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Inoculation of IAA producing endophytic bacteria on the growth of mung bean (*Vigna radiata* L.) Var. vima2

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Abstract : The interaction of endophytic bacteria with their host plants through a direct mechanism can stimulate growth by increasing nutrient absorption and modulating phytohormones such as IAA (Indole-3-acetic acid). A previous study revealed the IAA production ability by endophytic bacteria from Cinchona (*Cinchona Iedgeriana* Moens.) plant. The most optimum IAA producer from the previous study will be analyzed for the effect on plant growth. The purpose of this study was to test and analyze the effect of IAA-producing endophytic bacteria inoculation obtained from the roots of cinchona on the growth parameter of mung bean seedling. The highest IAA-producing bacteria, isolate with code a15 has been compared to the MC Farland 0.5 and MC Farland 1 standards are used to soak the mung bean seeds for 1 hour and being planted on sterile soil media in six replications. The inoculation of MC Farland 0.5 equivalent bacteria showed the increased growth parameters of mung bean seedling, such as height, wet weight, and the number of leaves. Furthermore, the concentration of bacterial suspension that has been equalized with the Mc Farland 1 shows the inhibition effect of the growth.

Keywords : *Cinchona Iedgeriana*; mung bean; endophyte; IAA; plant growth promotion bacteria.

INTRODUCTION

The knowledge of bacterial associated with the plant was established many years, and bacterial endophytes were revealed to have an essential role in supporting the plant growth (Chaturvedi & Singh, 2016). Most bacterial endophytes can produce various essential substances related to plant growth promotion and protection mechanism against pathogens such as IAA (Etesami et al., 2015). The ability to produce IAA hormone by bacterial endophytes is affecting many important events in supporting plant growth, such as tissue and morphological development, ACC deaminase trigger factors, and the involvement in secondary metabolite production (Zhao et al., 2011). Although the investigation of bacterial endophytes that produce IAA is quite establishing, the implementation of this potency should be more investigated. The implementation of bacterial endophytes that can stimulate the host plant growth could be used directly as a biofertilizer (Wong et al., 2015). Furthermore, this study will try to reveal the effect of IAA producer bacterial endophyte obtained from the Cinchona plant on the growth and physiological changes of the mung bean.

IAA produced in plants is called endogenous IAA, while IAA produced by organisms other than plants is called exogenous IAA (Ludwig-Müller, 2011). Similar to plants IAA product, the exogenous IAA produced by endophytes also have an important role on the plant to regulates plant growth and development through cell division and elongation, tissue differentiation, apical dominance, and response to light, gravity, and pathogens (Ahmed & Hasnain, 2010). In previous research (data not shown), most of the endophytic bacteria derived from quinine roots can produce IAA phytohormones. The isolate a15 was selected for the subsequent investigation due to the highest IAA production and stability during the culture period.

Moreover, the object of this research is the mung bean (*Vigna radiata* L.) var. vima2, which is one of the superior varieties released by BALITKABI on December 11, 2013. The Vima2 variety has been chosen due to the growth characteristics such as early maturity, consistent growth, and the widely used (Iswanto, 2014). This research was conducted to determine the effect of inoculation of IAA-producing endophytic bacterial suspension from quinine root on the growth of mung bean seeds. Furthermore, the effect of different concentrations of bacterial endophytes will be evaluated, especially the characteristics related with the growth parameter such as length, leaf number, dry and fresh weight.

MATERIALS AND METHODS

Preparation of Bacterial Suspension Concentration

The bacterial density used was balanced using standard solutions of 0.5 and 1 Mc Farland. The standard 0.5 Mc Farland solution was prepared by mixing 9.95 ml of 1% H2SO4 solution with 0.05 ml of 1% NaCl solution so that the volume became 10 ml. 1 Mc Farland standard solution was prepared by mixing 9.90 ml of 1% H2SO4 solution with 0.10 ml of 1% NaCl solution, then shaken until homogeneous. The absorbance value is measured with a 600 nm wavelength spectrophotometer. Mc Farland standard absorbance value 0.5 = 0.133, Mc Farland 1 = 0.342. The available bacterial suspensions were adjusted to the Mc Farland standard absorbance values of 0.5 and 1.

Green Bean Seed Sterilization

Mung bean seeds are obtained from BALITKABI. The seeds were sterilized using a fungicide solution plus two drops of 80% tween solution and then shaken for 30 minutes at a speed of 120 rpm. The seeds were rewashed with sterile distilled water, soaked in 10% Clorox solution, then shaken for 15 minutes, and washed with sterile distilled water three times. The seeds were then soaked in 70% alcohol for 1 minute and rinsed with sterile distilled water (Herlina et al., 2016).

Soil Media Sterilization

Organic growing media with a composition of humus, manure, and leaves were left for three days at room temperature and then sterilized using an autoclave for 1 hour with a pressure of 0.10 MPa and a temperature of 121^{o}C. The sterilized soil is added with distilled water so that it is not too dry. The growing media was then left for two days and then autoclaved once again to ensure the loss of microbes that might not have been perfect in the first autoclave process.

Inoculation of IAA-producing Root Endophytic Bacteria to Mung Bean Sprouts

Twenty sterile green bean seeds were soaked in each treatment as much as 100 ml and shaken using a rotary shaker for 1 hour in six replicates.

Observation of Growth Parameters on Green Bean Sprouts

Mung bean plants aged 21 days were removed from the growing media, and then their growth parameters were observed, including plant height, root length, wet weight, and dry weight of the plant. Measurement of plant length (from the base of the stem to the tip of the leaf) and wet weight using an analytical balance. All the data obtained were analyzed using the ANOVA method and the 5% BNT follow-up test.

RESULT

The result shows the bacterial treatment is significantly different in three measurements (Length, leave a number, and Fresh weight); see Table 1. All results were being analyzed using the ANOVA method and the 5% BNT follow-up test. However, the significant difference between treatments is significantly measured, but the follow-test indicates not significantly different from the control. According to the average value, the [0.5] concentration treatment is the most significant difference from other treatments.

Table 1. The measurement of mung bean growth parameters 21 days after inoculation

Measurement	Treatment concentration (Mc Farland standard)						
	Control	Std.	[0.5]	Std.	[1]	Std.	sig.
Length (cm)	24,90	+0.73	25,20	+1.74	22,08	+1.94	.013
Root length (cm)	10,57	+0.17	10,92	+0.31	10,70	+0.45	.896
Leave number	2,17	+0.4	5,00	0	5,00	0	.000
Fresh weight (gr)	0,837	+0.02	0,838	+0.01	0,755	+0.03	.000
Dry weight (gr)	0,095	+0.003	0,111	+0.008	0,098	+0.002	.253

cm = centimeters, gr = grams, LSD = $p \leq 0.05$

The biomass measurement shows a significant difference in the fresh weight of the mung bean. The highest fresh weight measured is the treatment concentration [0.5], even though the average value is higher than others, but the follow-up test shows no significant difference with control. This result still needs a further verification, and especially it should be having a strong correlation with external factors such as sunlight and gradual change in humidity. However, this

result is conformable with Devi et al., (2016) that bacterial with plant growth potential has an excellent effect on plant growth but still needs more investigation to explain the mechanism of influencing plant growth.

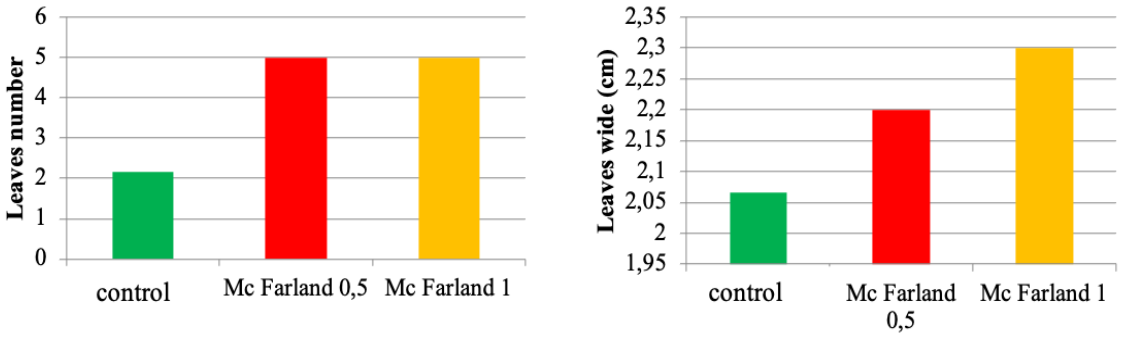

Figure 1. Leaves number and wide measurement of mung bean seedlings after 21 days treatment

Another interesting finding is the measurement result on the number of leaves and their morphological aspect (see Figure 1.). Both leaves number and its wide show the same trend that the bacterial IAA producer strongly correlates with the leave growth. This finding is also in accordance with Widowati & Sukiman, (2013) that also perform the treatment of IAA bacterial to enhance soybean growth.

DISCUSSION

IAA affecting cell division, extension, dan differentiation, one of which is to stimulate the seeds and germination (Díaz Herrera et al., 2016). The a15 isolate was tested for the ability to synthesis the IAA hormone. The number of bacteria concentrations is measured according to Mc Farland's standard concentrations of 0,5, and 1,0 for the treatment. Moreover, the bacterial diluted was used to soak the mung bean seed at the beginning of the imbibition process. However, this important step is the key to bacterial exposure to mung bean seeds (Goswami et al., 2016). The imbibition process becomes the excess for bacteria to enter the plant. By the imbibition process, the seed becomes larger in volume, causing the coat to break. Bacteria cells could entrance through this access and penetrate with the help of their locomotion properties such as flagella and philli or even using some enzymes (Sherameti et al., 2005).

There is no significant difference for some indicators measured, such as root length and dry weight. The most common indicator is the length of the plant, which becomes the main effect of the IAA hormone in the plant. Based on the result in Table 1, the similar pattern between control and treatment results is due to the endogenous IAA already high in mung bean seedling. However,

the additional IAA from bacteria could maximize the plant growth, such as the result of treatment concentration [0,5] on the length of the stem. The higher concentration of bacteria becomes a limiting factor of the plant growth because it might unbalance the indigenous auxin. The unbalance IAA concentration has a diverted effect compare to normal auxin and becomes a limiting growth rate. According to (Brígido et al., 2013), the high concentration IAA treatment on chickpea becomes the limiting factor. Moreover, the highest amount of IAA can cause ion imbalance that inhibits the growth of the plant.

The positive effect of IAA was found in leaves measurement and the fresh weight (see Table 1.). The number of leaves, water content, and leaves development increased accordingly to the highest bacterial concentrations. This becomes evidence of the direct effect of IAA on these factors. IAA receptors will receive the reason behind this, an exogenous IAA produced by bacteria in plant cells, namely ABP1 (Auxin Binding Protein), located in the endoplasmic reticulum or the extracellular space plants (apoplast). Auxin activates a proton pump located in the cell membrane, causing the excretion of protons from the cytoplasm into the space between the membrane and the cell wall so that the environment changes its pH to acid. Acidic pH will stimulate the activity of several enzymes, including cellulase, 1,4 beta-glucanase, and expansion. These enzymes synergize in softening the cell wall so that an imbibition process occurs, and there is an addition of cytoplasmic material that causes the cell growth. Auxin also activates the ion pump so that potassium ions enter the cytoplasm and increase water absorption. The water absorption process due to the activation of proton pumps and ion pumps will increase the turgor pressure (Ljung, 2013; Sauer & Kleine-Vehn, 2011).

High turgor pressure compresses the plasma membrane and causes the loosening of polysaccharides in the cell wall. The cell wall must modify and continue to add polysaccharides to expand the cell. Cell expansion increases cell size that accompanies the plant growth process (Majda & Robert, 2018). Auxin also plays a role in the cell cycle as a signal required to induce the G1 phase. The presence of auxin can reduce the expression of genes inhibiting the activation of complex compounds in the cell cycle to stabilize the reactions. Auxin also acts as a permissive signal to initiate cell division in the G1 and S phases that very important in developing leaves primordium (Perrot-Rechenmann, 2010).

CONCLUSION

On the one hand, the inoculation of a15 to enhance mung bean seeds and growth is observed. The concentration of bacterial suspension, which has been adjusted to the standard MC Farland 0.5, showed maximizing growth on the parameters of plant length and fresh weight. On the other hand, the concentration of the bacterial suspension, which has been adjusted to the standard Mc Farland

1, shows the results of inhibiting growth. Further study will reveal the molecular process and mechanism related to the bacterial IAA producer and the plant.

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