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Original Research Paper

Tendrils Growth of Pegagan (*Centella asiatica*) with the Application of Phosphorus and Methyl Jasmonate

Science

KEYWORDS :*Centella asiatica*, phosphorus fertilization, application of methyl jasmonate, number of primary tendrils and secondary tendrils

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ABSTRACT

Pegagan (Centella asiatica) is a medicinal plant which is very useful because it is believed to have a special quality of healing power. Researches show that pegagan contains such compounds as saponin, asiaticoside, madecasoside and asiatic acid. The objectives of this study were(1) to obtain proper doses of phosphorus and concentration of methyl jasmonate and (2)to find out the interaction dose of phosphorus and methyl jasmonate concentration for pegagan optimum growth for and production. This study used a Separate Plot Design with two factors consisting of a fertilization treatment of P2O5 with four levels i.e.F0 = 0 kg P2O5/ ha, F1 = 18 kg P2O5 /ha, F2 = 36 kg P2O5/ha, F3 = 54 kg P2O5 /ha. The application of methyl jasmonate consisted of two levels i.e. J0 = 0 μM and J1 = 100 μM, with a harvest time of 84 HST, repeated 3 times to determine the effects of the treatments on the growth components. The results showed that the treatments of methyl jasmonate and phosphorus fertilization did not increase the number of primary tendrils and secondary tendrils. Neither phosphorus fertilization alone nor the interaction of methyl jasmonate hormone and phosphorus fertilization affect the number of primary tendrils and secondary tendrils

Background

Pegagan plant is very useful because it contains several saponin compounds, including asiaticoside (Matsuda, et al., 2001). Accelerating a wound healing process and being useful in the treatments of leprosy and tuberculosis are among the benefits of bioactive compounds of asiaticoside (Mangas, et al., 2006; Mangas. et al.,2008; Mangas. et al., 2009). Pagagan has a cooling property, purifies blood, improves blood circulation, flows out urine (diuretics), relieves fever (antipiretics), stops bleeding (haemostatics), improves memory nerves, and contains of antibacteria, tonic, antispasm, anti-inflammatory properties, hipotensis, insecticides, hypo-allergenic substances and stimulants. Saponin can also inhibit the production of excessive scar tissue (inhibit keloids occurrence) (Mangas, et al., 2008).

Mostly, pegagan is still harvested from the wild. To develop pegagan on a large scale, cultivation efforts should be carried out. High-quality pegagan products require plant material with high productivity as well as quality (Ghulamahdi et al., 2007. Ghulamahdi et al., 2010. Noverita, 2006.Nurliana, et al., 2008).The demand for pegagan (*Centella asiatica*) reached 100 tons per year. For example, PT. Sidomuncul alone required 2-3 tons / month. The local manufacturers needed about 25 tons per year and in the mean time the supplies only reached 4 tons per year (Study Center for biomedicine of IPB. 2005; Editorial of Herba. 2003). Most people generally do not recognize medicinal plants and their benefits. However, the people in other eastern countries are very concerned about conserving medicinal plants. The Japanese pay a special attention to the sustainability of medicinal and aromatic plants and they strive for a sustainable harvest of the plants. China is the biggest exporter of medicinal and aromatic plants (Asian Scientist. 2012).

In agribusiness, pegagan as a commodity has a promising prospect. This is caused by the presence of a positive indication for the business opportunity in biomedicine; consequently,the demand for pegagan 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).

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 resulting from the accumulation of secondary metabolites. Methyl jasmonate (MJ) is one of the elicitors which is widely used and modulates many physiological occurances in higher plants. 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 peak at 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

High demand for simplisia collected from wild plants will cause the plants to become rare or even endangered. Due to its benefits and for a reason of standardized quality, simplisia cultivation is absolutely required.

The asiaticoside content and the production of pegagan through the application of phosphorus and methyl jasmonate should be further studied to obtain maximum results.

Research methods

This study used Separate Plot Design with two factors consisting of a fertilization treatment of P_2O_5 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, and F_3 = 54 kg P_2O_5 /ha. The treatment of applying methyl jasmonate consisted of was cunducted two levels: J0 = 0 μM and J1 = 100 μM, with a harvest time of 84 HST, repeated 3 times to determine the effects of the treatment on the growth components. The data were analyzed using variance analysis (F test) at alevel of 5%. If there was a significant effect, the study would be continued with Duncan's multiple range test and a relation pattern of regression equation.

Research Implementation

The research was implemented by conducting land preparation,calcification, plant preparation, planting, fertilizing, nursing, methyl jasmonate application, and harvesting. The harvest was conducted all at once when the plants had reached 12 WAP by dismantling all parts of the plants.

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Observation

The observation began from week 1 to week 12 by taking 2 sample plants from each plot. The morphological characters observed included the number of primary tendrils and secondary tendrils.

Results

1. Number of Primary Tendrils

The observation data on the number of primary tendrils at age 3-12 WAP showed that the treatments of Methyl Jasmonate (J) and phosphorus fertilization (F) and the two interactions (JxF) had no significant effects on the average number of secondary tendrils. The treatments of Methyl Jasmonate (J) and phosphorus fertilization (F) are shown in Tables 1 and 2.

Table 1.The Average Number of Primary Tendrils Age 3-12 WAP on the Treatments of Methyl Jasmonate and Phosphorus Fertilization

Treatment	Number of primary tendrils at age (WAP)									
	3	4	5	6	7	8	9	10	11	12
J_0= 0 µM	1.92	2.92	4.33	4.58	7.25	7.83	9.92	11.75	13.42	15.00
J_1 = 100 µM	1.71	3.25	5.17	5.08	8.17	8.58	11.25	13.33	12.83	14.00
Average	1.81	3.08	4.75	4.83	7.71	8.21	10.58	12.54	13.13	14.50
F_0= 0 kg/ha	1.92	3.00	5.33	6.17	8.83	9.17	12.67	15.50	17.33	18.33
F_1 = 18 kg/ha	2.00	3.00	4.67	4.17	9.00	7.83	9.67	14.17	12.33	14.00
F_2 = 36 kg/ha	1.75	3.17	4.83	4.17	6.33	7.00	10.17	10.83	11.83	13.33
F_3 = 54 kg/ha	1.58	3.17	4.17	4.83	6.67	8.83	9.83	9.67	11.00	12.33
Average	1.81	3.08	4.75	4.83	7.71	8.21	10.58	12.54	13.13	14.50

Table 1.The treatments of Methyl Jasmonate and Phosphorus fertilization were not significantly different statistically asthe growth chart of the number of primary tendrilsis shown in Figure 1

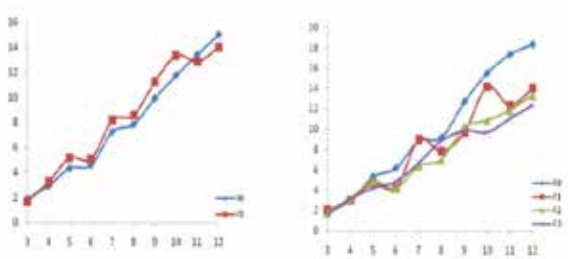

Figure 1. Growth Graph of Primary Tendrils Age 3-12 WAP on the Treatments of Methyl Jasmonate and Phosphorus Fertilization

Table 2. Interaction of the Treatments of Methyl Jasmonate and Phosphorous Fertilization to the Number of Primary Tendrils of Pegagan Age 3-12 WAP

Treatment	Number of primary tendrils at age (WAP)									
	3	4	5	6	7	8	9	10	11	12
J_0F_0	2.00	1.33	3.33	5.33	5.67	7.33	9.67	12.33	14.33	15.33
J_0F_1	2.33	3.67	4.67	4.33	10.00	7.00	9.67	14.00	15.00	15.67
J_0F_2	2.17	3.67	4.67	4.33	6.33	7.67	10.67	11.00	13.00	15.33
J_0F_3	1.17	3.00	4.67	4.33	7.00	9.33	9.67	9.67	11.33	13.67
Average	1.92	2.92	4.33	4.58	7.25	7.83	9.92	11.75	13.42	15.00
J_1F_0	1.83	4.67	7.33	7.00	12.00	11.00	15.67	18.67	20.33	21.33
J_1F_1	1.67	2.33	4.67	4.00	8.00	8.67	9.67	14.33	9.67	12.33
J_1F_2	1.33	2.67	5.00	4.00	6.33	6.33	9.67	10.67	10.67	11.33
J_1F_3	2.00	3.33	3.67	5.33	6.33	8.33	10.00	9.67	10.67	11.00
Average	1.71	3.25	5.17	5.08	8.17	8.58	11.25	13.33	12.83	14.00

2. Number of Secondary Tendrils

The observation data on the number of secondary tendrilsat age 5-12 WAP show that the treatments of Methyl Jasmonate (J) and phosphorus fertilization (F) and the two interactions(JxF) had no significant effects on the number of secondary tendrils. The treatments ofMethyl Jasmonate (J) and phosphorus fertilization (F) are shown in Tables 3 and 4.

Table 3. The Average Number of Secondary Tendrils Age 5-12 WAP on the Treatments ofMethyl Jasmonate and Phosphorus Fertilization

Treatment	Number of secondary tendrils at age (WAP)							
	5	6	7	8	9	10	11	12
J_0= 0 µM	0.75	1.00	6.92	8.50	14.67	19.33	24.00	28.17
J_1 = 100 µM	1.17	1.25	5.92	8.92	19.67	22.42	27.25	35.00
Average	0.96	1.13	6.42	8.71	17.17	20.88	25.63	31.58
F_0= 0 kg/ha	1.33	0.33	6.50	8.83	18.00	27.00	37.33	47.17
F_1 = 18 kg/ha	0.50	1.17	4.50	5.00	13.17	14.17	22.67	29.00
F_2 = 36 kg/ha	0.67	1.50	4.00	8.33	17.67	21.33	24.33	27.83
F_3 = 54 kg/ha	1.33	1.50	10.67	12.67	19.83	21.00	18.17	22.33
Average	0.96	1.13	6.42	8.71	17.17	20.88	25.63	31.58

Growth graphs of the number of secondary tendrils in Figure 2 below.

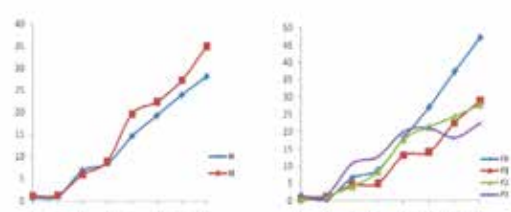

Figure 2. Growth graphs of the number of secondary tendrils at age 5-12 WAP on the Treatments of Methyl Jasmonate and Phosphorus Fertilization

Table 4. Interaction of Methyl Jasmonate and PhosphorusFertilizationTreatments to the Number of Secondary TendrilsofPegaganat Age 3-12 WAP

Treatment	Number of secondary tendrils at age (WAP)							
	5	6	7	8	9	10	11	12

J_0F_0	0.33	0.00	4.67	8.67	15.00	21.33	30.00	39.00
J_0F_1	0.67	0.00	5.67	4.67	10.33	19.00	23.67	31.00
J_0F_2	0.67	2.00	5.00	8.33	17.33	20.33	24.00	27.33
J_0F_3	1.33	2.00	12.33	12.33	16.00	16.67	18.33	15.33
Average	0.75	1.00	6.92	8.50	14.67	19.33	24.00	28.17
J_1F_0	2.33	0.67	8.33	9.00	21.00	32.67	44.67	55.33
J_1F_1	0.33	2.33	3.33	5.33	16.00	9.33	21.67	27.00
J_1F_2	0.67	1.00	3.00	8.33	18.00	22.33	24.67	28.33
J_1F_3	1.33	1.00	9.00	13.00	23.67	25.33	18.00	29.33
Average	1.17	1.25	5.92	8.92	19.67	22.42	27.25	35.00

General Discussion

Effects of the Methyl Jasmonate Hormone Treatment on-Pegagan Growth

Results of the analysis of variance for age 1-12 WAP showed that the treatment of Jasmonate Methyl hormone to the parameters of the number of primary and secondary tendrils was not significantly different.

Pegagan is an annual creeping herbal plant that grows and blooms throughout the year. It usually grows in the wild, especially in paddy fields, side by side with grass (weeds), propagated by stolon separation. Pegagan plants that grow in the wild generally produce branches to form new plants reaching a length of 10-80 cm (Januwati and Conan, 2005), but the change in the cropping pattern, namely from a wild cropping pattern to a cultured cropping pattern, including the application of Methyl Jasmonate hormone as much as 100 μM had apparently suppressed the growth of the vegetative organs of pegagan plants and inhibited the plant growth as evident from some of the parameters observed.

Methyl Jasmonate hormone is an organic compound that is formed from biosynthetic-free linoleic acid by lipoxygenase enzyme that functions to inhibit the growth of some parts of a certain plant and is very strong to encourage leaf aging (Salisbury and Ross, 1995). Methyl Jasmonate (MeJa) is an elicitor to modulate physiological events in higher plants such as defense response, flowering, and aging, so that methyl jasmonate is a new class of phytohormone with its derivatives, and it is a key signal to the accumulation of plant secondary metabolites (Lambert et al., 2011, Kim et al., 2004, Mangas et al., 2006 and Bonfill et al., 2011).

Effects of the Phosphorus Fertilization Treatment on Growth Pegagan Plant

Phosphorus fertilization should be done to each type of the plants. Phosphorus fertilization could be conducted through a number of treatments, namely F_0 = 0 kg / ha as control, F_1 = 18 kg / ha, F_2 = 36 kg / ha, and F_3 = 54 kg / ha. Actually, the use of phosphorus fertilizer as a treatment in this study did not significantly affect the morphological characters of pegagan plant growth.There are many factors affecting the growth and development of *Centella asiatica* such as P content in the soil, soil type, local climate and the adaptation process from growing in the wild to growing in there search area accompanied by disease disruption, making the application of P 18 P_2O_5 kg/ha, 36 P_2O_5kg/ha, 54 P_2O_5kg/ha as treatments have no effect on pegagan plants (Januwati and Yusron, 2005). Pegagan plants will thrive on suitable soil and environmental conditions because the growth of the plants cosmopolitan in nature. They grow in the wild in moist places under the low intensity of sunlight (shaded) and do not grow in the places that are too dry, because they have shallow roots (Winarto and Surbakti, 2004). Another possibility is the levels of P_20_5 from various treatment doses were used only to improve the content of pegagan asiaticoside, so that energy-rich phosphate compounds served as a medium for the phosphorylation of energy transfer to produce secondary metabolites (Kim, et al., 2010). P content of the soil affected the level of pegagan asiaticoside (Noverita, 2010).

Effects of the Interaction Treatment of Methyl Jasmonate Hormone and Phosphorus Fertilization on Pegagan Plant Growth

Both the individual treatment (Methyl Jasmonate or Phosphorus Fertilization alone) and the combined treatment (Methyl Jasmonate and Phosphorus Fertilization) had no significant effects on the parameters of growth components.

This happened because not all the treatments given to pegagan plants could affect the organs of vegetative growth significantly. Methyl Jasmonate hormone 100 uM had inhibited the development of several other parameters and the elicitation effect of methyl jasmonate only increased the content of secondary metabolites of pegagan triterpene saponin, affecting the expression of saponin biosynthetic genes (Kim et al., 2004, Mangas et al., 2006 and Bonfill et al., 2011).

Another possibility why the pegagan growth was inhibited and could not produce optimally was because the phosphorus level required in the plant was around 0.1-0.5% (Havlin, 2005). In addition, the change of a cropping pattern, from growing wild to growing in the cultivation area had made pegagan plants unable to quickly adapt to the new growing environment. Naturally, the growth of new branches forms new plants. In fact, pegagan produces new branches easily and dieseasily if the environment is not suitable.

CONCLUSION AND RECOMMENDATION

Conclusion

1. Methyl Jasmonate hormone did not increase the number of primary and secondary tendrils.
2. Phosphorus fertilization did not affect the number of primary and secondary tendrils.
3. The interaction of methyl jasmonate and phosphorus fertilization did not affect the number of primary and secondary tendrils.

Recommendation

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

It is recommended that the research be conducted on wet locations such as rice fields or on shaded locations to find out the maximum vegetative growth and the substance content with the same treatments.

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