The Effect of Seed Soaking with *Rhizobacteria Pseudomonas alcaligenes* on the Growth of Swamp Cabbage (*Ipomoea reptans* Poir)

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Abstract. The research 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. The research has been initially developed on tomatoes. In this research, Randomized Block Design was chosen as its model while the data analysis was performed by using SPSS v.17 for Windows. Three types of treatment were administered towards *P. alcaligenes*, namely isolating, soaking, and growing the medium. Some observed parameters were germination and growth. The results showed that seed soaking treatments with suspense *P. alcaligenes* fostered the germination 25% faster, enhanced the crop up to 24.4%, increased the number of leaves up until 23.15%, lengthen stems to 25%, lengthen the roots up to 46.90%, and increase the fresh weight of stems up until 67.07% and oven-dry weight of stem up to 84.21% compared to the control treatment. The best response of treatment for germination speed was soaking seeds with *P. alcaligenes* TrN2 for 20 minutes on both NB (*Natrium Broth*) and PDB (*Potato Dextrose Broth*) media.

1. Introduction

Swamp cabbage (*Ipomoea reptans* Poir) is a vegetable that has an economic value. It is widespread in Southeast Asia. Swamp cabbage is generally consumed by Indonesians and can be one of the restaurant's menu [1]. It is relatively resistant to drought and has a broad adaptability to various environmental conditions, low-maintenance, and has a short harvest period [2]. Swamp cabbage is commonly grown in home gardens. Yet, some are intensively planted on dry land to optimize the production of swamp. 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 herb to cure constipation and hemorrhoids, and soothe the nerves as well [4]. Its production in Indonesia could reach 50,000-60,000 kg ha\(^{-1}\) [5]. Cultivating swamp cabbage for 0,10 ha\(^{-1}\) needs approximately 16 kgs of seeds. However, slight results are found compared with other crops [6]. Considering the social and economic aspects, swamp has good prospects if it is developed towards agribusiness, but it does require more efforts in planting with efficiency.

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

Rhizobacteria is a group of bacteria with plant root zone habitat (rhizosphere) which has been researched and proven to improve soil fertility, increase plant resistance, and suppress plant pathogens. Rhizobacteria directly acts as a biological fertilizer and biological stimulant which produces essentials hormones to grow crops, such as IAA (Indoleacetic Acid), gibberellin, cytokinin, ethylene, dissolving minerals, and indirectly serves to prevent pathogenic microorganisms through the formation of siderophore and antibiotics. Furthermore, it has not been widely known can stimulate plant growth mechanism [9]. One of the Rhizobacteria which has already been investigated as PGPR and ISR is \textit{P. alcaligenes} isolate KtS1, TrN2, and TmA1. Widnyana \textit{et al.} [10] discover that it increases the growth and yield of tomatoes. Some problems investigated in this research are (1) how seed soaking with \textit{P. alcaligenes} suspend the growth of swamp cabbage and (2) when the best time of seed soaking in promoting the growth of swamp cabbage is, with the purpose of obtaining information related to the benefits of seed soaking for swamp cabbage and best soaking time for swamp cabbage growth.

2. Materials and Methods

2.1. Research Design

The research was designed using Randomized Block Design (RBD) with seed soaking treatments using suspense of \textit{P. alcaligenes} isolates KtS1, TrN2, and TmA1 and two different media, namely Dextrosa Potato Broth (PDB) and Natrium Broth (NB), where they were respectively soaked for 10, 20, and 30 minutes. There were 18 units plus one control treatment. Further, 19 types of treatment were repeated 3 times. Hence, 57 experimental units were involved in the research.

2.2. Preparation of Isolating the \textit{P. alcaligenes} Bacteria

\textit{P. alcaligenes} was isolated in the laboratory of Agro-technology at Faculty of Agriculture, Universitas Mahasaraswati Denpasar. Its effect has been previously investigated on tomatoes. It was then identified as \textit{P. alcaligenes} KtS1, \textit{P. alcaligenes} TrN2, and \textit{P. alcaligenes} TmA1 [11]. The suspension of \textit{P. alcaligenes} was isolated on PDP and NB media and cultured for 48 hours in a-100 ml Erlenmeyer to get a colony density of $5 \times 10^8$ cfu / ml.

2.3 Swamp Cabbage Plantation

Seeds of swamp cabbage which have been treated with \textit{P. alcaligenes} were planted into polybags that have already been filled with sterile planting medium (mixture of soil, sand, and organic fertilizer with a ratio of 1:2:1). Two units of swamp cabbage seeds which have been soaked in suspense \textit{P. alcaligenes} were planted into some polybags. Polybags were then placed in a distance of 10 cm x 10 cm. Each of them was watered with a volume of 100 ml every morning and afternoon.

2.4 Parameter Observation and Data Analysis

Observation on the seeds germination was done daily until sprouts appeared. Height of plants, number of leaves, and length of leaf blades were measured once a week. Length of root was measured at harvest time. Moreover, fresh and dry weight of the roots and stems were also measured. The data was analyzed using SPSS v.17 for Windows. Different tests were performed on average making use of \textit{Duncans Multiple Rings Test} (DMRT) at the level of 5%

3. Results and Discussion

3.1. Germination of Swamp Cabbage Seeds, Its Height, Length of Leaves, and Number of Leaves
Statistical analysis showed that the effect of seed soaking treatments with *P. alcaligenes* TrN2, KtS1, and TmA1 were not significant towards swamp cabbage height, leaf length, and number of leaves. Detailed results of the analysis are presented in Table 1.

Table 1 illustrates that seed soaking with *P. alcaligenes* TrN2, KtS1, and TmA1 with different soaking time showed a significant effect on the speed of seed germination. Seeds germination fastest time was found on TmA1.2PDB and TmA1.2NB i.e. on the 3rd day with a record of 0.75 days faster than the control (3.75 days). This is in accordance with Widnya na and Javandira [12] findings where soaking time length on tomatoes with a bacterial suspension of *Pseudomonas* sp. and *Bacillus* sp. has been discovered to have a good effect.

**Table 1.** The effects of soaking seed with *P. alcaligenes* TrN2, KtS1, and TmA1 on seed germination, height of plant, number of leaves, and length of leaves of swamp cabbage

| No | Treatment with *P. alcaligenes* | Average results of observations on some parameters of swamp cabbage |
|----|---------------------------------|---------------------------------------------------------------|
|    |                                 | Speed of germination (days) | Height of plant (cm) | Number of leaves (sheet) | Length of leaves (cm) |
| 1  | CONTROL                         | 3.75 ab                     | 20.9 ns              | 10.8 ns                  | 8.3 ns               |
| 2  | TmA1.2 PDB                      | 3.00 b                      | 22.9 ns              | 11.8 ns                  | 9.2 ns               |
| 3  | TmA1.2 NB                       | 3.00 b                      | 22.6 ns              | 11.8 ns                  | 8.9 ns               |
| 4  | TmA1.3 PDB                      | 3.25 ab                     | 21.8 ns              | 12.3 ns                  | 8.7 ns               |
| 5  | TmA1.3 NB                       | 3.50 ab                     | 24.0 ns              | 11.8 ns                  | 9.6 ns               |
| 6  | TmA1.4 PDB                      | 4.50 ab                     | 23.8 ns              | 11.8 ns                  | 9.6 ns               |
| 7  | TmA1.4 NB                       | 4.50 ab                     | 24.8 ns              | 11.8 ns                  | 9.0 ns               |
| 8  | KtS1. 2 PDB                     | 3.75 ab                     | 22.9 ns              | 11.3 ns                  | 8.3 ns               |
| 9  | KtS1. 2 NB                      | 4.25 ab                     | 22.6 ns              | 10.8 ns                  | 8.6 ns               |
| 10 | KtS1. 3 PDB                     | 3.75 ab                     | 21.8 ns              | 11.5 ns                  | 8.9 ns               |
| 11 | KtS1. 3 NB                      | 3.75 ab                     | 21.8 ns              | 11.5 ns                  | 8.5 ns               |
| 12 | KtS1. 4 PDB                     | 4.00 ab                     | 21.0 ns              | 10.3 ns                  | 8.4 ns               |
| 13 | KtS1. 4 NB                      | 4.75 a                      | 24.0 ns              | 11.3 ns                  | 9.5 ns               |
| 14 | TrN2. 2 PDB                     | 3.50 ab                     | 21.6 ns              | 11.8 ns                  | 8.6 ns               |
| 15 | TrN2. 2 NB                      | 3.50 ab                     | 22.8 ns              | 13.0 ns                  | 9.1 ns               |
| 16 | TrN2. 3 PDB                     | 4.25 ab                     | 23.3 ns              | 13.3 ns                  | 9.6 ns               |
| 17 | TrN2. 3 NB                      | 3.25 ab                     | 26.0 ns              | 13.3 ns                  | 10.3 ns              |
| 18 | TrN2. 4 PDB                     | 4.00 ab                     | 24.1 ns              | 11.5 ns                  | 9.3 ns               |
| 19 | TrN2. 4 NB                      | 4.00 ab                     | 25.0 ns              | 12.8 ns                  | 9.8 ns               |

**Note:** Same letters behind the numbers in the same column show that the difference was insignificant at the 0.05 level of DMRT
Soaking seeds of tomato with bacterial suspensions of *Pseudomonas* sp. and *Bacillus* sp. for 10 and 20 minutes influenced the seedlings to grow well compared to other treatments and control treatment for 87.50%. The swamp cabbage in TrN2.3NB treatment was 26.0 cm, followed by TrN2.4NB with 25.0 cm. These height was higher for 24.4% and 19.6% compared to control treatment (20.9 cm). The highest number of leaves were found on TrN2.3PDB and TrN2.3NB treatment respectively 13.3 pieces, followed by TrN2.2NB treatment of 13 pieces. These findings are 23.15% and 20.37% higher than the control treatment (10.8 pieces). The longest leaf was discovered on TrN2.3NB treatment of 10.3 cm, followed by TrN2.4NB treatment of 9.8 cm as its length. These leaves were 24.10% and 15.31% longer than those in the control treatment (8.3 cm). These results were consistent with results of previous studies where the treatment of *Pseudomonas* sp. promotes the growth of tobacco plants up until 14% [10].

3.2. Swamp Cabbage's Length of Stem, Fresh Weight of Stem, Length of Root, and Fresh Weight of Root.

Statistical analysis showed that the treatment effect of seed soaking with *P. alcaligenes* TrN2, KtS1, and TmA1 were insignificant (P ≥ 0.05) to both length and fresh weight of swamp cabbage stem, and length and fresh weight of swamp cabbage root (Table 2).

**Table 2.** The effect of seed soaking with *P. alcaligenes* TrN2, KtS1, and TmA1 on swamp cabbage length of stem, length of root, and fresh weight of stem and root

| No | Treatment with *P. alcaligenes* | Length of Stem (cm) | Length of Root (cm) | Fresh Weight of Stem (g) | Fresh Weight of Root (g) |
|----|---------------------------------|---------------------|---------------------|-------------------------|-------------------------|
| 1  | CONTROL                         | 20.8 ns             | 14.5 ns             | 1.673 ns                | 0.408 ns                |
| 2  | TmA1.2 PDB                      | 26.0 ns             | 15.3 ns             | 2.175 ns                | 0.483 ns                |
| 3  | TmA1.2 NB                       | 23.5 ns             | 12.5 ns             | 1.988 ns                | 0.513 ns                |
| 4  | TmA1.3 PDB                      | 23.0 ns             | 14.8 ns             | 2.120 ns                | 0.485 ns                |
| 5  | TmA1.3 NB                       | 23.3 ns             | 12.8 ns             | 1.990 ns                | 0.410 ns                |
| 6  | TmA1.4 PDB                      | 25.5 ns             | 14.5 ns             | 2.168 ns                | 0.465 ns                |
| 7  | TmA1.4 NB                       | 25.0 ns             | 21.3 ns             | 2.280 ns                | 0.663 ns                |
| 8  | KtS1. 2 PDB                     | 22.5 ns             | 13.0 ns             | 1.670 ns                | 0.420 ns                |
| 9  | KtS1. 2 NB                      | 21.5 ns             | 16.0 ns             | 1.718 ns                | 0.418 ns                |
| 10 | KtS1. 3 PDB                     | 21.3 ns             | 12.5 ns             | 1.763 ns                | 0.428 ns                |
| 11 | KtS1. 3 NB                      | 24.8 ns             | 11.3 ns             | 1.820 ns                | 0.423 ns                |
| 12 | KtS1. 4 PDB                     | 22.5 ns             | 12.5 ns             | 1.748 ns                | 0.338 ns                |
| 13 | KtS1. 4 NB                      | 24.3 ns             | 13.5 ns             | 2.240 ns                | 0.493 ns                |
| 14 | TrN2. 2 PDB                     | 22.0 ns             | 15.0 ns             | 1.558 ns                | 0.450 ns                |
| 15 | TrN2. 2 NB                      | 24.0 ns             | 12.5 ns             | 2.038 ns                | 0.430 ns                |
| 16 | TrN2. 3 PDB                     | 24.3 ns             | 16.5 ns             | 2.473 ns                | 0.608 ns                |
| 17 | TrN2. 3 NB                      | 27.0 ns             | 15.3 ns             | 2.495 ns                | 0.560 ns                |
| 18 | TrN2. 4 PDB                     | 25.0 ns             | 13.0 ns             | 1.905 ns                | 0.533 ns                |
Table 2 shows that the longest stem of swamp cabbage was found in TmA1.2PDB treatment of 26.0 cm and followed by TrN2. 4NB treatment of 25.8 cm, both were 25% and 24.04% longer than control treatment (20.8 cm). The longest root was found in TmA1.4NB and followed by TrN2.4NB, respectively 21.3 cm to 18.3 cm. Roots in both treatments were 46.90% and 26.21% longer compared to control treatment (14.5 cm). The highest fresh weight of stem was found in TrN2. 4NB of 2.795 g, followed by TrN2. 3NB of 2.495 g. Fresh weight of stem in both treatments were 67.07% and 49.13% higher compared to the control treatment (1.673 g). The highest fresh weight of roots found in TrN2.4NB treatment of 0.788 g, followed by TmA1.4NB treatment of 0.663 g. The fresh weight of root on both treatments were 93.14% and 62.50% heavier than the control treatment (0.408 g).

The data indicates that seed soaking treatment with \textit{P. alcaligenes} affected the growth of swamp cabbage, as \textit{P. alcaligenes} TrN2 caused weight gain for the stem of swamp cabbage up to 67.07%. These results are consistent with Gehardson's (2002) finding that the use of \textit{Pseudomonas} spp in plant roots promotes plant growth and protects plants from plant pathogens and pests. Rhizobacteria \textit{Pseudomonas} spp. brings some positive effects by occupying the surface of plant root tissues and provides compounds which are beneficial to plants. Some of these bacteria further enter the tissue and become endophytic without causing any damage or morphological changes in plants themselves [13].

3.3 Oven-Dry Weight of Plant, Stems, and Roots of Swamp Cabbage

Statistical analysis showed that the effect of seed soaking treatments with \textit{P. alcaligenes} TrN2, KtS1, and TmA1 were significant (P ≤ 0.05) on oven-dry weight of stem and root. The details can be seen in Table 3.

Table 3. The effect of seed soaking treatments with \textit{P. alcaligenes} TrN2, KtS1, and TmA1 on the oven-dry weight of stems and roots of swamp cabbage

| No | Treatment with \textit{P. alcaligenes} | Average results of observations on the parameters of swamp cabbage |
|----|--------------------------------------|-----------------------------------------------------------------|
|    |                                      | Oven-dry weight of stem (g) | Oven-dry weight of root (g) |
| 1  | CONTROL                              | 0.19 cd 0.04 b            | 0.04  b                     |
| 2  | TmA1.2 PDB                           | 0.26 abcd 0.07 ab         | 0.07  ab                    |
| 3  | TmA1.2 NB                            | 0.22 bcd 0.07 ab          | 0.07  ab                    |
| 4  | TmA1.3 PDB                           | 0.25 abcd 0.07 ab         | 0.07  ab                    |
| 5  | TmA1.3 NB                            | 0.21 cd 0.05 ab           | 0.05  ab                    |
| 6  | TmA1.4 PDB                           | 0.23 abcd 0.07 ab         | 0.07  ab                    |
| 7  | TmA1.4 NB                            | 0.26 abcd 0.09 ab         | 0.09  ab                    |
| 8  | KtS1.2 PDB                           | 0.19 cd 0.05 ab           | 0.05  ab                    |
| 9  | KtS1.2 NB                            | 0.18 d 0.06 ab            | 0.06  ab                    |
| 10 | KtS1.3 PDB                           | 0.18 d 0.06 ab            | 0.06  ab                    |
| 11 | KtS1.3 NB                            | 0.18 d 0.04 b             | 0.04  b                     |
| 12 | KtS1.4 PDB                           | 0.19 cd 0.05 ab           | 0.05  ab                    |
| 13 | KtS1.4 NB                            | 0.29 abcd 0.08 ab         | 0.08  ab                    |
| 14 | TrN2.2 PDB                           | 0.19 cd 0.06 ab           | 0.06  ab                    |
Table 3 illustrates the highest weight of oven-dried swamp cabbage stem found in TrN2.4NB treatment of 0.35 g, followed by TrN2.3NB of 0.34 g. These treatments were significantly different (P< 0.05) compared to the control treatment (0.19 g). These treatments had oven-dry weight of stem which were 84.21% and 78.95% higher than the control treatment. The highest oven-dried weight of swamp cabbage root was found in TrN2.4NB treatment of 0.10 g, which was significantly different from KtS1.3NB and control treatment, with oven-dry weight of root of 0.04 g respectively. TrN2.4NB treatment had oven-dry weight of root 150% higher than the control.

The oven-dry weight of stems and roots of swamp cabbage were found the highest in TrN2.4NB. Similarly, the fresh weight of swamp cabbage stems and roots was found the highest in TrN2.4NB treatment. This suggests that *P. alcaligenes* TrN2 treatment through seeds soaking for 60 minutes on Natrium Broth (NB) medium gave the best effect on plant growth of swamp cabbage.

In sum, the data displayed in Table 1 and Table 2 illustrates that even though the statistical analysis of soaking treatments shows no significant effect compared to the control treatment, results presented have shown that all seed soaking treatment with *P. alcaligenes* TrN2, KtS1 and TmA1 yielded higher values than control treatments on all parameters of observation, such as seed germination, height of plant, length of stem, weight of plants, numbers and length of leaves, fresh weight of stems and roots of swamp cabbage. This is in accordance with Tenuta's study [14] which states that the mechanism of PGPR in improving the health of plants occurs through three ways: 1) Pressing the development of pest and/or disease (bioprotectant) has a direct influence on the plant against pests and diseases; 2) Producing phytohormones (biostimulant), IAA (Indoleacetic Acid), cytokinins, gibberellin, and inhibiting the production of ethylene can increase the surface area of fine roots; and 3) Improving the availability of nutrients for plants (biofertilizer). McMilan and Gerhardson [15,16] further elaborates that several roles of PGPR in promoting the growth of plants are (1) increasing nitrogen fixation in legumes, (2) increasing the population of nitrogen-fixing bacteria, (3) increasing the supply of other nutrients, such as phosphorus, sulfur, iron, and copper, (4) producing hormones, (5) increasing the population of beneficial fungi or bacteria, (6) controlling the pathogenic fungus, (7) controlling pathogenic bacteria, and (8) controlling pest insects.

### 4. Conclusion

1. Seed soaking treatments by suspension of *P. alcaligenes* TrN2 for 60 minutes on Natrium Broth (NB) medium gave the best effect on plant growth of swamp cabbage.

2. All seed soaking treatments with *P. alcaligenes* TrN2, KtS1, and TmA1 gave higher impacts than the control treatment on all parameters of observation, such as seed germination, height of plant, length of stem, weight of plants, number and length of leaves, and fresh weight of stems and roots of swamp cabbage.

3. The results showed that seed soaking treatment with suspense *P. alcaligenes* caused germination 25% faster, yield of crop up to 24.4% higher, increased number of leaves up until 23.15%, made stems 25% longer, lengthened roots up to 46.90%, and enhanced fresh weight of stem up to 67.07% higher and oven-dry weight of stem up until 84.21% higher than the control treatment.
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