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 (rhizosphere) 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), gibberelin, 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 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 water               |
| 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 Mahasarawati 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 5x10^5 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%.
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) |
| 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       | 3.50b                         | 24.0ns                      | 11.8ns                      | 9.6ns                      |
| 6  | TmA1.6 PDB      | 4.50ab                        | 23.8ns                      | 11.8ns                      | 9.6ns                      |
| 7  | TmA1.6 NB       | 4.50b                         | 24.8ns                      | 11.8ns                      | 9.0ns                      |
| 8  | KtS1.2 PDB      | 3.75ab                        | 22.9ns                      | 11.3ns                      | 8.3ns                      |
| 9  | KtS1.2 NB       | 4.25b                         | 22.6ns                      | 10.8ns                      | 8.6ns                      |
| 10 | KtS1.4 PDB      | 3.75ab                        | 21.8ns                      | 11.5ns                      | 8.9ns                      |
| 11 | KtS1.4 NB       | 3.75b                         | 21.8ns                      | 11.5ns                      | 8.5ns                      |
| 12 | KtS1.6 PDB      | 4.00b                         | 21.0ns                      | 10.3ns                      | 8.4ns                      |
| 13 | KtS1.6 NB       | 4.75b                         | 24.0ns                      | 11.3ns                      | 9.5ns                      |
| 14 | TrN2.2 PDB      | 3.50b                         | 21.6ns                      | 11.8ns                      | 8.6ns                      |
| 15 | TrN2.2 NB       | 3.50b                         | 22.8ns                      | 13.0ns                      | 9.1ns                      |
| 16 | TrN2.4 PDB      | 4.25b                         | 23.3ns                      | 13.3ns                      | 9.6ns                      |
| 17 | TrN2.4 NB       | 3.25b                         | 26.0ns                      | 13.3ns                      | 10.3ns                     |
| 18 | TrN2.6 PDB      | 4.00b                         | 24.1ns                      | 11.5ns                      | 9.3ns                      |
| 19 | TrN2.6 NB       | 4.00b                         | 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 TmA1.2 PDB and TmA1.2 NB ie on 3rd day, 0.75 days faster than the control (3.75 days). 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 seedling of the most well compared with other treatments and control the same namely 87.50%. Swamp cabbage highest in TrN2.4 NB treatment is 26.0cm, followed by TrN2.6 NB is 25.0cm, higher 24.4% and 19.6% compared control (20.9cm). Highest number of leaves found on TrN2.4 PDB treatment and TrN2.4 NB respectively 13.3 pieces, followed by treatment TrN2.2 NB as 13 pieces. This amount is more 23.15% and 20.37% compared with control (10.8 pieces). The longest leaves are on treatment TrN2.4 NB is 10.3 cm, followed by treatment TrN2.2 NB is 9.8 cm. This leaves a longer 24.10% and 15.31% of the control (8.3 cm). 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 TmA1.2 PDB is 26.0 cm, followed by TrN2.6 NB is 25.8 cm, both longer 25% and 24.04% of the control (20.8 cm). The longest roots found in TmA1.6 NB is followed by TrN2.6 NB 21.3 cm to 18.3 cm. The
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 (0.408g).

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          | 1.908ns               | 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    | 25.5ns          | 12.5ns          | 1.748ns               | 0.383ns               |
| 13  | KtS1.6 NB     | 24.3ns          | 13.5ns          | 2.240ns               | 0.493ns               |
| 14  | TrN2.2 PDB    | 22.0ns          | 15.0ns          | 1.585ns               | 0.450ns               |
| 15  | TrN2.2 NB     | 24.0ns          | 12.5ns          | 2.038ns               | 0.430ns               |
| 16  | TrN2.4 PDB    | 24.3ns          | 16.5ns          | 2.473ns               | 0.606ns               |
| 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 endoﬁtk 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 weight of the stem (g) | Oven 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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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, Ks1 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 suspension *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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