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The Use of Rhizobacteria to Promote Buds Formation of Bulbil of Elephant Foot Yam

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Abstract
Elephant foot yams (*Amorphophallus muelleri* Blume) is one of tuber crops becoming popular among farmers in Indonesia as the tubers of this plant rich in carbohydrates, fats, protein, minerals, vitamins and fiber. However, the propagation of this plant can not be done easily as the seedlings materials such as tubers, bulbils and seeds have dormancy periode after harvested. This study was done to evaluate the capability of rhizobacteria from rhizospheres of graminous plants to promote the buds formation of bulbil of elephant foot yams. A total of 76 isolates of rhizobacteria were tested for their capability to produce indole acetic acid (IAA), and for those capable of producing IAA were tested for their ability to promote buds formation of elephant foot yams bulbils. Results of this strudy showed that, nine isolates of rhizobacteria were proven to produce IAA, namely Sr3, Sr16, Sr17, Sr18, Sr19, Sr21, Jg8, Rg1 and Pb2. Treatments with rhizobacteria significantly ($p<0.05$) increased the percentage of bulbils produced buds. Treatment with three isolates namely Sr21, Jg8 and Rg1 resulted in percentage of buds formation more than 60%, in which treatment with isolate Rg1 showed the highest percentage of buds formation. This results suggested that isolate Rg1 potentially can be used as a bio-agent to promote buds formation in bulbils of elephant foot yams to produce uniform and vigorous seedlings of this plant.
Keywords: elephant foot yams, rhizobacteria, buds formation, promotion, seedlings
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1. Introduction

Elephant foot yam (*Amorphophallus muelleri* Blume) belongs to the Araceae family and is one of the biological riches of Indonesian tubers. In Indonesia this plant is called *porang*. As a plant that produces carbohydrates, fats, proteins, minerals, vitamins and dietary fiber, elephant foot yam have long been used as food and as industrial raw materials (Saleh *et al*., 2015). This plant is widely cultivated commercially in India because it is well known as a root vegetable. The tubers of this plantare rich in starch and are used as a staple food. The tubers are consumed as vegetables after boiling, roasting and frying (Nedunchezhiyan *et al*., 2002; Nedunchezhiyan, 2008). Elephant foot yam tubers have several medicinal properties and have been shown to be effective in the treatment of hemorrhoids, dysentery, asthma, inflammation of the lungs, vomiting and gastrointestinal disorders (Raghu *et al*., 1999). Moreover, it is used in pharmaceutical raw materials, especially in ayurvedic medicines.

The elephant foot yam is starting to receive attention among farmers and the government because it has enormous potential to produce export products in the form chips and glucomannan flour. The area of elephant foot yam plantations in Indonesia in 2020 was 19,950 ha and in 2021 reached 47,461 ha spread over 15 provinces and is targeted to be a maximum of 100,000 ha in 2024 supported by processing industries and their markets (Ministry of Agriculture the Republic of Indonesia, 2021). Specifically for Bali, the area of porang plants in 2020 reached 942 ha spread over several regencies such as Jembrana, Tabanan, Buleleng, Klungkung and Karangasem (Media Indonesia, 2021).

Several factors affect the growth and yield of elephant foot yam (Ravi *et al*., 2011; Suja *et al*., 2012). Lack of sufficient plant material (tubers or bulbil) of uniform size, poor tuber quality and presence of dormancy are the main constraints limiting elephant foot yam production (Bhagawan *et al*., 2008; Misra *et al*., 2001). About 25% of the crop is usually used as seed in India. Sometimes it is difficult for farmers to access large quantities of good quality seeds. Shoot (apical) buds grow together on the planted tuber, regardless of size, after dormancy is broken. It takes 5.0 to 6.0 tonnes of tubers to plant one hectare of land and this makes transportation and storage difficult. Tubers are not suitable for immediate planting due to dormancy and also slow shoot development which takes about 3-4 months after harvest. After shoots emerge from the tuber, shoot differentiation and shoot growth takes almost 2 months after the shoots start to grow. In Indonesia elephant foot yam propagation is done with tubers and bulbil. If bulbil are used, it takes around 100 kg/ha or as many as 20,000 bulbil/ha (Ministry of Agriculture, The Republic of Indonesia, 2021).

As is the case in India, elephant foot yam cultivation in Indonesia is also experiencing problems with nurseries. It is very difficult to be able to plant elephant foot yam seedlingswith a uniform age because of the dormancy phase of the seedling materials, both tubers, bulbil and seeds. This condition causes elephant foot yam planting to not be

carried out whenever needed. It is necessary to find bio-agent that can promote the germination of seedling material particularly bulbil. This study was done in order to find rhizobacteria that can promote the shoot formation of bulbil of elephant foot yam.

2. Materials and Method

2.1. Rhizobacteria Isolates

A total of 76 isolates of rhizobacteriaobtained from the Laboratory of Biopesticide, Faculty of Agriculture, Udayana University, Bali Indonesia were used in this study. These isolates were isolated from rhizospheres of the plants belong to the family Graminae (Poaceae) grown in Bali.

2.2. Test for Indole Acetic Acid Production

All 76 isolates of rhizobacteria were tested for their ability to produce indole acetic acid (IAA). The ability of rhizobacteria isolates to produce IAA was based on the method developed by Gusmiaty *et al*. (2019) with modification. Bacterial isolates were grown in 100 ml of nutrient broth medium added with 1 mM L-tryptophan, incubated in the dark at room temperature (28±2ºC). A total of 1.5 ml of the rhizobacteria culture was centrifuged for 10 minutes at 8,000 rpm. A total of 1 ml of the supernatant was added with 4 ml of Salkowski reagent and this mixture was incubated in the dark at room temperature for 24 hours. Absorbance measurements were carried out using microplate reader RT-2100C (P.R. China) with a wavelength of 520 nm. The concentration of IAA was determined based on a pre-prepared standard curve.

2.3. Test for the Ability of IAA-producing Rhizobacteria Isolates to Promote the Buds Formation The seedling material used in this study was bulbil with average diameter of 3.1 cm and average weight of 7.81 g. A total of 9 IAA-producing isolates were tested in this experiment, namely Sr3, Sr16, Sr17, Sr18, Sr19, Sr21, Jg8, Rg1 and Pb2. The experimental design used was a completely randomized design (CRD) with a total of 10 treatments (9 isolates and 1 control), and each treatment was repeated 3 times so that there were 30 experimental units consisting of 30 bulbils each. The treatment was carried out by immersing the bulbil in an isolate suspension with a density of 10^6 CFU/ml for 60 minutes. Bulbils for control were immersed in sterile water. The treated bulbil were placed on a plastic tray which had been covered with wet tissue to keep moisture in, then wrapped in plastic wrapping. These trays were then placed in a dark place for 14 days at room temperature (28±2ºC). The number of bulbil that produced shoots was then counted to determine the percentage of shoot formation.

2.4. Data Analysis

Data obtained from this study were analysed using analysis of variant (ANOVA) and continued by significance test using the Duncan's multiple range test at 5%. Statistical analysis was conducted with the help of SPSS software forWindows version 17.0 in 2009.

3. Results and Discussion

3.1. Rhizobacteria with IAA Production

Of 76 isolates of rhizobacteria tested for their capability to produce IAA, only nine isolates produced obvious quantity of IAA namely Sr3, Sr16, Sr17, Sr18, Sr19, Sr21, Jg8, Rg1 and Pb2 as presented in Table 1.

Table 1. List of Isolates of rhizobacteria capable of producing IAA

No	Isolates	Rhizospheres of plants	Location of sampling	IAA production
1	Sr3	*Cymbopogon citratus*	Denpasar	+
2	Sr16	*Cymbopogon citratus*	Bangli	+
3	Sr17	*Cymbopogon citratus*	Bangli	+
4	Sr18	*Cymbopogon citratus*	Jembrana	+
5	Sr19	*Cymbopogon citratus*	Tabanan	+
6	Sr21	*Cymbopogon citratus*	Buleleng	+
7	Jg8	*Zea mays*	Karangasem	+
8	Rg1	*Pennisetum purpureum*	Denpasar	+
9	Pb2	*Amorphophallus muelleri*	Jembrana	+

+: indicates positive for IAA production

Data in Table 1 indicates that most of rhizobacteria (66.67%) that capable of producing IAA from rhizospheres of *Cymbopogon citrates.*

3.2. Buds Promotion by IAA-producing Rhizobacteria

This study proved that treatment with rhizobacteria from rhizospheres of the plants of the family Graminae

significantly ($p<0.05$) promoted the formation of buds from bulbil of elephant foot yams as presented in Table 2. Except for treatments with isolates Sr19 and Pb2, all treatments with other isolates showed significant higher percentage of bulbil produced shoot. Among others, isolate Rg1 showed the highest percentage of bulbil produced buds (92.84%). Two treatments with other isolates namely Sr21 and Jg8 resulted in percentage of bulbil produced buds 61.29 and 73.49% respectively.

All bulbils used in this study produced only one bud after treatment as shown in Fig. 1. This condition is suitable for seedling material, as the buds will not compete each others and will be able to grow become vigorous shoot when transplanted into the field.

Propagation by using seedling material in the form of tubers or pieces of tubers that have growing points (apical meristems) is the most common method. Tubers used as seedling material should be quite large, because if they are too small, to grow and produce large tubers requires 2-3 growing seasons. According to Mondal and Sen (2004), a high percentage of shoot formation (98%) if the seedlings are obtained from the upper half of the tuber cut, while from the lower half of the tuber, will result in lower shoot formation. The base of the tuber is generally not good for use as seedlings. According to Santosa *et al*. (2006a), seedlings with intact apical buds germinated faster and produced larger plants than seedlings with split apical buds or seedlings without apical buds. Cutting apical shoots encourages growth of lateral shoots which will delay germination. Whole and upper half seedlings with intact apical buds produce larger tiller tubers than seedlings with injured shoots. The low yields obtained using seed slices with sliced apical buds are supported by the fact that seed cutting reduces the size of the leaves that grow during growth. Kumar *et al*. (1998) reported that treating sweet potato pieces with chemicals such as thiourea (200 ppm), potassium nitrate (1000 ppm), kinetin (5 ppm), was quite effective in increasing sweet potato germination from 24.3 to 92%, 17.8% and 13.4 %. However, this treatment did not significantly increase the yield of sweet potatoes.

Tabel 2. Percentage of bubil of elephant foot yams produced buds without (control) and with treatments of isolates of IAA-producing rhizobacteria

No	Treatments	Percentage of bulbil produced buds
1	Control	29,15 g*
2	Sr3	46,44 de
3	Sr16	44,52 de
4	Sr17	48,25 d
5	Sr18	39,54 ef
6	Sr19	33,87 fg
7	Sr21	61,29 c
8	Jg8	73,49 b
9	Rg1	92,84 a
10	Pb2	31,32 fg

Means followed by the same letters are not signicicantly ($p<0.05$) different According to the Duncan's Multiple Range Test at 5% level

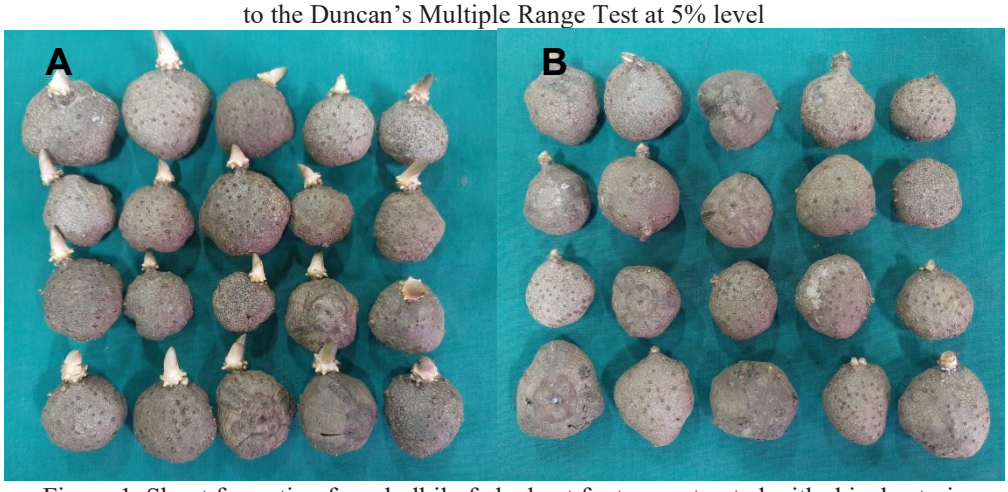

Figure 1. Shoot formation from bulbil of elephant foot yams treated with rhizobacteria isolate Rg1 (A) and Control (B)

Mohankumar and Ravi (2001) also reported that smoking whole tubers for 6 hours/day for six weeks increased buds formation by 58.4% compared to no smoking. Similar results were obtained by exposing tubers to

a temperature of 45ºC for 6 hours/day for three weeks, increasing buds formation by 83.3%. It was also concluded that the heating treatment at 32ºC and the immersion treatment in thiourea solution for 20-30 minutes had a significant effect on breaking seed dormancy. Treatment of dark conditions had a negative effect on seed germination. Likewise, the use of abscisic acid (ABA) 10 mg/l and ferulic acid (400 mg/l) actually inhibited seed germination. The size of the sweet potato or the pieces of sweet potato used as seedlings has an effect on plant productivity. In general, seedlings weighing 500 g, planted with a spacing of 90 x 90 cm are ideal conditions for producing elephant foot yams tubers.

According to Sumarwoto and Maryana (2011), medium-sized (5 g) and large (10 g) bulbils are equally good when used as seedlings, while small bulbils (1.5 g) can be used as seeds if they have undergone special maintenance first. In natural propagation occurs through bulbils that fall scattered around the mother plant. Elephant foot yams can also be propagated by using seeds. Seeds are taken from ripe fruit. Seeds are spread evenly in the seedbed with sand or soil that is crumbly and fine, protected from direct sunlight and kept moist by watering. Not all seeds that are sown can grow, generally around 40%, depending on growing environmental conditions and the level of maturity of the fruit. When the seedlings have grown and reached a height of 10-15 cm, the seedlings are ready to be transferred to the field. The tubers harvested from the seedling are not big enough and not yet fit for harvest. Seed sowing is more intended to prepare seedlings for the next season.

4. Conclusion

IAA-producing rhizobacteria isolated from rhizospheres of plants belong to the family Graminae (Poaceae) obviously promoted the formation of buds of bulbils of elephant foot yams. Three isolates of rhizobacteria namely Sr21, Jg8 and Rg1 resulted in percentage of buds formation more than 60%, and treatment with isolate Rg1 showed the highest percentage of buds formation. This results suggested that isolate Rg1 potentially can be used as a bio-agent to promote buds formation in bulbils of elephant foot yams. This isolate should be further tested in a large scale of sedlings production, to prove whether isolate Rg1 shows its stability and consistency.

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