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Effect of N_2-fixing and IAA synthesis endophytic bacteria on growth of *Vanda* under greenhouse condition

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ABSTRACT

Vanda is one of the orchid genera that are important commercial crops. It was traded in both cut flower and plant form. In 2018, the *Vanda* export of Thailand had a marketable value of about 0.13% as cut flowers and 8.9% as orchid plants. Orchid cultivation is usually used a large number of fertilizers for growth and development, which lead to high cost and environmental problems. Therefore, this research was aimed to study N_2-fixing and IAA produced by endophytic bacteria as biofertilizers under greenhouse conditions. This research was conducted in a completely randomized design (CRD), and *Vanda* plantlets from tissue culture were inoculated with isolated 3S19 endophytic bacteria compared with deionized water (as a control treatment). Each treatment had twenty replications. The results showed that there were no significantly different between isolate 3S19 inoculation plants and with sterilized deionized water in plant height and leave number at 2, 3, and 4 months after inoculation. In contrast, root length and the number of roots were significantly different at 4 months after inoculation. The data from this research is beneficial as primary data for biofertilizer application to *Vanda* orchid.

Keywords: *Vanda*, orchid, endophytic bacteria, biofertilizer

INTRODUCTION

Orchids are an important commercial crop as an ornamental plant, potted plant, medical material, and industrial material (Hinsley et al., 2018). In 2018, Thailand exported cut flower and orchid plants for about 6.81 million US dollars (Department of International Trade Promotion, 2020). *Vanda* is one of the orchid exporting products with a market value of about 0.13% as cut flower form and 8.9% as an orchid plant (De et al., 2015; Panjama, 2018). Orchid cultivation has many factors to promote quality and yield, such as water, fertilizer, greenhouse, microorganism, etc. Generally, Thai farmers use a large number of fertilizers as water-soluble fertilizer forms, i.e. 20-20-20 or 21-21-21 (balance fertilizer) for the vegetative stage, 10-52-17 during floral stimulation, and 16-21-27 for growth and development (Department of Agriculture, 2017). The above information indicated that Thai growers used large amounts of fertilizers, resulting in high costs for orchid production. In addition, using chemical fertilizer leads to chemical accumulation in the environment because chemical fertilizer is slowly disintegrating (Ray et al., 2018. Micro-organisms, including bacteria or fungi, are one of the alternative products to promote plant growth through organic matter synthesis and are eco-friendly to the environment.

Endophytic bacteria is one micro-organism that lives inside the tissue of the plant and does not cause plant disease to the host plant (Lacava and Azevedo, 2013). It can promote host plant growth and development through many mechanisms such as plant growth promoter substance synthesis, e.g., Indole-3-acetic acid (IAA), gibberellic acid, and cytokinin substance, promoting nutrient or water absorbance, nitrogen fixation, and biological control (Lacava and Azevedo, 2013).

Therefore, this research was aimed to study N_2-fixing and IAA synthesis by endophytic bacteria to promote *Vanda* growth under greenhouse conditions. This basic information may be useful for biofertilizer production in the future.

MATERIALS AND METHODS

This research was conducted with two treatments. *Vanda* plants were inoculated with isolate 3S19 at a ratio of 1:1 (endophytic bacterial suspension: sterilized water) compared with deionized water (as control treatment). There were 20 replications per treatment.

Preparing of endophytic bacteria suspension

The isolate 3S19 of endophytic bacteria, isolated from *Vanda* 'Manuvadee'which has the capability to fix nitrogen and synthesize IAA. (Inkaewpuangkham et al., 2021a; Inkaewpuangkham et al., 2021b). The assessment of nitrogen-fixing was conducted according to Weaver and Danso (Weaver and Danso, 1994),

by acetylene reduction assay and an IAA synthesis estimating according to a modified version of the Gordon and Weber method (Gordon and Weber, 1951). Firstly, the isolate 3S19 was cultured in a nutrient agar (NA) medium for seven days. Then, a single colony of isolate 3S19 was cultured in nutrient broth (NB) medium at 25 C° and shaken at 120 rpm for three days. Finally, endophytic bacteria suspension was estimated at an optical density of 0.5 at 600 nm (Lertjantarangkool et al., 2017) (approximately 10^8 cfu/ml) before use.

Inoculation in Vanda plantlet

Vanda hybrid 6 months old plantlets from tissue culture were immersed in isolate 3S19 endophytic bacteria suspension compared with sterilized deionized water for 30 minutes. Then, inoculated plantlets were transferred to the basket and cultivated under greenhouse conditions for four months. The average temperature in the greenhouse was about 28˚C and 70% RH.

Data collection

The data collection were plant height, number of leaves, root length, and number of roots at 2, 3, and 4 months after inoculation. The data was collected once times per month.

Statistical analyses

The data were analyzed by T-test independent using the Sxw program (Analytical Software version 8.0) with a significant difference at 95% probability level.

RESULTS

Growth of plantlet

The height of inoculated plantlets was not significantly different from control treatments at 2, 3, and 4 months after inoculation (Table 1).

Table 1. Height (cm.) of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water (control).

Treatments	Months after inoculation (month)		
	2	3	4
Inoculated *Vanda*	8.3	8.5	8.6
Control plant	7.9	8.1	8.3
T-test	ns	ns	ns

ns = not significant

The number of leaves per plant of *Vanda* increased 4 months after inoculation. However, there was no significantly different between treatments at 2, 3, and 4 months (Table 2).

Table 2. Number of leaves of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water (control)

Treatments	Months after inoculation (month)		
	2	3	4
Inoculated *Vanda*	4.2	4.5	5.1
Control plant	4.2	4.7	5.3
T-test	ns	ns	ns

ns = not significant

There was no significantly different in root length of *Vanda* between treatments at 2 and 3 months after inoculation. However, there was a significantly different root length between treatments at 4 months. The inoculation treatment with isolate 3S19 showed higher root length than the control plant (Table 3.).

Table 3. Root length (cm.) of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water (control)

Treatments	Months after inoculation (month)		
	2	3	4
Inoculated *Vanda*	1.1	2.0	2.9[a]
Control plant	1.0	1.8	2.2[b]
T-test	ns	ns	*

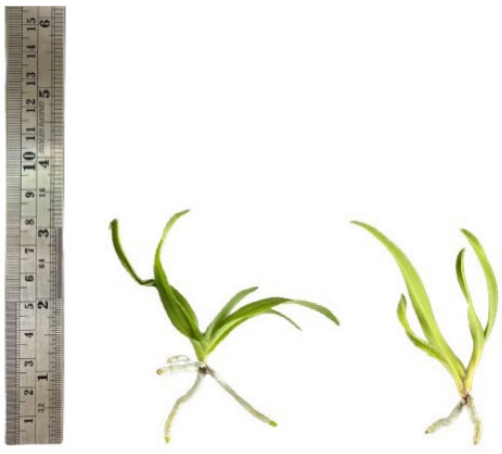
Data analysis by T-test
ns = not significant
* = significant difference at 95% (P< 0.05)

At 2 and 3 months after inoculation, there was no significantly different between treatments. Nevertheless, inoculated *Vanda* with isolate 3S19 had more roots than control plants at 3.8 and 3.3 roots per plant, respectively (Table 4.).

Table 4. Number of roots of *Vanda* at 2, 3, and 4 months after inoculation with bacterial endophytes or sterilized deionized water

Treatments	Months after inoculation (month)		
	2	3	4
Inoculated *Vanda*	2.8	3.5	3.8[a]
Control plant	2.9	3.2	3.3[b]
T-test	ns	ns	*

Data analysis by T-test
ns = not significant
* = significant difference at 95% (P< 0.05)



A B

Figure 1. Growth of *Vanda* plantlet after 4 months inoculation with isolate 3S19 (A), with sterilized deionized water (control) (B).

DISCUSSION

Plant height and leaf number of *Vanda* plantlet during 2-3 months after inoculation were not significantly different. It might be associated with host plant and bacteria interaction. The success of plant-bacteria associate depends on several factors. The condition of the environment is one factor for endophytic bacteria colonization, including season, temperature, latitude, longitude, nutrient, soil conditions, and so on (Chiellini et al., 2014; Yang et al., 2017; Wu et al., 2021). For example, the study of Ou et al. (2019) revealed that in the spring season, Actinobacteria and Proteobacteria of endophytic bacteria were found to be the most abundant in mulberry (*Morus* L.), but in the fall season found only Proteobacteria. In addition, the host plant is the main factor for endophytic bacteria colonization, consists of the plant growth stage, plant physiology, plant tissue type: Santos et al. (2018) showed that the plant tissue type leads to the different community of endophytic bacteria in host plant, plant species that similar Engelhard et al. (2000) reported that wild rice species and traditional race rice had a population of *Azoarcus* sp. higher than modern varieties of rice since the influence of plant species. Likewise, the plant defense process is associated with plant-bacteria interaction: in some genera bacteria can induce plant defensive process that results in colonization difficult by other endophytic bacteria into the host plant (Santos et al., 2018). Furthermore, micro-organism species are an important factor for entry to plants. Research by Nogueira et al. (2001) reported that plant responses involve in plant-gene expression which was found associated with bacteria and a specific bacterial species.

Root length and number of roots of inoculated *Vanda* at 4 months after inoculation showed better growth than the control treatment. It might be because of abundant endophycic bacteria in the root organ. Typically, endophycic bacteria start entering into host plant via the roots because the root plant produces exudates to interact with bacteria. After endophytic bacteria entry to the root, they can spread to colonize above ground tissue by the movement of bacteria (bacterial flagella) and plant transpiration stream (Afzal et al., 2019). So, the 3S19 endophytic bacteria might be more abundant in roots than in other organs resulting in the root zone had more IAA synthesis by endophytic bacteria than the above zone. The property of isolate 3S19 could produce indole-3-acetic acid (IAA) without and with L-tryptophan medium at 9.48 and 17.38 mg IAA/l, respectively (Inkaewpuangkham et al., 2021a). Indicating that this isolate was active to produce IAA without exogenous tryptophan. Similarly, the previous report found that orchid roots are associated with fungal and bacterial symbionts, and they can synthesize IAA into orchids (Novak et al., 2014). Furthermore, a similar result was revealed by Khalid et al. (2004) that microorganisms isolated from plant rhizoferic soil could synthesize IAA by using exudate containing tryptophan substrate from the plant root released. Since IAA is a plant growth regulator classified in the auxin group, it is predominant for promoting root formation or root initiation and enhancing root growth (Novak et al., 2014). Thus, isolating 3S19 could effectively promote root growth. Similar Tsavkelova et al. (2007) research reported that bacteria were isolated from root terrestrial orchids and epiphytic tropical orchids. These bacteria can produce IAA and stimulate root formation of kidney bean cutting.

The above reasons indicate that most bacteria often enter through roots and spread to the above plant part, and isolate 3S19 might have an intensive bacteria population in roots than leaves. So, isolate 3S19 might affect root growth than leave growth in this stage of *Vanda*.

CONCLUSIONS

In conclusion, *Vanda* plantlets inoculated with isolate 3S19 of endophytic bacteria at a ratio of 1:1 (endophytic bacterial suspension: sterilized water) affected the growth of *Vanda* roots in both root length and the number of roots per plant than with sterilized deionized water inoculation. However, the data from this research is basic information to develop into a biofertilizer product. In the next step, the researcher will assess aspects of this isolate, such as phosphate solubilization and siderophore synthesis.

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