Caraka Tani: Journal of Sustainable Agriculture
ISSN 2613-9456 (Print) 2599-2570 (Online)

Caraka Tani: Journal of Sustainable Agriculture, 36(2), 283-294, 2021
URL: https://jurnal.uns.ac.id/carakatani/article/view/46882
DOI: http://dx.doi.org/10.20961/carakatani.v36i2.46882

Seedling Growth Analysis of Papaya Cultivated on Several Planting Media Enriched by Plant Growth Promotor Microbes

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Abstract

There are factors contributed to the growth and development of fruit crop seedling. Microbes are well known as plant growth promotors such as symbiotic mycorrhizae and antagonist fungi, *Trichoderma* spp. The main objective of this experiment is to find out the best medium composition enriched by beneficial microbes to improve papaya seedling growth. The experiment was conducted at Sumani Experimental Station, Indonesian Tropical Fruit Research Institute, Solok, West Sumatera, Indonesia from August until December 2017. The experiment was arranged in a Randomized Complete Block Design with ten treatments and three replicate blocks. The treatments were ten combinations of media for papaya seedling growth enriched by plant growth promotor microbes. In this experiment, medium soil with additional manure, rice husk charcoal and compost (single or combination) combined with mycorrhizae were used. Also, the effect of *Trichoderma* sp. enrichment into media composition were tested in this experiment. Treatments SCRMc and SCRMcT; with its complexity; were the best media composition to promote papaya seedling growth. These treatments resulted in best performance of plant height, stem diameter and number of leaves of papaya seedlings. The additional of *Trichoderma* sp. into medium did not show beneficial effect for all parameters in this experiment.

Keywords: fruit crop; growth promotor; symbiotic mycorrhizae; Trichoderma

Cite this as: Fatria, D., Sparta, A., Emilda, D., Hariyanto, B., Budiyanti, T., Martias, & Iztianto, M. (2021). Seedling Growth Analysis of Papaya Cultivated on Several Planting Media Enriched by Plant Growth Promotor Microbes. *Caraka Tani: Journal of Sustainable Agriculture*, *36*(2), 283-294. doi: http://dx.doi.org/10.20961/carakatani.v36i2.46882

INTRODUCTION

Papaya is an important tropical fruit in Indonesia (rank 7th). In recent years, there has been an increasing demand for papaya fruits. As the fourth of the most consumed fruits, papaya was consumed around 2.25 kg capita^{-1} in 2015 and increased at approximately 0.60 kg capita^{-1} in 2016. Papaya consumption in Indonesia has increased significantly in the past eleven years (2007 to 2017); in 2017 the papaya consumption was consumed around 2.45 kg capita^{-1}. The increasing demand for papaya fruits also followed by the increase of national production every year. Compared to data in 2017, papaya production growth was around 1.43% higher in 2018 (Pusat Data dan Sistem Informasi Pertanian, 2019).

The growth and development of the tropical fruit crop business needs support from many sectors. Nursery production of seedlings is one of the main factors in this business. The success of crop production starts from the use of high-quality seedlings and followed by the application of good agricultural practices (Murugesan et al., 2016). The introduction and development of new superior varieties also depend on the availability of seedlings of this variety. So, the availability of seedlings will determine the future of that variety.

* *Received for publication December 22, 2020*
Accepted after corrections April 8, 2021

Many factors contributed to the growth and development of fruit crop seedlings, such as temperature, relative humidity, nutrients and environmental condition (Ozturk and Serdar, 2011). The availability of nutrients depends on the composition of the planting medium. Conventionally, farmers used the mixture of soil and manure as the composition of the planting media in the fields. Planting media is important to increase the growth and development of the root system, as the sources or providing water and nutrients for plants (Soepardi, 1983). An appropriate planting medium is required to support nutrients availability for plant growth, maintain the humidity of the rhizosphere, permit oxygen diffusion, provide healthy and high-quality plants (Ge et al., 2012; Bhardwaj, 2014; Meng et al., 2018).

Lately, the application of fungal biofertilizer is getting more attention in supporting sustainable agriculture. Several microbes are well known as plant growth promotors such as symbiotic mycorrhizae and antagonist fungi, *Trichoderma* spp. Both of those fungi are considered fungal biofertilizers (Odoh et al., 2020) that have beneficial effects on plant growth and development. The symbiotic arbuscular mycorrhizal fungi (AMF) increase the nutrient uptake and nitrogen use efficiency, reduce the adverse effect of heavy metal, stimulate plant growth, improve phosphorus uptake in low fertile soil, provide better carbon sequestration, improve fruit quality, enhance resistance to water shortages and suppress soil pathogens (Camprubi and Calvet, 1996; Dutra et al., 1996; Fortuna et al., 1996; Matsubara et al., 1996; Muas, 2003, 2004, 2005; Miransari et al., 2009; Talanca., 2010; Yadav et al., 2013; Wang et al., 2016; John et al., 2017; Tarraf et al., 2017; Verzeaux et al., 2017; Begum et al., 2019; Magallon-Servin et al., 2020).

Several studies show the positive effects of *Trichoderma* sp. for plant growth. The application of *Trichoderma* spp. promotes plant growth, increases plant survival rates and suppresses the virus and soil-borne disease (Gutiarrez-Miceli et al., 2008; Martinez-Medina et al., 2011; Ferrigo et al., 2014; Barari, 2016; Murunde et al., 2018). These antagonist fungi control various pathogenic fungi due to their strong ability of mycoparasitic and antagonists to pathogenic fungi. *These fungus groups also release* elicitors that induce transmission signals and lead plants to express resistance proteins (Nawrocka and Małolepsza, 2013). The ability of compost added with *Trichoderma* sp. to inhibit pathogenic fungi is not only due to the bio-control effect of the biological agent but also because of the ability of this biological agent to change the biotic and abiotic characteristics of the planting media (Blaya et al., 2013). With those abilities, this kind of microbes could suppress several plant diseases and promote better growth above and below ground.

Based on these findings, the application of these two types of microbes or fungi has the potential to improve plant growth of papaya seedlings. Several experiments were conducted to test the potential of microbes in papaya seedlings. Cruz et al. (2017) investigated the optimum levels of nitrogen and phosphorus in combination with AMF for papaya seedlings growth. While, Aguilar et al. (2018) applied MYKOVAM (biological fertilizer that contained more than 10 species AMF) in order to increase biomass production in papaya seedlings. Another study tried to evaluate the application of AMF in papaya seedlings under salt-stress condition (Oliveira Filho et al., 2020). However, the experiment studying combination medium of papaya that enriched with plant promotor microbes is not available yet. Therefore, we conduct research with the main objective to find out the best medium composition enriched by beneficial microbes to improve papaya seedling growth.

MATERIALS AND METHOD

Experimental site

The experiment was conducted at Sumani Experimental Station, Indonesian Tropical Fruit Research Institute, Solok, West Sumatera, Indonesia; from August until December 2017. The experimental site is located at 0°44’17” S and 100°37’20” E and approximately 425 m above sea level. Indonesian Tropical Fruit Research Institute is a part of the Indonesian Ministry of Agriculture that focused on tropical fruit research in Indonesia.

Plant and microbe materials

The papaya cultivar used in this study was Merah Delima which is the superior papaya cultivar released by the Indonesian Tropical Fruit Research Institute. In this experiment, the certified seed (white label) of papaya Merah Delima was used. Papaya var. Merah Delima is one of papaya variety that has high production potential with sweet taste of fruit. However, this variety still has a problem with germination process. Until now, the germination value of this variety is around 75%. The Mycorrhizae and *Trichoderma* sp. formulations were produced by ITFRI. In order to improve papaya productions in Indonesia, the Indonesian Tropical Fruit Research Institute have to support the farmer in the availability of papaya seedlings, especially papaya var. Merah Delima.

Seed treatment

Papaya seeds were soaked in warm water for ± 24 hours. The sunken seeds were selected and the floating seeds were discarded. Then these seeds were drained and placed on soft tissues/cloth, rolled and moistured. The seeds were stored in glass jars and covered with plastic and tightly tied. In addition, the seeds were stored in a room exposed to sunlight with temperatures of approximately 30°C for 7 days. It was required to spray these seeds with clean water to maintain the seed's moisture. The seeds were germinated after 7 days. After that, the papaya seedlings were transferred into different planting media (polybag) based on the treatment.

Experimental design

The experiment was arranged in a Randomized Complete Block Design with ten treatments, three replicate blocks and ten samples for each replication. The treatments were a combination of media for papaya seedling growth that consisted of:

1. Soil + manure = 1 : 1 (v/v) + 5 g mycorrhizae (SMMc);
2. Soil + compost = 1 : 1 (v/v) + 5 g mycorrhizae (SCMc);
3. Soil + rice husk charcoal = 1 : 1 (v/v) + 5 g mycorrhizae (SRMc);
4. Soil + manure + rice husk charcoal = 1 : 1 : 1 (v/v/v) + 5 g mycorrhizae (SMRMc);
5. Soil + compost + rice husk charcoal = 1 : 1 : 1 (v/v/v) + 5 g mycorrhizae (SCRMc);
6. Soil + manure = 1 : 1 (v/v) + 5 g mycorrhizae and 5 g *Trichoderma* sp. (SMMcT);
7. Soil + compost = 1 : 1 (v/v) + 5 g mycorrhizae and 5 g *Trichoderma* sp. (SCMcT);
8. Soil + rice husk charcoal = 1 : 1 (v/v) + 5 g mycorrhizae and 5 g *Trichoderma* sp. (SRMcT);
9. Soil + manure + rice husk charcoal = 1 : 1 : 1 (v/v/v) + 5 g mycorrhizae and 5 g *Trichoderma* sp. (SMRMcT);
10. Soil + compost + rice husk charcoal = 1 : 1 : 1 (v/v/v) + 5 g mycorrhizae and 5 g *Trichoderma* sp. (SCRMcT).

Ultisol soil was used in this experiment as the common soil type in this area. The initial conditions of soil in this experiment are pH condition (H_2O) 4.1, organic carbon 1.8%, total nitrogen 0.07%, phosphorus 0.92 ppm and potassium 0.53 cmol(+)kg^{-1}.

Data collection

Media properties

Cation exchange capacity, pH, organic carbon, nitrogen, phosphorus, potassium, calcium and magnesium of each medium composition were measured at the beginning of the experiment.

Measurement of plant growth

Plant growths were measured destructively and non-destructively. Ten plant samples per replication were measured non-destructively at two months after planting. These samples were used to determine plant height, number of leaves, stem diameter and root length. Plant height was measured from the level ground to the point of plant growth. A number of leaves were counted for all leaves that fully opened. Stem diameter was measured at 1 cm from ground level. Root length was measured at the longest root.

Five samples per replication were measured destructively at 60 days after planting. These samples were used to determined fresh matter, dry matter and nutrients contents (nitrogen, phosphorus, potassium, calcium and magnesium). The plant samples were dried for three days at 80°C and then weighted for the plant dry matter data.

Nutrient contents on plant tissues

Nutrient contents on plants were assessed at the end of the experiment. The measured parameters were N, P, K, Ca and Mg contents of the plant.

Population of Trichoderma sp. *propagules*

The population of *Trichoderma* sp. propagules was collected monthly (the first month and the second month). The amount of *Trichoderma* sp. propagules in the media were calculated by using 1 ml of the planting media solution at two dilutions (10^{-2}) per petri dish and poured with PDA media (Potato Dextrose Agar).

Data analysis

Data were analyzed using the software SPSS 17 software package (SPSS Inc., Illinois, USA). Analysis of variance was used to analyze the data and followed by orthogonal contrast.

RESULTS AND DISCUSSION

Several factors affect the growth and development of plants in nursery conditions. These factors divided into genetic factors (seed quality) and environmental factors (light, temperature, humidity, water and nutrition) (Ozturk and Serdar, 2011). Those factors could limit the growth and development of plants. To grow up normally, it would depend on plant's abilities to combine those factors effectively and efficiently.

Nutrition is one of the limiting factors for plant growth and development. This external factor would determine the key success for the seedlings quality. This experiment focused on nutrition management by combining different medium compositions in order to provide suitable nutrition for the growth of plants. Suhaila et al. (2013) found that a medium containing a combination of several materials tends to provide better plant growth than a single medium. A combination of media could support nutrient availability in each other and improve deficiency nutrient of each planting medium. It is a common knowledge that planting mediums is the only nutrient sources for papaya seedlings in nursery conditions.

At the beginning of the experiment, we analyzed the soil properties and soil chemicals that were used in the experiment. The soil type was clay; with 80.36% fraction of clay, 9.03% fraction of dust and 5.83% fraction of sand. The initial conditions of soil in this experiment are pH condition (H_2O) 4.1, organic carbon 1.8%, total nitrogen 0.07%, phosphorus 0.92 ppm and potassium 0.53 cmol(+)kg^{-1}. Media properties after a mixture of media components as described on treatments were analyzed for pH, carbon, nitrogen, phosphorus, potassium, calcium, magnesium contents and cation exchange capacity. The results of media properties were shown in Tables 1 and 2. The pH conditions, carbon, nitrogen, calcium, magnesium and cation exchange capacity were varied among medium compositions. While phosphorus and potassium contents on all medium combinations were almost similar.

Neutral pH conditions were found on treatments SCMc, SCRMc and SCRMcT (Table 1). While other treatments resulted in pH conditions that ranged from acidic to a slightly alkaline condition. Carbon, nitrogen, calcium, magnesium and cation exchange capacity levels of planting mediums were distributed from low to very high levels (Tables 1 and 2). Soil analysis showed a very high level of phosphorus contents in all combination media, as well as the potassium contents.

Table 1. Analysis of pH, carbon, nitrogen and phosphorus in ten different medium compositions of papaya seedlings

Treatment code	pH		Carbon (%)		Nitrogen (%)		Phosphorus (ppm)	
	H_2O	Criteria	WB	Criteria	Kjeldahl	Criteria	Olsen	Criteria
SMMc	5.16	Acid	2.41	Medium	0.26	Medium	430.03	Very high
SCMc	7.32	Neutral	3.16	High	0.26	Medium	281.44	Very high
SRMc	4.95	Acid	1.21	Low	0.11	Low	48.74	Very high
SMRMc	5.79	Slightly acid	2.91	Medium	0.33	Medium	542.97	Very high
SCRMc	6.75	Neutral	3.85	High	0.26	Medium	236.58	Very high
SMMcT	5.88	Slightly acid	3.94	High	0.33	Medium	600.01	Very high
SCMcT	7.85	Slightly alkaline	4.17	High	0.26	Medium	440.71	Very high
SRMcT	4.95	Acid	1.29	Low	0.26	Medium	89.52	Very high
SMRMcT	5.95	Slightly acid	3.62	High	0.26	Medium	681.31	Very high
SCRMcT	6.79	Neutral	5.74	Very high	0.26	Medium	744.08	Very high

Table 2. Analysis of potassium, calcium, magnesium and cation exchange capacity in ten different medium composition of papaya seedlings

Treatment code	Potassium (me 100g^{-1})		Calcium (me 100g^{-1})		Magnesium (me 100g^{-1})		Cation exchange capacity (me 100g^{-1})	
	Value	Criteria	Value	Criteria	Value	Criteria	Value	Criteria
SMMc	3.17	Very high	7.11	Medium	3.73	High	13.33	Low
SCMc	11.18	Very high	9.44	Medium	3.34	High	11.13	Low
SRMc	1.13	Very high	2.19	Low	0.89	Low	14.25	Low
SMRMc	3.44	Very high	8.04	Medium	4.52	High	13.15	Low
SCRMc	5.77	Very high	9.36	Medium	3.03	High	12.22	Low
SMMcT	5.59	Very high	8.89	Medium	4.77	High	19.68	Medium
SCMcT	14.01	Very high	12.19	High	4.50	High	19.89	Medium
SRMcT	1.14	Very high	2.38	Low	1.01	Low	8.97	Low
SMRMcT	4.03	Very high	9.60	Medium	5.24	High	15.45	Low
SCRMcT	6.23	Very high	8.92	Medium	3.92	Medium	11.17	Low

The application of different planting media showed significant differences in the plant growth of papaya seedlings in some vegetative characters. Analysis of variance on plant height, stem diameter and the number of leaves showed significant differences between treatments at P ≤ 0.01 (Table 3; Figures 1, 2 and 3). The addition of *Trichoderma* sp. into planting media did not affect these parameters. The highest seedling's height was found in treatments SCRMc and SCRMcT; while treatments SRMc and SRMcT produced the shortest seedlings.

Table 3. Orthogonal contrast of growth parameters

	Manure vs compost vs rice husk charcoal (less complex media composition)	Manure + rice husk charcoal vs compost + rice husk charcoal (Most complex media composition)	Non trichoderma vs trichoderma	Less complex media composition vs most complex media composition
Plant height	**	**	ns	**
Number of leaves	ns	**	ns	**
Stem diameter	*	**	ns	**
Root length	ns	**	ns	ns
Fresh matter of root	ns	**	ns	**
Dry matter of root	ns	**	ns	**
Fresh matter of stem	ns	**	ns	**
Dry matter of stem	ns	**	ns	**

Note: Significant differences are represented by: (*) at P < 0.05 and (**) at P < 0.01 and ns (not significant)

All treatments in this experiment also resulted in various number of papaya seedling leaves. In line with the result of papaya seedling height, the highest number of leaves was also found in treatments SCRMc and SCRMcT. The lowest number of leaves were also found in SRMc and SRMcT treatments. For stem diameter, the highest stem diameter of papaya seedlings was found in treatment SCRMc. According to Nakasone and Paull (1999), the growth rates of stem diameter are influenced by the availability of nitrogen and phosphorus, irrigation and temperature. In the seedling stage, stem diameter is an important parameter to ensure the continued growth of seedlings. Seedlings with larger stem diameters had better vigor and deal with unfavorable field conditions (Sumartuti, 2004). The most complex media composition (with or without *Trichoderma* sp.) showed the superior results in plant height, a number of leaves and stem diameter (at P ≤ 0.01) compared to less complex media composition.

The addition of *Trichoderma* sp. into planting media did not affect root length, root fresh weight, root dry matter, stem fresh weight and stem dry weight of papaya seedlings (Table 3;

Figures 4, 5, 6, 7 and 8). The longest root of papaya seedling was found in treatment SMMc, while the shortest one was found in treatment SRMc. Significant difference only found at the comparisons between treatment with the most complex media compositions (SMRMc vs SCRMc and SMRMcT vs SCRMcT) at P ≤ 0.01. The most complex media composition (with or without *Trichoderma* sp.) also showed better root fresh weight, root dry matter, stem fresh weight and stem dry weight (at P ≤ 0.01) compared to less complex media composition.

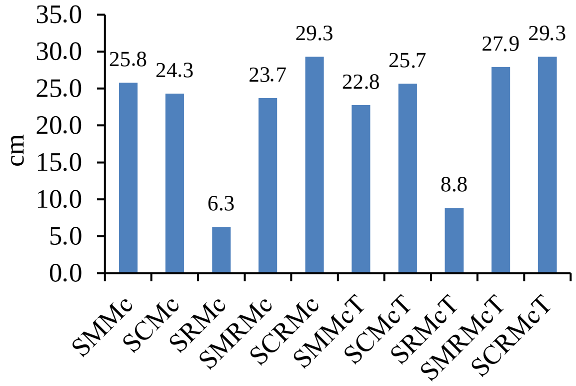

Figure 1. Plant height of banana seedling in ten different media composition at two months after planting

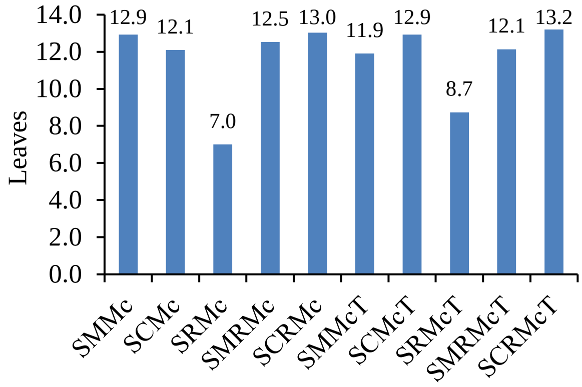

Figure 2. Number of leaves of banana seedling in ten different media composition at two months after planting

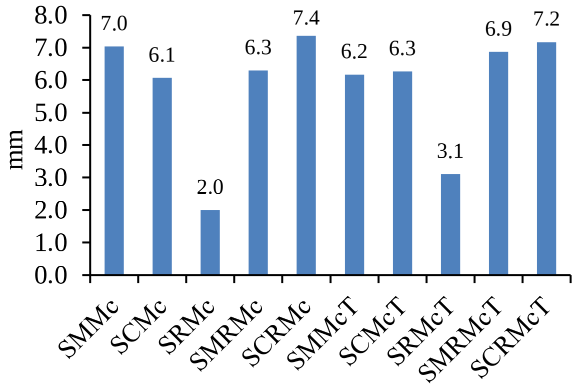

Figure 3. Stem diameter of banana seedling in ten different media composition at two months after planting

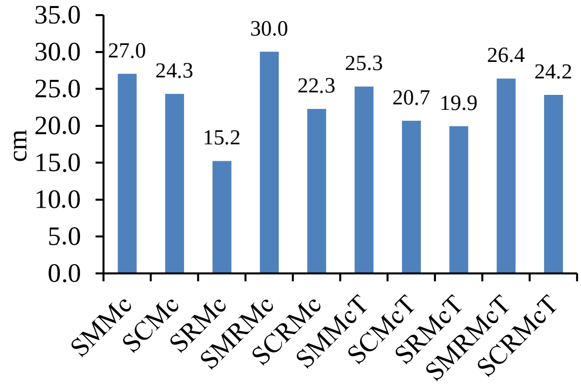

Figure 4. Length of root of banana seedling in ten different media composition at two months after planting

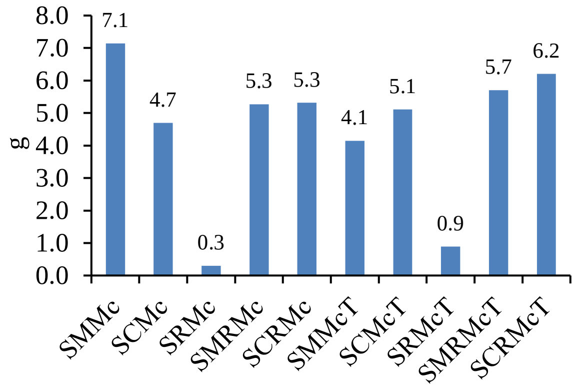

Figure 5. Fresh weight of root of banana seedling in ten different media composition at two months after planting

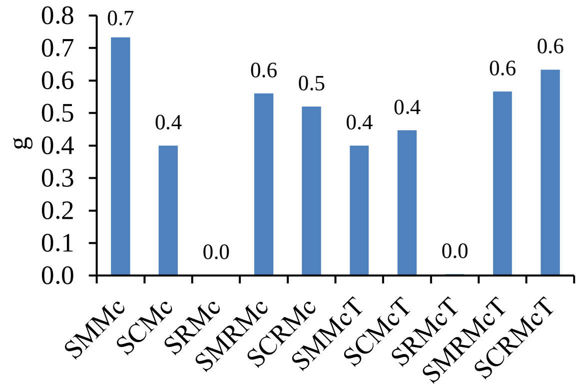

Figure 6. Dry weight of root of banana seedling in ten different media composition at two months after planting

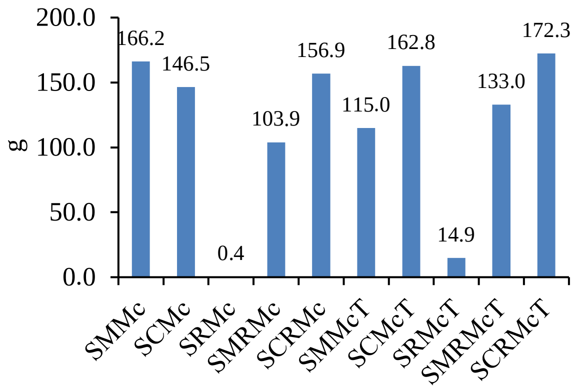

Figure 7. Fresh weight of shoot of banana seedling in ten different media composition at two months after planting

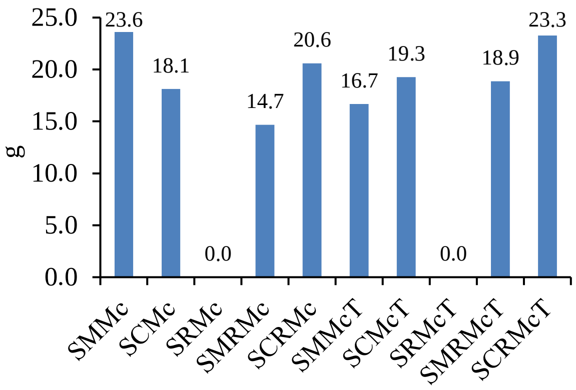

Figure 8. Dry weight of shoot of banana seedling in ten different media composition at two months after planting

Table 4 showed the data of analysis of nitrogen, phosphorus, potassium, calcium and magnesium contents in papaya seedlings. The nitrogen contents of papaya seedlings were around 1.72–2.94%. The highest nitrogen content was found in treatment SCMc and followed by treatments SCMcT and SCRMc; while the lowest nitrogen contents were found in treatments SMMc and SMRMcT. The result of phosphorus content of papaya seedlings in this experiment was almost similar, with the range 0.37–0.58%. On the contrary with the result of nitrogen uptake, treatment SMRMcT produced the highest potassium content compared to other treatments. Furthermore, treatment SMRMcT also resulted in the highest magnesium content. In line with the result of phosphorus content, all the treatments showed similar results in calcium contents; the range was around 0.22–0.4%.

The population of *Trichoderma* sp. in planting media of papaya seedling was observed to determine the population density of *Trichoderma* sp. in various medium combinations. *Trichoderma* sp. which was added into planting medium as a treatment led to the increase in the population of this antagonist fungus. In the first month, the population of *Trichoderma* sp. increased to around 4-folds; and in the second month the increase of this fungus was around 11-fold compared to combination media without *Trichoderma* sp. (Figure 9). Figure 9 showed that planting media without *Trichoderma* sp. lead to a lower amount of fungus propagules than planting media with the addition of *Trichoderma* sp. However, the addition–of *Trichoderma* sp. into the medium did not show any beneficial effect in all parameters.

The result in this experiment showed that the complexity in planting media combinations (SCRMc and SCRMcT) promotes better plant growth in several plant properties than other treatments. This condition probably due to the complexity of medium which could provide sufficient nutrients that allow papaya seedling grew in favorable conditions. However, one of the simplest treatment (SMMc-soil, manure and mycorrhizae) provided the same results as the complex treatments in some of the parameters. The lower papaya seedling growth was found in planting media without additional manure or compost (treatments SRMc and SRMcT). Those treatments resulted in lower growth performances in several plant growth parameters. Probably, these combinations resulted in denser and less fertile media; therefore caused limitation in plant growth. The nutrient contents in those two treatments were not enough to promote plant growth of papaya seedlings as well as media with additional manure or compost. Based on our findings, the addition of manure or compost into papaya planting media is required in order to provide sufficient seedling growth.

Apart from medium composition, several microbes were also used in order to promote plant growth of several plants. Several experiments showed the effectiveness of mycorrhizae and *Trichoderma* sp. in promoting plant growth and development and other benefits in several plants (Gutiarrez-Miceli et al., 2008; Martinez-Medina et al., 2011; Ferrigo et al., 2014; Barari, 2016; Wang et al., 2016; Murunde et al., 2018;

Begum et al., 2019; Magallon-Servin et al., 2020). Those microbes have potential to increase plant growth, photosynthesis rate, enzyme activity and nutrient uptake (Guler et al., 2016; López-Coria et al., 2016; Mo et al., 2016; Chen et al., 2017; Lin et al., 2017; Ait-El-Mokhtar et al., 2019; Zhang et al., 2019).

Preceding experiments showed mycorrhizae promote plant growth even in unfavorable conditions (Tedersoo and Bahram, 2019). Mycorrhizae applications are beneficial in improving plant tolerance against environmental stress conditions; for example, drought stress (Mo et al., 2016) and salt-alkali stress (Lin et al., 2017). Mycorrhizae also support plant growth in a wide range of pH, including in acid soil (Clark and Zeto, 1996). Further, Yano and Takaki (2005) found that plant growth-promoting function of mycorrhizae only significant in lower pH condition.

Table 4. Analysis nutrients content of nitrogen, phosphorus, potassium, calcium and magnesium in papaya seedlings

Treatment code	N (%)	P (%)	K (%)	Ca (%)	Mg (%)
SMMc	1.72	0.37	3.71	0.27	0.70
SCMc	2.94	0.52	6.09	0.22	0.76
SRMc	2.02	0.36	2.13	0.39	0.82
SMRMc	1.88	0.43	3.69	0.25	0.76
SCRMc	2.49	0.38	4.42	0.28	0.71
SMMcT	1.88	0.38	3.71	0.27	0.72
SCMcT	2.78	0.58	5.96	0.23	0.72
SRMcT	2.02	0.44	2.35	0.40	0.80
SMRMcT	1.72	0.48	6.63	0.23	1.04
SCRMcT	1.89	0.44	4.35	0.25	0.62

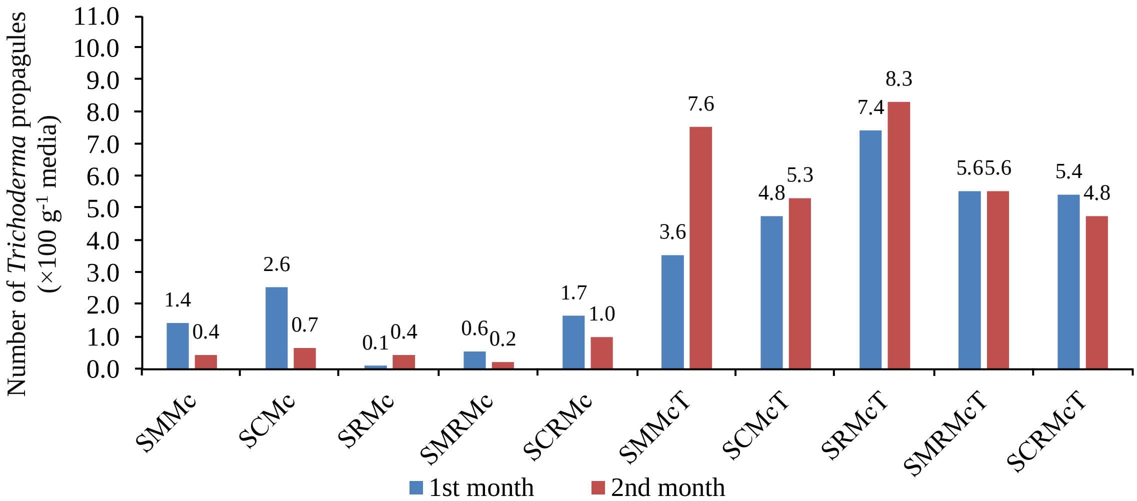

Figure 9. The average numbers of *Trichoderma* sp. propagules in planting media of papaya (propagules g^{-1} media)

CONCLUSIONS

Treatments SCRMc and SCRMcT were the best media compositions to promote papaya seedling growth. This treatment resulted in the best performance of plant height, stem diameter and number of leaves of papaya seedlings. The addition of *Trichoderma* sp. into the medium did not show a beneficial effect for all parameters in this experiment.

ACKNOWLEDGEMENT

This project was financially support by Indonesian Ministry of Agriculture.

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