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The Pattern of Stomatal Opening through the Exposure of High-Frequency Sound Wave with the Different Duration and Age of Soybeans (*Glycine max* (L.) Merril)

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Abstract

Since the productivity of soybean in Indonesia is still low, a new innovation is needed to improve it. The objectives of this study are to determine a pattern of the stomatal opening of soybean's leaves which exposed by high-frequency sound waves with different duration time and age of soybean plants. This study was carried out at the Experimental Farm Agriculture Faculty and Biology Laboratory of Science Faculty of the Islamic University of Malang, East Java in April to August 2013.The first factor was duration time of the exposure which consist of three levels: 20 minutes (D_1), 40 minutes (D_2) and 60 minute (D_3).The second factor was the age of the soybean which consist of three levels: 15 days after planting (dap) (A_1), 25 dap (A_2) and 35 dap (A_3). The soybean plants were exposure by the 5000 hertz frequency. The variables measured consisted of stomatal opening width, plant height, leaf area, fresh weight of pods, fresh weight of seed, oven dry weight of beans and harvest index. Increase of the duration time of exposure of high frequency sound waves by 20 to 60 minutes, tends to decrease the width of stomatal opening. The treatment of duration of exposure by 40 minutes at the age of 15 dap had the highest soybean grain yield by 24.10 g.plant^{-1}, equivalent to 3.93 ton.ha^{-1}. The relationship between the widths of the stomatal opening with soybean production showed no significant correlation. The results of this study suggest that maintaining stomatal opening at optimum width through the manipulation of high-frequency sound waves will increase the yield of soybean plants.

Keywords: soybean, high frequency sound waves, stomatal width, duration exposure, the age of application

1. Introduction

Soybean (*Glycine max* (L.) Merril) is one of the important agricultural commodities in addition to rice and corn. Soybean is the main source of vegetable protein and oils of the world as well asin Indonesia. However, 70% of soybean needs of Indonesia are still met from imports. It means the domestic soybean needs can only meet 30%. Soybean productivity of Indonesia in the last 10 years has reached 1.27 ton ha^{-1}. The productivity is still low when compared with the soybean-producing countries such as Japan and China (Antara, 2008). Therefore, the efforts to improve the productivity is greatly needed. The various soybean cultivation technology has been applied to improve productivity such as the use of high yielding varieties, a balance fertilization, integrated pest and disease control. However, these efforts have not been able to increase the productivity of soybean and the fulfillment of national need. Therefore, it is neededa new break through effort to improve the soybean production of Indonesia. One of them can be done through the application of sonic bloom.

Sonic Bloom is a technology which combines high-frequency sound waves and liquid nutrients intended to improve the plant growth so that it's the plant productivity can be increased. This technology utilizes the natural sound waves with high frequency which stimulate the mouth of leaves (stomata) remain open. It can increase the speed and efficiency of fertilizer absorption through leaves that are beneficial to the plant. This technology can also improve the efficiency of photosynthesis so the quantity and quality of production can enhance. Itcan increase the plant metabolism when followed by sprayingof nutrients, so that the plant growth take place quickly (Iriani *et al*., 2005; Widyawati, Kadarisman, & Purwanto, 2011). This technology was introduced by Dan Carlson (United States) in 1986. The results of his research for fifteen years at the University of Minnesota concluded that the sounds of certain frequencies can help plants breathe better and absorb more nutrients. He found sounds like a bird chirping in the morning with a frequency of 3000 to 5000 hertz will open wider stomata (Nahesson, 2012). The sonic bloom technology enabled when the mouth leaves open between 04:00 and 10:00 a.m.and then at 04.00 to 08.00 p.m. The opening of leaves stomatal is affected by light, temperature, and the other factors. However, the scattered sound waves can open stomatal maximally (Haryanti & Meirina, 2009).

Environmental factors that affect stomatal opening is often difficult to be manipulated. Therefore, the application of sonic bloom technology is needed to maximize the stomata opening. This way will increase the amount of CO_2 that enter so the photosynthesis process occured quickly (Baldocchi, 1997). The stomatal opening was also affected by cells turgor pressure associated with the metabolism of ions uptake, especially K^+ ions (Roelfsema, Steinmeyer, Staal, & Hedrich, 2001). A low turgor pressure causes the stomatal closing (Zhao, Kong, Escobedo, & Gao, 2010). The sonic bloom technology has been proven to increase productivity pepper plant, rice, barley and soybean plants. Through this technology, the production of soybeans increased by 31%, protein content also increased by 15% (Agriculture Research and Development Agency, 2005; Carlson, 2008).

The purpose of this research are (1) to determine the pattern of the relationship between the width of the stomatal opening with the duration of exposure of sound waves at different ages of soybean plants; (2) to describe the influence of the stomatal opening on growth and yield of soybean plant. The hypotheses are (1) the width of the stomatal opening correlated positively with the duration of exposure of sound waves at a younger age plants; (2) the stomatal opening width will increase the soybean production.

2. Material and Methods

2.1 Study Location and Time

A pot experiment was conducted in the experimental farm of Agrotechnology Departement, Faculty of Agriculture, Islamic University of Malang with altitude of 540 meters above sea level with a geographical location at 07^{o}59'S and 112^{o}36' E, the average air temperature 24.14^{o}C, humidity 72% with a rainfall average of 1,883 mm per year. This study started from April to August 2013. Observations of stomata carried out in the Laboratory of Biology, Faculty of Science, Islamic University of Malang.

2.2 The Treatments and Experimental Design

This experiment used randomized block designs consisting of two factors. The first factor: the duration of the exposure, consisting of three levels: D_1 = 20minutes, D_2 = 40 minutes, D_3 = 60 minutes.The second factor was age of the soy plant consisting of three levels: A_1 = 15 days after planting (dap), A_2 = 25 dap, A_3 = 35 dap. The combination of these two factors produces the nine treatments and repeated threetimes.

2.3 Preparation of Growing Media, Planting and Maintenance

Planting medium used consisted of soil, sand and compost in the ratio 1:1:1. The planting medium was weighed by10 kg and put into polybag. The pots were watered with a same volume. Then, seeds were planted. Each pot contained three uniform seeds. Watering, fertilization and pest and disease control were done to maintain an optimum growth of soybean. Watering is done in accordance with the conditions of the media, if it does not rain the same amount of water were watered in to the polybag. Fertilization was applied at 21 days after plantingby 0.12 g urea polybag^{-1} (equivalent to 50 kg urea.ha^{-1}), 0.18g tricalsium phosphate polybag^{-1} (equivalent to 75 kg ha^{-1}) and 0.18g KCl polybag^{-1} (equivalent to 75 kg ha^{-1}). Pests and diseases were controlled at the age of 7 days after planting when there is an attack aphid, sprayed with Decis 20 EC (active ingredient Deltamethrin 25 g l^{-1}) at a concentration of 2 ml l^{-1}.

2.4 The Exposure of Sound Wave

The exposure of sound waves was conducted for 20 minutes, 40 and 60 minutes. The exposure was conducted in the morning at 08:00 a.m. to 03:00 p.m.when the stomatal optimally opened. To test the sensitivity of stomatal guard cell conditions, the exposure is done at the age of the soybean 15 days after planting (dap), 25 dap and 35 dap. The applied frequency ranged from 4270-4601 hertz.

2.5 Observation of Stomata

The stomatal opening was measured after soybean plants were exposed by high-frequency sound waves through semi-permanent preparations. The steps of preparations of replicas as followed: (1) lower surface of leaves were spread by colorless nail polish and left for 10 minutes, (2) a colorless tape was taped on the surface of the nail polish, (3) the preparate was placed on a glass objects and labeled, and then observed under a microscope (Haryanti & Meirina, 2009).

2.6 Measurement of Plant Growth and Yield

The measurement of plant growth and yield include: plant height (cm), leaf area (cm^2), fresh weight of pods (g), fresh weight of seed (g), oven dry weight of seeds per plant (g), and harvest index (%).

2.7 Statistical Analysis

The collected data were analyzed using the Analysis of Variance (ANOVA) (F-test) at level ($p \leq 0.05$) and differences in each treatment were adjudged by DMRT (Duncan Multiple Range Test) and LSD (Least Significant Difference) at level ($p \leq 0.05$). For quantitative data of stomatal opening width were analyzed using regression analysis and correlation.

3. Results

3.1 The Pattern of Stomatal Opening

Results of analysis of variance showed no interaction effect of age and duration of exposure on the width of stomata, but separately the duration of the sound wave exposure significantly affect stomatal opening width. In general, it can be explained that the duration of exposure of high-frequency sound waves are negatively correlated with the optimization of stomatal opening. It means the longer the duration of exposure, the lower the width of stomatal opening (Figure 1).

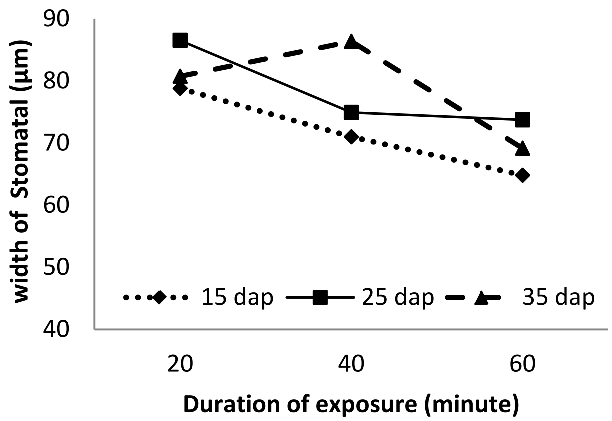

Figure 1. The relationship between the duration of sound wave exposure with width stomatal opening

3.2 The Growth of Soybean Plant

The interaction between exposure duration and age affected plant height age 22, 43, 50 and 57 days after planting (dap) and leaf area age 43 dap. But separately, the duration of exposure and age did not significantly affect plant height. The significant effect of duration of exposure onthe leaf area occurred at the age of 57, 64 and 71 dap, while the effect of age occurred at age 64 dap (Figure 2).

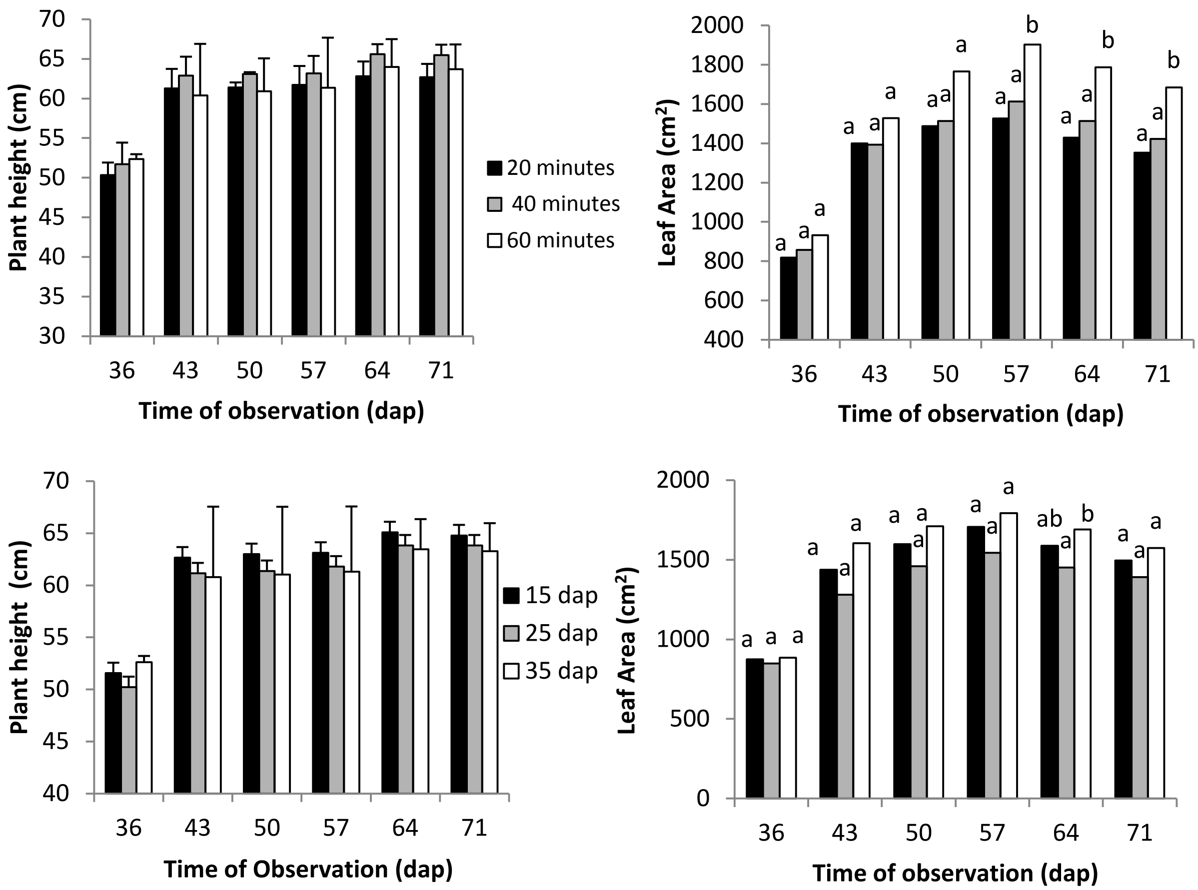

Figure 2. The effect of exposure duration of wave sound and age on plant height and leaf area

There is a trend that the longer the duration of exposure,the higher soybean leaf area, while at the treatment of age of soybean plants did not show the tendency that the older the age of the plant exposed by high-frequency waves, the higher the leaves area of soybean plants. However, the largest leaf area was found at the age of 35 dap exposure of soybean plants.

3.3 Yields of Soybean Plant

The interaction between duration and age of exposure affected significantly on the fresh weight of pods, fresh weight of seed, oven dried seed weight, but not significantly on harvest index. The highest pod fresh weight was obtained on exposure with a duration of 20 minutes performed at the age of 35 dap, the duration of 40 minutes performed at the age of 35 dap, while for the duration of 60 minutes maximum pod weight obtained at the age of 15 dap exposure (Figure 3A).

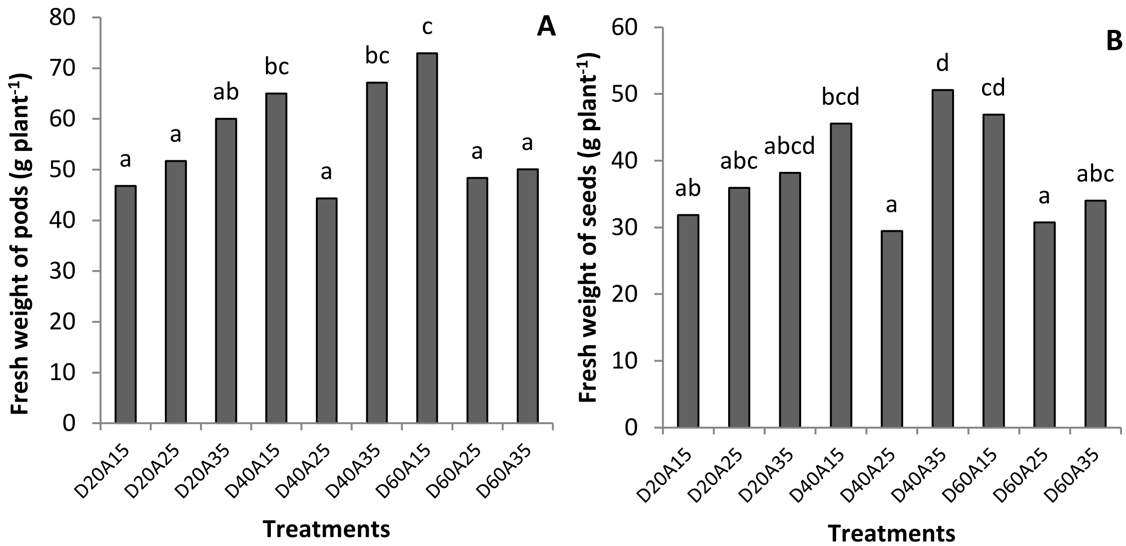

Figure 3. Interaction effect between duration of wave sound exposure and age of soybean plant on the fresh weight of pods and fresh weight of seeds

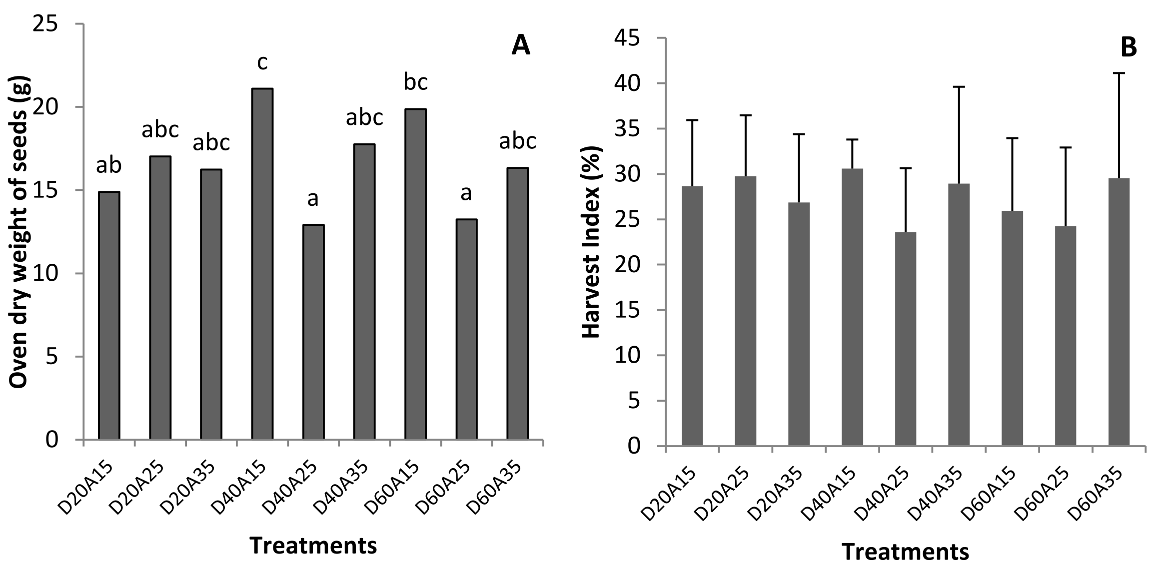

Figure 4. Interaction effect between exposure duration of sound waves and the age of the soybean onoven dried seed weight and harvest index

The highest seed fresh weight was obtained in the combination treatment duration of 40 minutes of exposure applied at age 35 dap ($D_{40}A_{35}$), not significantly different from the exposure duration of 60 minutes at 15 dap ($D_{60}A_{15}$) (Figure 3B).On the oven dried seed weight showed that the highest yield wasfoundon the treatment of high-frequency sound wave exposure with a duration of 40 minutes at 15dap ($D_{40}A_{15}$) (Figure 4).

The highest harvest index for the duration of the 20-minute exposure with the exposure was achieved at the age of 25 dap ($D_{20}A_{25}$), for the duration of the 40 minute exposure was achieved at ages 15 dap ($D_{40}A_{15}$) and for the duration of the 60minute exposure was obtained at the age of 35 dap exposure ($D_{60}A_{35}$).

4. Discussion

4.1 Effect of Duration of Exposure Sound Waves on Stomatal Opening Width

This research result that exposure of high-frequency sound waves has proven able to optimize soybean stomatal opening.This is consistent with the research conducted by Rohmah (2012) in which the leaves stomatal of soybean plant opened wider when there is noise exposure, when compared with before and after exposure to a broad sound. According to Van Doorne (2000) the wider stomatal opening is caused by (1) a certain sound frequencies can activate certain genes in cells that affect cell growth and expression. Expression of the cell is a process by which codes the information in genes is converted into proteins that operate in the cell, (2) the frequency of the sound resonate with the object. According to Carlson (2008) sound frequencies resonate with the stomatal cavity, thereby increasing water uptake. Collins and Foreman (2001) reported that the sound frequencies resonate with cell organelles. A certain sound frequency resonate increases movement inside the cell cytoplasm. Tyree and Sperry (1989) explained that (3) the phenomenon of cavitation is a phenomenon caused by a sound in the liquid. The sound which comes from the sound source toward the cytoplasm which led to the emergence of micro-bubbles. Furthermore, the micro-bubbles resonate with the sound and push the guard cell wall. Therefore, turgidity pressure increased and the stomatal will open maximum. However, it is not yet known to the size and number of micro-bubbles needed to push the guard cell wall. (4) The sound propagates through the liquid. Thus, it stimulates the movement of molecules such as diffusion processes. Collins and Foreman (2001) reported that the resonant sound can affect protein biosynthesis.

The results of this study indicate that the duration of exposure of high-frequency sound waves are negatively correlated with the optimization of stomatal opening. The longer the exposure of sound waves, the lower the width of the stomatal opening. However, the plants treated with sound waves have a higher stomatal width than the plants not given sound stimuli such as control plants were not exposed to high-frequency sound waves average had the average stomatal opening by 60 µm. This is because the sound wave exposure can cause the stomata of the leaves will open more optimal than the normal conditions. The longer the duration of exposure, the wider the stomatal opening so that the transpiration rate increases resulting in decreasing the water content in the plants. Decrease of the water content of the guard cells lose the turgor pressure so that the the width of the stomatal opening will shrink. Closing stomata is the most direct means by which plants prevent celluler water loss. Thus, stomatal closure simultaneously slows transpiration and the diffusion of CO_2 into substomatal cavity. This process is determined by stomatal conductance which describes the first step in the diffusion path from the atmosphere to substomatal cavities (Warren, 2008). However, the plant will attempt to balance the water content in the body by absorbing water through the roots, but this is not necessarily able to stabilize the water content in the plant. To minimize the negative effects of water stress the plants respond by changing their growth pattern, producing stress proteins and chaperones, up-regulation of anti-oxidants, accumulation of compatible solutes, increasing the amount of transposters involved in water and ion uptake snd transport and by closing the stomata (Arve, Torre, Olsen, & Tanino, 2011).

4.2 Effect of Duration of Sound Wave Exposure on Soybean Growth and Yield

The treatment of exposure for 60 minute at 15 dap ($D_{60}A_{15}$) generally provided the highest plant height and leaf area. This is because at the age of 15 dap soybean plants entering the vegetative phase-2 (V_2) is characterized by imperfect leaves have opened the book into two branches and roots begin to develop, at this time the availability of nutrients required to meet the needs of the plant (Arsyad & Syam, 1995). The $D_{60}A_{15}$ treatment was capable to supply nutrients to the soybean during this V_2, so the plant growth was better. However, the plant productivity on this treatment ($D_{60}A_{15}$) was lower than the treatment $D_{40}A_{15}$.This wasdue to the treatment $D_{60}A_{15}$had a low harvest index by 25.95%, while the treatment $D_{40}A_{15}$hada highharvest index by 30.59%. The harvest index described the efficiency of the use of assimilation. Although the treatment $D_{40}A_{15}$ performed a good plant growth, it had a lower harvest index than the treatment $D_{40}A_{15}$. The treatment $D_{40}A_{15}$had the highest yield.

The treatment duration of exposure significantly ($P <0.05$) affected on the fresh weight of pods, fresh weight of seeds, oven dried seed weight and harvest index. The exposure duration for 40 minutes at 15 dap generally provided the best results for all the observed variables. This is because the application of fertilizer at the age of 15 dap soybean plants can be perfectly absorbed through the stomata are opened so optimally supported the plant growth. A good performance of the plant growth will be able to increase the productivity of plants. The exposure duration for 40 minutes will open the wider stomatal than the exposure duration for 60 minutes so that the absorption of nutrients through the leaves can be more optimally, as well as with the absorption of nutrients through the roots. The wider stomatal opening will increase the plants transpiration, so to balance the water content the plant will absorb water and dissolved nutrients from the growth medium (Brittlate, 2007). The plants exposed for 20 minutes, despite having a wider stomatal opening but growth and yield less than optimal. This is because an increase in the rate of absorption of nutrients through the leaves and roots are not equal to the magnitude of the rate of transpiration with increased transpiration rate.The too high transpiration can cause the absorbed nutrients evaporate through the stomatal of the leaves in the transpiration process of plants.

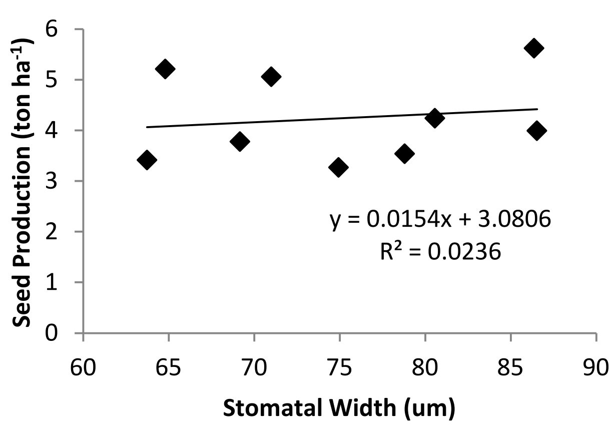

Figure 5. The relationship between the width of stomata and soybean production

4.3 Relationship between Widths of Stomatal Opening with Soybean Yield

The relationship between the width of stomata and soybean yielddid not show a clear pattern, but there is a trend ofthat the wider the stomatal, the higher the yield of soybean (Figure 5). The yield of soybean on $D_{40}A_{15}$ treatment reached 24.10 g.plant^{-1}, equivalent to 3.93 t.ha^{-1}, while the average production of soybean varieties *Anjasmoro* according to specifications issued by breeding centers ranging from 2.25 to 2.30 t.ha^{-1} or increased production of ± 70.87%. Suwardi (2010) had reported that the influence of the frequency of sound wave on the growth of soybean, showed that the frequency of 10 kHz sound wave is able to optimize the growth of soybeans. The stomatal are open will result in an increase in the optimal rate of absorption of nutrients through the leaves and an increase in the rate of nutrient uptake by the roots is also able to compensate for the rate of transpiration that occurs as a result of the widening of stomatal opening.

5. Conclusion

The results of this study showed that increasing the exposure duration of high frequency sound waves of 20 to 60 minutes decrease the width of the stomatal opening. The relationship between the width of stomata and soybean yield did not show a significant correlation. The treatment combination of exposure duration of 40 minutes at the age of 15 daphad the highest yield by 24.10 g.plant^{-1}, equivalent to 3.93 t.ha^{-1}, with an increase in production of 70.9% compared to the average production of soybean varieties *Anjasmoro*.It is thus suggested that maintaining the stomatal opening at optimum width through the manipulation of high-frequency sound waves will increase the yield of soybean plants. However, the further research is needed to describe the relationship pattern of the stomatal opening with a loss of moisture in the plant in the transpiration process related to a given level of nutrient uptake through the stomatal.

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