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The Effect of Soaking Seed with Rizobacteria *Pseudomonas* Alcaligenes to the Growth of Swamp Cabbage *Ipomoea Reptans* Poir

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Abstract

Research of "The effect of soaking seeds with the bacteria *Pseudomonas alcaligenes* to the growth of swamp cabbage (*Ipomoea reptans Poir*)" was conducted to determine the effect of seed soaking with suspense of P. *alcaligenes isolate* KtS1, TrN2 and TmA1 to the growth of swamp cabbage. This research is the development of research that has been done before on tomato plants. This study was designed with a Randomized Block Design and data analysis using SPSS v.17 for windows. The treatments were three types of isolates of P. *alcaligenes*, soaking time, and growing medium. The parameters observed were germination and other growth parameters. The results showed that seed soaking treatment with suspense P. *alcaligenes* cause germination 25% faster, higher crop up 24.4%, number of leaves more up to 23.15%, longer stems to 25%, longer roots up 46.90%, fresh stem weight higher up to 67.07%, dry weight oven stem higher up 84.21% if compared with control. The best treatment response was TrN2.6NB, soaking seeds of swamp cabbage with P. *alcaligenes* TrN2 for 60 minutes on medium NB (Natrium broth).

Keywords: Seed soaking; *Pseudomonas alcaligenes; Rizobacteria*; Swamp cabbage

Introduction

Swamp cabbage (*Ipomoea reptans Poir*) is a vegetable that has economic value and widespread in Southeast Asia. Swamp cabbage is generally consumed by the people of Indonesia and can be one of the restaurant's menu [1]. Swamp cabbage is a plant that is relatively resistant to drought and has a broad adaptability to various environmental conditions plants, easy maintenance and has a short harvest period [2]. Swamp cabbage commonly grown in home gardens and some small intensively planted on dry land, so as to optimize the production of swamp is still lacking. Swamp cabbage contains complete nutrition, including protein, fat, carbohydrates, fiber, calcium, phosphorus, iron, sodium, potassium, vitamin A,B,C, and carotenoids [3]. Additionally, swamp cabbage serves as medicinal plants to cure constipation, soothe the nerves and the drug hemorrhoids [4]. Swamp cabbage production in Indonesia can reach 50.000-60.000 kg ha-1 [5]. Cultivated Swamp cabbage for 0.10 ha spent 16kg of seed planting swamp cabbage however slightly results are compared with other crops [6]. From the social and economic aspects, swamp has good prospects if developed towards agribusiness, but it does require effort in planting efficiency.

The use rizobacteria (Plant Growth Promoting Rhizobacteria (PGPR)) as a biological fertilizer is the contribution of biotechnology in crop productivity improvement efforts. This was achieved by nutrient mobilization, growth hormone production, nitrogen fixation or activation of the mechanism of disease resistance [7,8]. Therefore, the evaluation of the ability of local Rizobacteria as bacterial growth driver needs to be done. If proven effective, the local Rizobacteria can be used as an alternative biological fertilizer (biofertilizer) on the cultivation of swamp cabbage in Indonesia. Efforts to reduce the use of synthetic fertilizers and pesticides are needed in moving towards environmentally sustainable agriculture. Lately, attention has focused on biological resources in improving health (resistance) of plants, through the role of beneficial soil microbes. Microbes that are beneficial to plants, such as *Pseudomonas spp* of the rizobacteria group can serve as fertilizer, as a means of biological control of plant pathogens and improve plant resistance (induced systemic resistance (ISR) [9].

Rizobacteria is a group of bacteria with the plant root zone habitat (rizosphere) which has been researched and proven to

improve soil fertility, increase plant resistance and can suppress plant pathogens. Rizobacteria act directly as a biological fertilizer and biological stimulants to produce hormones to grow crops such as IAA (indole acetic acid), gibberilin, cytokinin, ethylene, dissolving minerals and indirectly also serves to prevent pathogenic microorganisms through formation of siderophore, and antibiotics. Besides, it can stimulate plant growth mechanism is not widely known [9]. One of the Rizobacteria already been investigated as PGPR and ISR is P. *alcaligenes isolate* KtS1, TrN2 and TmA1 has been shown to increase the growth and yield of tomatoes [10]. The issues examined are how will soak the seeds with P. *alcaligenes* suspense to the growth of swamp cabage and how best to seed soaking time in promoting the growth of swamp cabage, with the purpose of obtaining information related to the benefits of soaking seeds of swamp cabage and best soaking time for swamp cabage growth.

Materials and Methods

Research design

This Research was designed using Randomized Block Design (RBD) with seed soaking treatment use suspense of P. *alcaligenes* isolates KtS1, TrN2, and TmA1 and using two different media which Dextrosa Potato Broth (PDB) and Natrium Broth (NB), respectively soaked 20, 40, 60 minutes so there are 18 units plus one control treatment. Thus there are 19 types of treatment were repeated 3 times so that there are 57 experimental units. The 19 types of treatment are as presented in Table 1.

Table 1: Type of treatment observed in their effects on growth of the swamp cabbage.

Number	Code	Treatment
1	Control	Soaking seed with sterilized watter
2	TmA1.2PDB	Soaking seed with *P. alcaligenes* TmA1 20 minute on PDB medium
3	TmA1.2NB	Soaking seed with *P. alcaligenes* TmA1 20 minute on NB medium
4	TmA1.4PDB	Soaking seed with *P. alcaligenes* TmA1 30 minute on PDB medium
5	TmA1.4NB	Soaking seed with *P. alcaligenes* TmA1 30 minute on NB medium
6	TmA1.6PDB	Soaking seed with *P. alcaligenes* TmA1 60 minute on PDB medium
7	TmA1.6NB	Soaking seed with*P. alcaligenes* TmA1 60 minute on NB medium
8	KtS1.2PDB	Soaking seed with *P. alcaligenes* KtS1 20 minute on PDB medium
9	KtS1.2NB	Soaking seed with *P. alcaligenes* KtS1 20 minute on NB medium
10	KtS1.4PDB	Soaking seed with *P. alcaligenes* KtS1 30 minute on PDB medium
11	KtS1.4NB	Soaking seed with *P. alcaligenes* KtS1 30 minute on NB medium
12	KtS1.6PDB	Soaking seed with *P. alcaligenes*KtS1 60 minute on PDB medium
13	KtS1.6NB	Soaking seed with *P. alcaligenes* KtS1 60 minute on NB medium
14	TrN2.2PDB	Soaking seed with *P. alcaligenes* TrN2 20 minute on PDB medium
15	TrN2.2NB	Soaking seed with *P. alcaligenes* TrN2 20 minute on NB medium
16	TrN2.4PDB	Soaking seed with *P. alcaligenes* TrN2 30 minute on PDB medium
17	TrN2.4NB	Soaking seed with *P. alcaligenes* TrN2 30 minute on NB medium
18	TrN2.6PDB	Soaking seed with *P. alcaligenes* TrN2 60 minute on PDB medium
19	TrN2.6NB	Soaking seed with *P. alcaligenes* TrN2 60 menit pada NB medium

Preparation isolate the bacteria P. *Alcaligenes*

Isolates of *P. alcaligenes* obtained in the laboratory of Agro Technology Faculty of Agriculture, University Mahasaraswati Denpasar previously been investigated its effect on tomato plants and have been identified as *P. alcaligenes* KtS1, P. alcaligenes TrN2 and P. alcaligenes TmA1 [10]. The suspension isolates of P. *alcaligenes* isolated on PDP and NB media and cultured for 48 hours in a 100ml Erlenmeyer to get a colony density of $5x10^8$cfu/ml.

Planting swamp cabbage

Planting seeds of swamp cabbage that had been treated with P. alcaligenes into polybag that already filled sterile planting medium (mixture of soil, sand and organic fertilizer with a ratio of 1:2:1). Planting is done by 2 units swamp cabbage seeds that have been soaked in suspense P. alcaligenes according to treatment in a polybag. Polybag then placed with a distance of 10cmx10cm. Watering every morning and afternoon with a volume of 100ml for each polybag.

Parameter observation and data analysis

Observation on the germination of seeds is done daily until sprouts appear, for plant height, leaf number, leaf blade length was measured once a week. Root length was measured at harvest. Besides, do also measuring the fresh weight and oven dry weight of the roots and stems of plants swamp cabbage. Data analysis was performed with SPSS v.17 for windows and different test performed on average by Duncans Multiple Rings Test (DMRT) at the level of 5%.

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Results and Discussion

Germination of seeds, plant high, long leaf and leaf number swamp cabbage

Table 2: Effect of seed soaking with the bacteria P. *alcaligenes* TrN2, KtS1, and TmA1 on seed germination, plant height, leaf number and length leaf of swamp cabbage.

No	Treatment	Average Results of Observations on the Parameters of Swamp Cabbage			
		Speed of Germination (Days)	Plant Height (cm)	Number of Leaves (Sheet)	Leaf Length (cm)
	Leaf Length (cm)				
1	CONTROL	3.75ab	20.9ns	10.8ns	8.3ns
2	TmA1.2 PDB	3.00b	22.9ns	11.8ns	9.2ns
3	TmA1.2 NB	3.00b	22.6ns	11.8ns	8.9ns
4	TmA1.4 PDB	3.25ab	21.8ns	12.3ns	8.7ns
5	TmA1.4 NB	3.50ab	24.0ns	11.8ns	9.6ns
6	TmA1.6 PDB	4.50ab	23.8ns	11.8ns	9.6ns
7	TmA1.6 NB	4.50ab	24.8ns	11.8ns	9.0ns
8	KtS1. 2 PDB	3.75ab	22.9ns	11.3ns	8.3ns
9	KtS1. 2 NB	4.25ab	22.6ns	10.8ns	8.6ns
10	KtS1. 4 PDB	3.75ab	21.8ns	11.5ns	8.9ns
11	KtS1. 4 NB	3.75ab	21.8ns	11.5ns	8.5ns
12	KtS1. 6 PDB	4.00ab	21.0ns	10.3ns	8.4ns
13	KtS1. 6 NB	4.75ab	24.0ns	11.3ns	9.5ns
14	TrN2. 2 PDB	3.50ab	21.6ns	11.8ns	8.6ns
15	TrN2. 2 NB	3.50ab	22.8ns	13.0ns	9.1ns
16	TrN2. 4 PDB	4.25ab	23.3ns	13.3ns	9.6ns
17	TrN2. 4 NB	3.25ab	26.0ns	13.3ns	10.3ns
18	TrN2. 6 PDB	4.00ab	24.1ns	11.5ns	9.3ns
19	TrN2. 6 NB	4.00ab	25.0ns	12.8ns	9.8ns

Note: The same letters behind the numbers in the same column shows the difference was not significant at the 0.05 level of DMRT.

Statistical analysis shows that the effect of seed soaking treatment with the bacteria *P. alcaligenes* TrN2, KtS1, TmA1 not significant effect on plant height, leaf length and number of leaves of swamp cabbage, detailed results of the analysis are presented in Table 2.

Data shown in Table 2 show that soaking seeds with P. *alcaligenes* TrN2, KtS1, and TmA1 with different soaking time showed a significant effect on the speed of seed germination. Seeds germinate fastest found on TmA1.2PDB and TmA1.2NB ie on 3rd day, 0.75 days faster than the control (3.75days). This is in accordance statement Widnyana & Javandira [11]. Which states that based on the observations of the length of time soaking of tomato plants with a bacterial suspension of *Pseudomonas sp*. and *Bacillus sp.* give a good effect. Soaking seeds of tomato plants with a bacterial suspension of Pseudomonas spp and Bacillus sp. for 10 minutes and 20 minutes gave the influence of tomato seedlings grown in the seeding of the most well compared with other treatments and control the same namely 87.50%. Swamp cabbage highest in TrN2.4NB treatment is 26.0cm, followed by TrN2. 6NB is 25.0cm, higher 24.4% and 19.6% compared control (20.9cm). Highest number of leaves found on TrN2.4PDB treatment and TrN2.4NB respectively 13.3 pieces, followed by treatment TrN2. 2NB as 13 pieces. This amount is more 23.15% and 20.37% compared with control (10.8 pieces). The longest leaves are on treatment TrN2.4NB is 10.3cm, followed by treatment TrN2.6NB is 9.8cm. This leaves a longer 24.10% and 15.31% of the control (8.3cm). These results are consistent with results of previous studies that the treatment of *Pseudomonas* spp. can promote the growth of tobacco plants with up to 14% [12].

The length of the stem and stem fresh weight, length and fresh weight root swamp cabbage

Statistical analysis showed that the treatment effect of soaking seeds with the bacteria P. alcaligenes TrN2, KtS1, and TmA1 not significant (P≥0.05) to the length of the stem and stem fresh weight, as well as length and fresh weight root swamp cabbage, details are presented in Table 3.

Table 3 shows the longest swamp cabbage stem found in TmA1.2PDB is 26.0cm, followed by TrN2. 6NB is 25.8cm, both longer 25% and 24.04% of the control (20.8cm). The longest roots found in TmA1.6NB is followed by TrN2.6NB 21.3cm to 18.3cm. The

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roots in both the treatment is longer 46.90% and 26.21% compared with control (14.5cm). The fresh weight stem highest in TrN2. 6NB is 2795g, followed by TrN2. 4NB is 2.495g. The fresh weight stem in both treatments was higher 67.07% and 49.13% compared with control (1,673g). Weight of fresh roots that is highest in the treatment TrN2.6NB is 0.788g, followed by treatment TmA1.6NB is 0.663g. The fresh weight root on both treatments is higher 93.14% and 62.50% compared with the control (0408g).

Table 3: Effect of soaking seeds with the bacteria P. *alcaligenes*. TrN2, KtS1, and TmA1 on stem length, root length, fresh weight, and root fresh weight of swamp cabbage.

No	Treatment	Average results of observations on the parameters of swamp cabbage			
		Stem Length (cm)	Root Length (cm)	Stem Fresh Weight (g)	Root Fresh Weight (g)
1	Control	20.8ns	14.5ns	1.673ns	0.408ns
2	TmA1.2 PDB	26.0ns	15.3ns	2.175ns	0.483ns
3	TmA1.2 NB	23.5ns	12.5ns	1.988ns	0.513ns
4	TmA1.4 PDB	23.0ns	14.8ns	2.120ns	0.485ns
5	TmA1.4 NB	23.3ns	12.8ns	1.990ns	0.410ns
6	TmA1.6 PDB	25.5ns	14.5ns	2.168ns	0.465ns
7	TmA1.6 NB	25.0ns	21.3ns	2.280ns	0.663ns
8	KtS1. 2 PDB	22.5ns	13.0ns	1.670ns	0.420ns
9	KtS1. 2 NB	21.5ns	16.0ns	1.718ns	0.418ns
10	KtS1. 4 PDB	21.3ns	12.5ns	1.763ns	0.428ns
11	KtS1. 4 NB	24.8ns	11.3ns	1.820ns	0.423ns
12	KtS1. 6 PDB	22.5ns	12.5ns	1.748ns	0.338ns
13	KtS1. 6 NB	24.3ns	13.5ns	2.240ns	0.493ns
14	TrN2. 2 PDB	22.0ns	15.0ns	1.558ns	0.450ns
15	TrN2. 2 NB	24.0ns	12.5ns	2.038ns	0.430ns
16	TrN2. 4 PDB	24.3ns	16.5ns	2.473ns	0.608ns
17	TrN2. 4 NB	27.0ns	15.3ns	2.495ns	0.560ns
18	TrN2. 6 PDB	25.0ns	13.0ns	1.905ns	0.533ns
19	TrN2. 6 NB	25.8ns	18.3ns	2.795ns	0.788ns

Note: The same letters behind the numbers in the same column shows the difference was not significant at the 0.05 level of DMRT.

The data indicate that treatment of soaking seeds with the bacteria *P. alcaligenes* give good influence in the growth of swamp cabbage, especially *P. alcaligenes* TrN2 that cause weight gain swamp cabbage stem up to 67.07%. These results are consistent with the statement Gehardson [13] that the use of these *Pseudomonas spp* in plant roots can promote plant growth and protect plants from plant pathogens and pests. Rizobacteria Pseudomonas spp. have a positive effect by occupying the surface of plant root tissues and provides compounds that are beneficial to plants. Some of these bacteria entrance further into the tissue and become endofitik without causing damage or morphological changes in plants [14].

Oven dry weight of plants, stems, and roots swamp cabbage

Statistical analysis showed that the treatment effect of soaking seeds with the bacteria *P. alcaligenes* TrN2, KtS1, and TmA1 significant (P≤0.05) on oven dry weight of stem and root, the details are presented in Table 4.

Table 4 shows that the weight of oven dried swamp cabbage stem highest in TrN2.6NB is 0.35g, followed by TrN2. 4NB is 0.34g. Both of these treatments significantly different (P<0.05) with the control (0.19g). Both of these treatments have the oven dry weight of stem is higher 84.21% and 78.95% compared to the control. Swamp cabbage root oven dry weight highest in TrN2.6NB is 0.10g significantly different with KtS1.4NB and control, with roots oven dry weight 0.04g respectively. Treatment TrN2.6NB has oven dry weight of 150% higher than the control.

Table 4: Effect of soaking seeds treatment with bacterial isolates of P. *alcaligenes* TrN2, KtS1, and TmA1 to the oven dry weight of stems, and roots swamp cabbage.

No	Treatment	Average Results of Observations on the Parameters of Swamp Cabbage	
		Oven dry weight the stem (g)	Oven dry weight of the root (g)
1	Control	0.19cd	0.04b
2	TmA1.2 PDB	0.26abcd	0.07ab
3	TmA1.2 NB	0.22bcd	0.07ab
4	TmA1.4 PDB	0.25abcd	0.07ab
5	TmA1.4 NB	0.21cd	0.05ab
6	TmA1.6 PDB	0.23abcd	0.07ab
7	TmA1.6 NB	0.26abcd	0.09ab
8	KtS1. 2 PDB	0.19cd	0.05ab

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9	KtS1. 2 NB	0.18d	0.06ab
10	KtS1. 4 PDB	0.18d	0.06ab
11	KtS1. 4 NB	0.18d	0.04b
12	KtS1. 6 PDB	0.19cd	0.05ab
13	KtS1. 6 NB	0.29abcd	0.08ab
14	TrN2. 2 PDB	0.19cd	0.06ab
15	TrN2. 2 NB	0.25abcd	0.06ab
16	TrN2. 4 PDB	0.32abc	0.09ab
17	TrN2. 4 NB	0.34ab	0.09ab
18	TrN2. 6 PDB	0.26abcd	0.07ab
19	TrN2. 6 NB	0.35a	0.10a

Note: The same letters behind the numbers in the same column shows the difference was not significant at the 0.05 level of DMRT.

The oven dry weight stems and roots of swamp cabbage are highest in TrN2.6NB, as well as fresh weight swamp cabbage stems and roots is highest in TrN2.6NB treatment. This suggests that the bacterium P. alcaligenes TrN2 treatment by soaking the seed for 60minutes at medium Natrium Broth (NB) gives the best effect on plant growth swamp cabbage. In fact, according to the data that is already displayed in Table 2&3, it can be said that even though the statistical analysis soaking treatment no significant effect compared to control, but on the value presented shows all seed soaking treatment with the bacteria P. alcaligenes TrN2 , KtS1 and TmA1 give higher values than the control on all parameters of observation, such as seed germination, plant height, stem length and weight of plants, number and length of leaves, stems and roots fresh weight swamp cabbage. This is in accordance with the opinion of Tenuta [15], the mechanism of PGPR in improving the health of plants can occur through three ways, is:

i. Pressing the development of pest/disease (bioprotectant): has a direct influence on the plant against pests and diseases.

ii. Producing fitohormon (biostimulant): IAA (Indole Acetic Acid); cytokinins; giberellin; and inhibiting the production of ethylene: can increase the surface area of fine roots, and

iii. Improve the availability of nutrients for plants (biofertilizer). While according McMilan [16], several roles PGPR in promoting the growth of the plants:

a. Increase nitrogen fixation in legumes,

b. Increase the population of bacterial nitrogen-fixing more,

c. Increase the supply of other nutrients, such as phosphorus, sulfur, iron and copper,

d. The production of hormones,

e. Increase the population of beneficial fungi or bacteria,

f. Control of fungal pathogens,

g. Controlling bacteria pathogens, and

h. To control insect pests

Conclusion

Soaking the seed treatment by suspension of *P. alcaligenes* TrN2 for 60 minutes at medium Natrium Broth (NB) gives the best effect on plant growth swamp cabbage. All seed soaking treatment with the bacteria *P. alcaligenes* TrN2, KtS1 and TmA1 give higher values than the control on all parameters of observation, such as seed germination, plant height, stem length and weight of plants, number and length of leaves, stems and roots fresh weight swamp cabbage. The results showed that seed soaking treatment with suspense *P. alcaligenes* cause germination 25% faster, higher crop up 24.4%, number of leaves more up to 23.15%, longer stems to 25%, longer roots up 46.90%, fresh stem weight higher up to 67.07%, dry weight oven stem higher up 84.21% if compared with control.

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