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Production of Asiaticoside in Pegagan (*Centella Asiatica*) With Phosphorus and Methyl Jasmonate Treatment

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ABSTRACT *Pegagan is one of the plants that is utilized from the nature and contains several saponin componds, including asiaticoside. The research objectives were to obtain (1) an appropriate dose of phosphorus and concentration of methyl jasmonate hormone, and (2) the interaction of the phosphorus dose and concentration of methyl jasmonate to obtain optimum production containing high asiaticoside. This study used a Separate Plot Design with two factors consisting of a fertilization treatment of P2O5 with four levels i.e. 0, 18, 36, 54 kg P2O5 /ha and the application of methyl jasmonate treatment consisting of two levels i.e. J0 = 0 µM and J1 = 100 µM, with a harvest time of 84 DAP, repeated 3 times to determine the effects of the treatment on the asiaticoside production. The results showed that there was an interaction of methyl jasmonate and phosphorus fertilization. The interaction of phosphorus dose and the right concentration of methyl jasmonate intended to obtain high asiaticoside content and production both in the petiole leaf and the vine roots was 54 kg P2O5 / ha without methyl jasmonate. When using methyl jasmonate,it could be applied a concentration of 100 µ M with phosphorus 18 kg P2O5 / ha.*

KEYWORDS : Centella asiatica, phosphorus fertilization, application of methyl jasmonate, asiaticoside content in leaves and petioles, asiaticoside content in roots and tendrils.

Background

Pegagan (*Centella asiatica* L. Urban) is a tropical plant that contains several saponins, including asiaticoside (Matsuda, et al., 2001). Bioactive compounds ofasiaticoside can accelerate a wound healing process and are useful in the treatment of leprosy and tuberculosis (Mangas, et al., 2006; Mangas. et al., 2008; Mangas. et al., 2009). Pagagan can purify blood, improve blood circulation, flow outurine (diuretics), relieve fever (antipiretics), stop bleeding (haemostatics), improve memory nerves, and contains antibacteria, tonic, antispasma, anti-inflammatory properties, hipotensis, insecticides, hypo-allergenic subtances and stimulants. Saponin can also inhibit the production of excessive scar tissue (inhibit keloids occurrence) (Mangas, et al., 2008). In Indonesia, pagagan is still harvested from the nature. To develop pagagan on a large scale,cultivation efforts should be carried out. High-quality pagagan products require plant material withhigh productivity as well as quality (Ghulamahdi et al., 2007. Ghulamahdi et al., 2010. Noverita, 2006. Nurliana, et al., 2008). The demand for pagagan (*Centella asiatica*) reached 100 tons per year. For example, PT. Sidomuncul alonerequired 2-3 ton / month. The local manufacturers needed about 25 tons per year and in the meantimethe supplies only reached 4 tons per year (Study Center for biomedicine of IPB. 2005; Editorial of Herba. 2003). In agribusiness, pagagan as a commodity has a promising prospect. This is caused by the presence of a positive indication for the business opportunity inbiomedicine, so the demand for pagagan will increase every year for drug production at home and abroad (Study Center for Biomedicine of IPB. 2005; Ghulamahdi, et al., 2007; Editorial of Herba, 2003; Editorial of Agromedia, 2008).

Methyl jasmonate (MJ) is one of the elicitors which is used widely and modulates many physiological occurances in higher plants. Elicitor is the term used in chemicals from various sources, biotic or abiotic, as well as physical factors, which can trigger a response in a living organism obtained in the accumulation of secondary metabolites. Methyl jasmonate and its derivatives have been proposed as a precusor compound in the elicitation process toward the accumulation of secondary metabolites (Lambert et al., 2011).

Kim et al., (2005) showed that the development level of mRNA CabAS (*C. asiatica*. -amyrin Synthase) in leaves reached its peakat the age of 2-3 weeks and decreased after 4 weeks. However, the asiaticoside content of the leaves increase from time to time.

According to a health food manufacturer Herba Penawar Al-Wahida (HPA), which produces Health-B, the pegagan they use is quite old, but not too old, and harvested at the age of 2 months and 15 days to get a high content of active ingredients (Herba Penawar Al wahida. 2011).

Problems

1. The need to obtain good-quality pagagan plants.

2. The need to examine the optimization of asiaticoside content and pegagan production through the application of phosphorus and methyl jasmonate.

Research methods

This study used Separated Plot Designwith two factors consisting ofa fertilization treatment of P2O5 with 4 levels i.e.F_0 = 0 kg P_2O_5/ha, F_1 = 18 kg P_2O_5 /ha, F_2 = 36 kg P_2O_5/ha, dan F_3 = 54 kg P_2O_5 /ha. The treatment of applying methyl jasmonate consisted of two levels: J0 = 0 µM and J1 = 100 µM, with a harvest time of 84 DAP, repeated 3 times to determine the effects of the treatment on the content of asiaticoside. The data were analyzed using variance analysis (F test) at a level of 5%. If there was a significant effect, the study would be continued with Duncan's multiplerange test and a relation pattern of regression equation.

Research Time

The research was conducted from March to July 2014. The land measured 1m x 1m with a plant spacing of 40 cm x 40 cm, and there were 4 plants per plot. The plant used was a plant that had 3 leaves. The fertilization was carried out according to treatments. The application of methyl jasmonate was done when reaching 50 DAP (days after planting), and the harvest was conducted all at once when the plant had reached 12 WAP (weeks after planting), by dismantling all parts of the plant.

Observation

The observations were conducted in week 12 and it was found that the leaves, petiole, roots and tendrils contained asiaticoside.

Results

The content of asiaticoside in leaves and petioles

The observation data on the asiaticoside content of the leaves showed that the treatments of methyl jasmonate (J) and phosphorus fertilization (F) as well as their interactions significantly affected the asiaticoside content of the leaves and petioles. The effects of methyl jasmonate treatment (J) and phosphorus fertilization (F) as well as their interactions on the average asiaticoside content of the leaves and petioles are presented in Table 1. In Table 1 below, it can be seen that based on observation, the highest asiaticoside content in the leaves and petioles was obtained at a treatment without the application of methyl jasmonate with a fertilization phosphorus dose of 54 kg P_20_5/ha (J_0F_3), which was significantly different from the other treatments. As for the interaction relation of the two treatment factors with the content of the leaves and petioles can be seen in Figure 1 below.

Production of asiaticoside in leaves and petioles

Based on the observation, the production data of asiaticoside in leaves and petioles showed that the treatment of methyl jasmonate (J) and phosphorus fertilization (F) as well as its interactions affected the asiaticoside production in leaves and petioles. The effects of methyl jasmonate treatment (J) and phosphorus fertilization (F) as well as their interactionson the average production of the asiaticoside in theleaves and petioles can be seen in Table 1. Table 1 below shows that the observation on the highest production in the leaves and petioles was obtained at the treatment without the application of methyl jasmonate and a phosphorus fertilization dose of 54 kg P_20_5 / ha (J0F3), which was also significantly different from the other treatments. The interaction of the two treatment factors to the asiaticoside production in the leaves and petioles are presented in Figure 2 below.

The Content ofAsiaticoside in Roots and Tendrils

The observation data of the asiaticoside content of roots and shoots showed that the treatment of methyl jasmonate (J) and phosphorus fertilization (F) as well as its interaction had an effect on the asiaticoside content of roots and tendrils. The effects of methyl jasmonate treatment (J) and phosphorus fertilization (F)as well astheir interactionson the average asiaticoside content of roots and tendrils are depicted in Table 1. Table 1 above revealed that the observation of the highest asiaticoside content of the roots and tendrils was obtained at the treatment of methyl jasmonate 100 μM and a phosphorus fertilization dose of 36 kg P205 / ha (J1F2), which was significantly different from the other treatments. As for the interaction relation of the two treatment factors with the asiaticoside content of the roots and tendrils, it can be seen in Figure 3 below.

The Production of Asiaticoside in Roots and Tendrils

The observation data of the asiaticoside production of roots and shoots showed that the treatment of methyl jasmonate (J) and phosphorus fertilization (F) as well as their interactions had an effect on the asiaticoside production of roots and tendrils. The effects of methyl jasmonate treatment (J), phosphorus fertilization (F), and their interaction on the average asiaticoside production of roots and tendrils are depicted in Table 1. Table 1 above revealed that the observation of the highest asiaticoside production of the roots and tendrils was obtained at the treatment without methyl jasmonate and a phosphorus fertilization dose of 54 kg P_20_5/ha (J_0F_3), which was significantly different from the other treatments. As for the interaction relation of the two treatment factors with the asiaticoside production of the roots and tendrils, it can be seen in Figure 4 below.

Figure 4 below demonstrates that the asiaticoside production of roots and tendrils increased with the application of 100 μM methyl jasmonate to a fertilization dose of P2O5 0 kg / ha, 18 kg / ha and 36 kg / ha. However, unlike the fertilization of P2O5 56 kg / ha, there was a decline in the asiaticoside production of roots and tendrils with the application of 100 μM methyl jasmonate.

Discussion

Effects ofthe Interaction Treatment of Methyl Jasmonate Hormone and Phosphorus Fertilization on the Content and Production of Asiaticoside

The results of the. study shown in Table 1 revealed the effects of the interaction treatment of methyl jasmonate hormone and phosphorus fertilization on the content and production of asiaticosid both in the petiole leaf and in the root of tendrils. Figure 1 and Figure 3 show the trend of the asiaticoside content on the leaves and roots where the asiaticoside content in the leaf increased, but the asiaticoside content in the root decreased both in the treatment without phosphorus fertilization and in the treatment of applying a phosphorus dose of 18 kg P2O5 / ha, 36 kg P2O5 / ha, 54 kg P2O5 / ha.

A different thing was in the parameter of asiaticoside production. The fertilization of 54 kg P2O5 / ha, with the application of 100 μM methyl jasmonate caused the asiaticoside production both in the leaves and the roots decreased. It is associated with a reduction in biomass produced due to the application of methyl jasmonate, and this compound was an organic compound formed through biosynthesis by the enzyme and functions to inhibit the growth of some parts of certain plants and was very strong to make leaves grow older (Salisbury and Ross, 1995). The previous research can be seen in the picture below. Figure 5 below shows that in vitro growth of pegagan without a treatment of methyl jasmonate and with a treatment of methyl jasmonate after 4 weeks (Mangas et al., 2009). The figure above also shows that the application of methyl jasmonate supressed the vegetative growth of pegagan.

The asiaticoside production of leaves decreased without phosphorus fertilization as well as 100 μM methyl jasmonate, while the asiaticoside production increased both in leaves and roots with a phosphorus fertilization treatment of P2O5 18 kg / ha and a methyl jasmonate application of 100 μM. The lowest asiaticoside production was in the treatment of the phosphorus fertilization of P2O5 36 kg / ha with or without the application of methyl jasmonate. In this research, the highest asiaticoside production was in the treatment with a phosphorus fertilization of 54 kg P2O5 without the application of methyl jasmonate. When applying 100 μM methyl jasmonate, the highest asiaticoside production was in the application of phosphorusP2O5 as much as 18 kg / ha.

CONCLUSION AND RECOMMENDATION

Conclusion

1. The appropriate phosphorus dose to obtain the high asiaticoside content as well as high production of pegagan leaveswas 54 kg P2O5 / ha.

2. Without methyl jasmonate hormone, the high content as well as high pagagan production was obtained.

3. The interaction of the appropriate phosphorus dose and concentration of methyl jasmonate to obtain pagagan with high asiaticoside content and production both in the leaves and roots was 54 kg P2O5 / ha with or without methyl jasmonate. When using methyl jasmonate, the application included a concentration of 100 μ M with a phosphorus dose of 18 kg P2O5 / ha.

Recommendation

The research can be continued with the same treatment in a different location and soil type by first adapting pegagan.

Table 1. Mean and the test of mean difference in the parameters of the asiaticoside content of leaves (KAD), the production of leaf asiaticoside (PAD), the asiaticoside content ofroots (KAA), the production of root asiaticoside (PAA); and in the treatment of methyl jasmonate interaction with phosphorus fertilization, and in the treatment of methyl jasmonate and phosphorus fertilization

Treatment		KAD		PAD		KAA		PAA	
J_0	F_0	18.26	b	5.51	b	15.32	f	4.63	cd
J_0	F_1	15.53	c	4.05	d	17.60	d	4.64	cd
J_0	F_2	15.93	c	2.66	f	18.49	c	3.48	e
J_0	F_3	20.54	a	6.08	a	17.85	cd	6.87	a
J_1	F_0	15.10	c	3.47	e	16.63	e	4.83	c
J_1	F_1	15.81	c	4.40	c	16.09	e	6.03	b
J_1	F_2	15.40	c	2.67	f	21.37	a	4.31	d
J_1	F_3	18.01	b	2.98	f	19.50	b	3.13	e
J_0		17.56	a	4.58	a	17.31	b	4.91	a
J_1		16.08	b	3.38	b	18.40	a	4.58	b
F_0		16.68	b	4.49	a	15.97	d	4.73	c
F_1		15.67	c	4.22	b	16.84	c	5.34	a
F_2		15.66	c	2.67	c	19.93	a	3.90	d
F_3		19.27	a	4.53	a	18.68	b	5.00	b

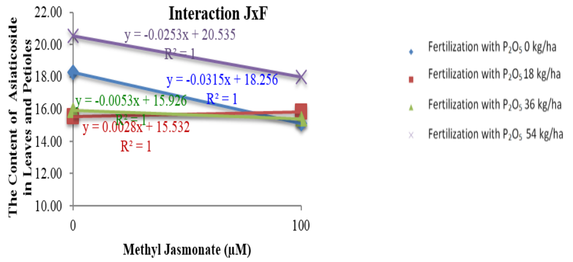

Figure 1. Effects of methyl jasmonate application to various doses of phosphorus fertilization on the asiaticoside fertilization of the leaves and roots

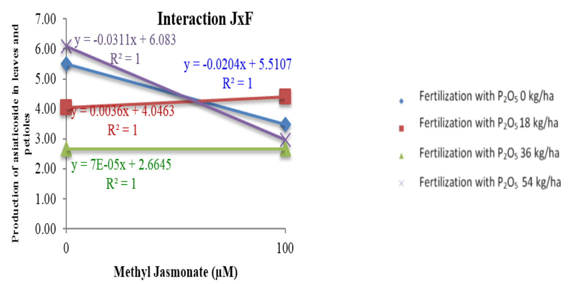

Figure 2. Effects of methyl jasmonate application to various doses of phosphorus fertilization on the asiaticoside production of the leaves and petioles

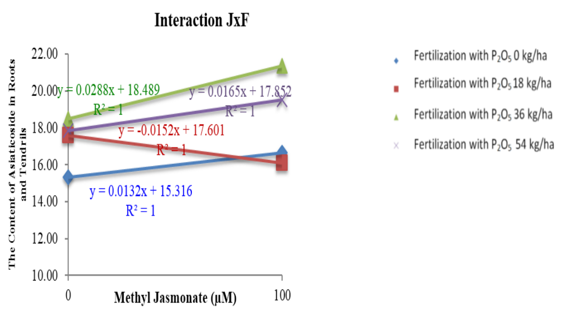

Figure 3. Effects of methyl jasmonate application to various doses of phosphorus fertilization on the asiaticoside content of the roots and tendrils

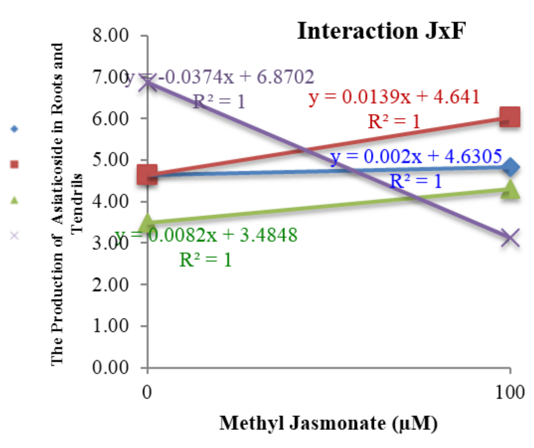

Figure 4. Effects of methyl jasmonate application to various doses of phosphorus fertilization on asiaticoside production of roots and tendrils

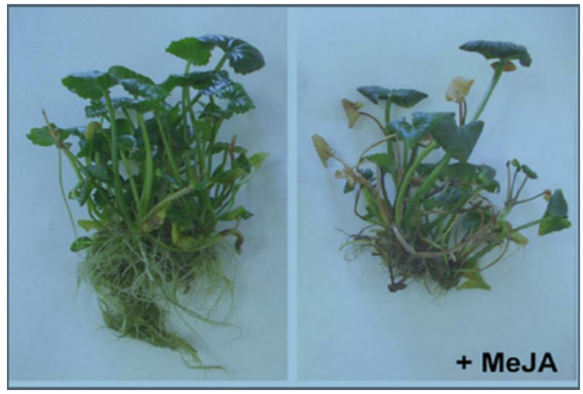

Figure 5. In vitro growth of pegagan without a treatment of methyl jasmonate and with a treatment of methyl jasmonate.

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