maintain aeration, abundantly available, cheap, and can be utilized as an alternative growth media (Putri, 2008). However, this research was conducted to find out the most effective seed media for tomato seedling for the grafting method and their effect on the intensity bacterial wilt disease.

MATERIALS AND METHODS

This study was conducted in December 2018 until September 2019. Coir and rice husk charcoal were mixed with a certain composition A (10:0), B (9:1), C (8:2), D (7:3), E (6:4), F (5:5) (coir : rice husk charcoal, v/v) (Table 1) to select the best composition for seedling media of tomato plants. After the media were mixed then H-7996 seeds were sown in the media (A, B, C, D, E, F), for the without grafting treatment H-7996 were sown in media C and after 21 das (days after sowing) the seedlings were grafted and observed agronomically while at 28 das tomato was inoculated with 10^8 CFU/ml of *R. solanacearum* (race 1 biovar 3 phylotype 1) by pouring 5 ml of the suspension to each plant, to know the disease intensity. This research was designed using a Completely Randomized Design (CRD) with 6 treatments and 15 replications for the observing media seedling and 7 treatments and 9 replications for the disease intensity.

Table 1. The volume ratio of six media seedlings

Media seedling	Coir	Rice husk charcoal
A	10	0
B	9	1
C	8	2
D	7	3
E	6	4
F	5	5

Observation on Seedling Media

Bulk Density

Bulk density was measured by putting the media into a measuring cup (100 ml) up to 50 ml. After that, the measuring cup was tapped to be solid. If the soil loosed when it was tapped, then added again to 50 ml. The soil was weighed on an analytical scale and then recorded. The result was then divided by 50 ml thus the units become g/cm^3 (Ciptaningtyas & Suhardiyanto, 2016).

pH

The pH of the seedling media was measured using a pH meter at a ratio of 1:5. Ten gram of soil samples were weighed, put into a glass and added 50 ml of distilled water, stirred until homogeneous. After that, the mixture was incubated until formed

sediment and then measured with a pH meter. The pH meter must not be exposed to the soil (Irfan, 2014).

Conductivity

The conductivity was measured using Saturated Media Extract (SME) method according to Landis & Dumroese (2006). The seedling media was mixed with distilled water, stirred and incubated until saturate. After saturation, the mixture was filtered using filter paper and the conductivity of the filtered-water was measured using an EC meter (EZ-1).

Porosity

The media porosity was measured according to Day (1964) *cit.* Ciptaningtyas & Suhardiyanto (2016) by putting the media into a measuring cup up to 500 ml without tapping. Then add water until the mixture was saturated. The addition of water was conducted as long as it did not change the volume of seedling media in the measuring cup and then stopped when the volume of seedling media started to change. The porosity of the media was calculated using this equation:

$$\text{Porosity} = \frac{\text{Volume of water mixed until the media was saturated}}{\text{Volume of seeding media}} \times 100\%$$

Observation on Tomato Seedling

Germination rate

Seedling media A (10:0), B (9:1), C (8:2), D (7:3), E (6:4), F (5:5) (coir : rice husk charcoal, v/v) were placed in Petri dishes then 10 seeds of H-7996 were buried in Petri dishes, each treatment contained 3 Petri dishes with 10 seeds in each petri dish, hence there were 30 replications. After the seed was immersed, the seedling media was watered using sprayer to maintain the moisture. On the 7th day, the number of germinated seeds was calculated using this formula by Awasthi *et al.* (2016):

$$\text{GP (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100$$

Plant height

The plants aged 21 dap (day after planting) was measured their height from the ground surface to the apical bud using a ruler.

Leaf Area

Leaf area was measured after the plants aged 21 dap using Leaf Area Meters, model A3 Light box gCL Bubble Etch Tanks. The leaves of each plant were separated from the stem and the roots were collected and laid out on the paper. After the samples were placed in front of the Leaf Area Meter camera, the tool would detect the leaf area automatically with its unit area (mm).

Fresh and Dry Mass of Canopy and Root

The ratio of fresh and dry mass was measured by cutting the root and canopy and then separated. For the fresh mass of the root, the roots were weighed in a fresh state after being cut as well as the fresh mass of the canopy. For root dry mass, the cut roots were dried with an oven at temperature of 60–80°C and then weighed until the dry mass was constantly produced. Histogram was made to determine the ratio of fresh and dry mass of root and plant canopy.

Inoculation of *Ralstonia solanacearum*

Grafted tomatoes and non grafted tomatoes were transplanted in the polybag. After one week being transplanted, tomato roots was injured with scalpel and then inoculated with *R. solanacearum* suspension (10⁸ CFU/ml) by bacterial suspension were poured into soil 5 ml/plant.

Disease Intensity

After the inoculation of *R. solanacearum* with a concentration of 10⁸ CFU/ml, the symptoms were observed. If the symptoms have appeared, then the disease intensity was observed every week. Disease intensity was observed by scoring the wilting symptoms of plants according to Arwiyanto & Hartana (1999):

Description Score

0 = All leaves are healthy

1 = 1– <10% leaves wilt

2 = 10– <30% leaves wilt

3 = 30– <60% leaves wilt

4 = 60– <100% leaves wilt

5 = 100% leaves wilt (the death of entire plants)

After scoring the symptoms, the disease intensity of was calculated according to Arwiyanto & Hartana (1999) using the formula:

$$I = \frac{\sum(n \times v)}{N \times Z}$$

I = disease intensity

n = number of plants infected by certain categories
v = score value (category) in each disease intensity category

Z = highest score

N = number of plants observed at each intensity

The disease intensity was used to determine plant resistance to *R. solanacearum*. According to Janaki & Putturaju (2012), there are 5 levels of plant resistance against bacterial wilt caused by *R. solanacearum* (Table 2).

Table 2. Resistance level to bacterial wilt disease (*Ralstonia solanacearum*)

Disease intensity (%)	Resistance level
0	Very resistant
1–5	Resistant
5–20	Medium resistant
21–51	Medium susceptible
>51	Susceptible

Source: Janaki & Putturaju (2012)

AUDPC (The Area Under the Disease Progress Curve)

The determination of AUDPC was carried out to determine the progression of the disease over time. After obtaining the intensity value of bacterial wilt disease from each observation in each treatment, then an area calculation was used to make disease progression curve (AUDPC), based on the severity of the disease over time using this formula (Roelfs *et al.*, 1992):

$$= \frac{1}{n} \sum_{i=1}^{n-1} (Y_i + Y_{i+1}) (t_{i+1} - t_i)$$

Y_i = incidence or intensity of disease in the i observation

Y_{i+1} = incidence or intensity of disease at the i+1 observation

t_i = observation time at i

t_{i+1} = observation time at i + 1

n = total number of observation

The AUDPC value of each treatment was combined into one graph, where AUDPC was calculated with disease intensity value. The data were analyzed by ANOVA and if there are significant differences

between treatments followed by LSD test at a 95% confidence level.

RESULTS AND DISCUSSION

Observation on Seedling Media

The seedling media of A had the highest soil density (0.24 g/cm³), while B and C had the lowest bulk density (0.19 g/cm³) (Table 3). The denser the soil, the higher the bulk density, more difficult the soil to pass the water or to be penetrated by plant roots. Denser soil has greater bulk density than less one, though from the same type of soil (Hardjowigeno, 2003). This finding showed that the seedling media of B and C would be easier to pass on water and easier to be penetrated by the plant roots hence it will be more effective as a tomato seedling media. The porosity value of A (69.3%) has the lowest porosity compared with the other seedling media, followed by B (72%), C (72.67%), and D (72.67%), and E and F media have the same porosity (75.33%) (Table 3). Planting media that has good porosity is planting media with large porosity because plant roots are easy to penetrate in search of nutrients. In addition, the growing media is able to hold rainwater thus plants do not lack water. However, if the porosity is too high, the water absorbed by the soil dropped directly to the next layer of the soil (Buckman & Brady, 1992). Based on the porosity value in this experiment, the better seedling media for tomato is B, C, and D media because the porosity value is not low but not too high.

The pH values of highest to lowest are E (6.9), A (6.5), F (6.4), D (6.1), C (6.0), and B (5.6). The pH

Table 3. Bulk density, porosity, pH, dan electrical conductivity of six seedling media

Media seedling*	Bulk density (gr/cm ³)	Porosity (%)	pH	EC (mS/m)
A (10:0)	0.24a	69.33b	6.5b	0.66a
B (9:1)	0.19e	71.33ab	5.6e	0.60a
C (8:2)	0.19e	72.00ab	6.0de	0.64a
D (7:3)	0.20d	72.67ab	6.1cd	0.64a
E (6:4)	0.21c	75.33a	6.9a	0.40b
F (5:5)	0.22b	75.33a	6.4bc	0.39b

Remarks: *(coir : rice husk charcoal), EC = Electrical Conductivity. Means followed by the same letter in the same column were not significantly different according to LSD (Least Significant Differences) (p = 0.05).

of A was similar to a study by Ghehsareh *et al.* (2011) with (6.7). The lower the pH of the growing media, the more difficult the plant to grow because the planting media is acidic and contains toxic (poison). On the other hand, if the pH of the planting medium is high then it is alkaline and contains lime. A good pH value for plants is approaching neutral, 6–7 (Rusdiana & Lubis, 2012). Based on the pH values obtained in this experiment, all the seedling media is good for tomato seedlings except media B because the pH value is below 6. The electrical conductivity in seedling media from the highest to lowest are A (0.66 mS/m), B (0.60 mS/m), C (0.64 mS/m), D (0.64 mS/m), E (0.40 mS/m), and F (0.39 mS/m). There is a trend that the electrical conductivity in all seed media is relatively low and low in salinity as well. Therefore from the value of electrical conductivity the six seedling media are considered could be used as seedling media.

Observation on Tomato Seedling

According to Hamidan (1983)–, a good seed has a germination percentage of 90% or more and according to Murniati & Suminar (2006), one of the factors influence seed germination is germination media. In certain seeds, the germination substrate causes the dormancy of the seeds (enforced dormancy). The highest germination percentage was in C (94%) thus it could be used to germinate the tomato seed H-7996. C treatment (8:2) (coir : rice husk charcoal, v/v) has the highest value for plant height (8.40 cm); leaf area (20.09 cm²); fresh mass of root and canopy (68.67 mg and 622 mg, respectively); and dry mass of root and canopy (11.51 mg and 48.83 mg, respectively) in six seed media (Table 4). These findings indicated that C is the most effective seed media for the growth of tomato seed.

Disease Intensity

The disease intensity of bacterial wilt was higher in the treatment without grafting, which reached 90% in the last observation compared to tomato plants grafted with *R. solanacearum* resistant rootstock (Figure 1). In the treatment without grafting, bacterial wilt symptoms were seen in the first observation, while in the treatment with grafting the only appeared in the second observation. Grafting is an effort to improve the growth of plants from unfavorable conditions including to be resistance to disease (Nurcahyanti, 2015).

Table 4. Germination style, plant height, leaf area, fresh mass and dry mass of root and plant canopy after 21 dap (day after planting)

Media seedling*	Germination percentage (%)	Plant height (cm)	Leaf area (cm ²)	Fresh mass (mg)		Dry mass (mg)	
				Canopy	Root	Canopy	Root
A (10:0)	90.0a	7.44b	17.58a	552.00a	61.33ab	44.81a	8.71b
B (9:1)	84.4a	7.78ab	17.79a	539.33a	48.67bc	47.30a	9.61ab
C (8:2)	94.4a	8.40a	20.09a	622.00a	68.67a	48.83a	11.51a
D (7:3)	80.0a	6.32c	13.89b	299.33b	34.67cd	28.20b	7.55b
E (6:4)	80.0a	5.13d	6.05c	185.33c	23.47de	20.30bc	5.39c
F (5:5)	88.9a	4.87d	6.85c	129.33c	16.67e	17.31c	5.01c

Remarks: *(coir : rice husk charcoal). Means followed by the same letter in the same column were not significantly different according to LSD (Least Significant Differences) ($p = 0.05$).

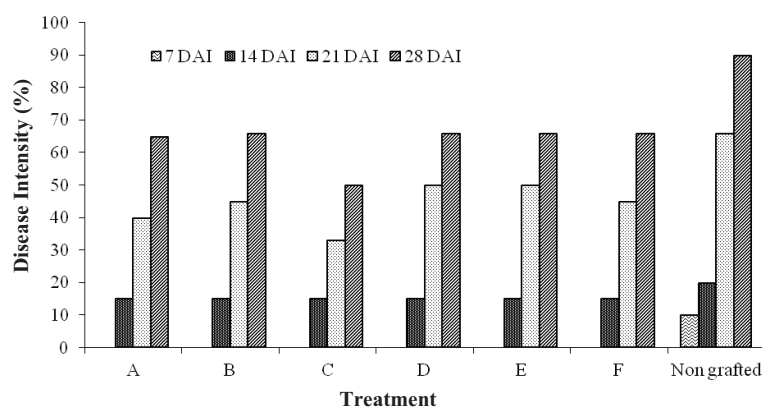


Figure 1. The disease intensity of bacterial wilt in the grafted tomato plants that the root of the tomato was grown in six media seedlings; (DAI, days after inoculation)

The grafting with the rootstock H-7996 sown of C seedling media 8:2 (coir : rice husk charcoal, v/v) has the lowest bacterial wilt intensity among tomato plants whose rootstock was grown in other media. In the first observation, the disease intensity of bacterial wilt was the same as in other media treatments (0%) as well as in the second observation (15%), however in the third observation was increased (33%), while in other media treatments was slightly higher (40–50%). In the last observation, the disease intensity of bacterial wilt in C was only 50% while other media reached 65–66% (Figure 1). These results revealed that the H-7996 tomato plant sown in C media and grafted with tomato varieties of Servo has the lowest disease intensity compared to other seedling media with grafting. The lower disease intensity in C media may be due to healthier seedlings produced thus it can survive against bacterial wilt infections caused by *R. solanacearum*. However, although the intensity of the disease is

lower among other treatments, H-7996 tomato plants sown in C then grafted with Servo have a medium susceptible level because the intensity of the disease is 50% at the last observation according to Janaki & Putturaju (2012).

AUDPC (The Area Under The Disease Progress Curve)

AUDPC value continue to increase from the first to the last observation in all treatments and from the first observation the highest area under the progress curve of bacterial wilt was in tomato plants without grafting to the resistant rootstock compared to the treatment of grafting with the rootstock resistant to *R. solanacearum* (Figure 2). The lowest value of area under the progress curve of bacterial wilt was in the C media, i.e. the grafting with H-7996 rootstock sown in the media C (8:2) (coir : rice husk charcoal, v/v). The low value of the area under the progress curve of bacterial wilt showed that the

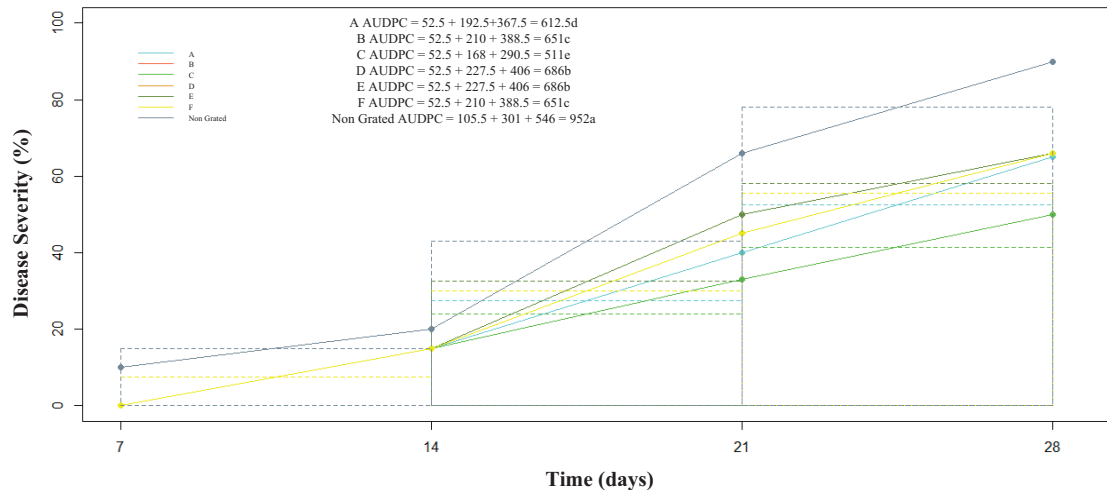


Figure 2. The Area Under the Disease Progress Curve (AUDPC) of bacterial wilt disease in the grafted tomato plants that the root of the tomato is grown in six media seedling

disease intensity is low, due to the C media produced healthier seedlings hence it can survive against bacterial wilt infections caused by *R. solanacearum*.

CONCLUSIONS

The grafting treatment with resistant rootstock could suppress bacterial wilt by reducing the disease intensity to (33–40%) compared to without grafting with resistant rootstock. The best media for the growth of tomato seedlings is media C (8:2) (coir : rice husk charcoal, v/v) which showed the lowest disease intensity compared with the other treatment. This might occur because the seedlings produced on C are healthier hence they can survive against bacterial wilt infections caused by *R. solanacearum*. The combination of the best treatment is the grafting with the resistant rootstock H-7996 was sown on media with the ratio 8:2 (coir : rice husk charcoal, v/v).

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