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The Effect of Harmonic Frequency and Sound Intensity on the Opening of Stomata, Growth and Yield of Soybean (*Glycine max* (L.) Merrill)

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ABSTRACT

Seventy percent of Indonesia's soybean demands and consumption are met from imports, and therefore, it is necessary to increase its local production. This study assessed the effects of harmonic frequency and sound intensity levels on the opening of stomata, the growth and yield of soybean. Experiments were conducted in a split plot design, with frequency harmony (F_4: 4 kHz, F_8: 8 kHz, F_{12}: 12 kHz) being the main plots and sound intensity (A_{50}: 50 decibels (dB), A_{80}: 80 dB and A_{110}: 110 dB) used as sub plots. The results showed there was no significant effect of frequency and intensity on the measured response (stomata opening). However, if they were compared with those at 0 frequency and 0 intensity, the stomata openings were significantly different based on t-test at p = 0.05. This means the opening of stomata was affected by resonance. In general, the sound level pressure attempted in the range of 50-110 dB had no effect on the width of stomata opening, but it affected to the growth and yield of soybean. The best growth of the leaf area and relative growth rate were in the presence of sound waves at a frequency of 4 kHz. Likewise, the best result of the average fresh weight of seed, dry weight of seed and harvest index were at a frequency of 4 kHz sound waves. The leaf area, seeds fresh and dry weight, and harvest index were also significantly highest at sound intensity of 50 dB. Therefore, to improve the productivity

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of soybean plants, exposure at a frequency of 4 kHz and sound intensity of 50 dB, followed by an application of leaf fertiliser according to recommended dosage is the best combination of treatment in growing soybean in Indonesia.

Keywords: Harmonic frequency, resonance, sound intensity, soybean

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the most important agricultural commodity after rice and maize in Indonesia. Soybean is a legume that contains vegetable protein and oil. The Central Statistics Agency data shows that soybean production fell by 780.16 thousand tons between 2012 and 2013, which is a decrease of 63 thousand tons (7.47%) compared to 2012. The decline in soybean production was due to decreased productivity by 0.07 t/hectare (4.65%) and a decrease in the harvested area of 16.83 thousand hectares (2.96%). Thus, strategic effort is needed to increase domestic soybean production. One of the strategies is through the application of sonic bloom technology.

Sonic bloom is a stimulation technology targeting wider stomata opening by using high frequency of sound waves. This is followed by an application of liquid fertiliser through leaves (Carlson, 2013). When the plants are at the stage of fruit bearing, and with the exposure of sound wave of 3.5 - 5 kHz, they are sprayed with foliar fertilisers. By a wider opening of the stomata, the absorption of fertiliser increases by the leaves (Carlson, 2013). The opening of stomata is affected by light, temperature, and other factors. However, by using scattered sound waves, the stomata can be maximally opened despite the above factors (Dalimunthe, 2004).

According to Weinberger and Graefe (1973), the stimulation of stomata opening by sound waves may be due to one or more of the following mechanisms: (1) certain sound frequencies can turn on certain genes in cells that affect growth and cell expression, (2) the frequency of resonating sound improves the movement of cytoplasm in the cell, (3) the cavitation phenomenon, a phenomenon caused by noise in the liquid. Sound waves coming from a cell's wall hits the cytoplasm. Certain frequencies affecting the cytoplasm cause micro-bubbles. The micro-bubbles resonate with the sound and spur the rise of guard cells turgidity, which causes maximal stomata opening. Additionally, the fourth (4) mechanism is the propagating sound through the liquid stimulates the movement of molecules such as in the diffusion process. However, until now, the proper mechanism of sound effect on plant is not fully understood. Better understanding is necessary to establish a mechanism and to develop models for application of this potential technology in addition to experiments to find the best sound frequencies and intensities for growth and yield of soybean.

Frequency is the number of vibrations in one second. Harmonic frequency is the integer multiples frequency of basic frequency (Satira, 2013). The basic frequency used in this study was 4 kHz, so that the harmonic frequencies used were at 8 kHz and 12 kHz. Pujiwati and Djuhari (2011) suggested exposure of sound wave frequency of 4-5 kHz causes an increase in the width of stomata opening. Furthermore, Pujiwati and Djuhari (2014) found that the 15-day old Anjasmoro soybean variety when exposed to sound waves with a duration of 40 minutes produced the best results with seed production reaching 3.93 t/ha, or increased by 71%, compared with the average production of 2.25 to 2.30 t/ha.

Exposure to high frequency sound waves is proven to optimise the stomatal opening of plant leaves (Collins & Foreman, 2001; Haryanti & Meirina, 2009). Rohmah (2012) reported that the stomata of soybean plant leaves opened wider when exposed to noise. The sound waves affect several growth processes, such as seed germination, growth of callus in tissue culture techniques, growth and development of vegetables, fruits and plantation crops (Hassanien et al., 2014). There is a biological effect of sound waves on the growth of rice seeds when exposed to sound frequency and intensity of 0.4 kHz and 106 dB respectively (Baldocchi, 1997). Their germination index, plant height, and root development are significantly increased by sound.

A tissue culture of *Actinidia chinensis* exposed to sound wave at frequency and intensity of 1 kHz and 100 dB respectively at a distance of 0.2 meters had resulted in an increase of ATP (soluble protein) and the activity of superoxide dismutase (SOD) enzyme of the plant. The SOD enzyme catalyses superoxide into oxygen and hydrogen peroxide (Yang et al., 2002), and it acts as an antioxidant and protects cell components from oxidation by reactive oxygen species (ROS) due to drought, deficiency of nutrients, heavy metals, reaction of ultra-violet or gamma rays and the metabolic activities of some plants (Alscher et al., 2002; Raychaudhuri & Deng, 2000).

At an Agricultural Engineering Research Centre in China, research was conducted on several kinds of vegetables using Plant Acoustic Frequency Technology (PAFT) with a frequency and a sound pressure level of 0.06-2 kHz and 50-120 dB respectively at a distance of 50-100 meters (Jun-ru et al., 2011; Meng et al., 2011). The results obtained in cucumber, tomatoes, watermelon, cowpea and eggplant showed significant increases in chlorophyll content, net photosynthetic rate, and the number of flowers and fruit. It was found that PAFT stimulates endogenous production of some hormones, such as IAA and GA. Sound wave technology is also effective in increasing the plant's immune system against disease and insect pests (Hou et al., 2010; Yu et al., 2013). In the case of cotton plants that were grown in an open area, a minimum yield increase of 5.2% was noted when crop received sound waves of 75-110 dB at a distance of 30 meters while the highest of 18.6% was recorded at the sound pressure of 70-75 dB (Hou et al., 2010).

This study was aimed at finding the initial proof that resonance events that might have caused the stimulation of stomata opening related to selected growth and yield parameters of soybean with the sound wave frequency and intensity, especially under the Indonesia's growing condition.

MATERIALS AND METHODS

Study Site

The experiment was conducted from June to September 2016 at the experimental station of Brawijaya University located at the Faculty of Agriculture, Jatimulyo village, Lowokwaru District Malang, East Java Province, Indonesia at the height of 540 meters above sea level. It lies at 07°59'S and 112°36'E. Its average temperature was 24.1°C with air humidity of 72%. The average rainfall was 1883 mm per year. The intensity of solar radiation ranged from 327.0 x 10^5 to 603.4 x 10^5 lux.

Experimental Design and Treatments

The experiments were conducted in a split plot design. The main plot was the frequency of sound waves with three levels (F_4, F_8, and F_{12} for 4, 8, and 12 kHz respectively). The sub plot was the sound level pressure (sound intensity) consisting of three levels (A_{50}, A_{80}, and A_{110} representing 50, 80, and 110 decibels [dB], respectively). The main plot and sub plot were repeated three times. The control was the samples that were not exposed to frequency and sound intensity.

Soybean plants were exposed to sound waves at the above levels of frequency and sound intensity from age 20 to 70 days after planting (DAP) with 10-day intervals. Each exposure lasted for 20 minutes. Subsequently, soybean plants were sprayed with Growmore liquid fertiliser at a dose of 2 g/litre. The observation of stomata width opening was made by using the replica method with a clear nail polish fixation. The recorded and computed growth parameters were plant height, leaf area, and relative growth rate (RGR = $(\ln W_2 - \ln W_1)/(t_2-t_1)$) (Sitompul and Guritno, 1995). The fresh weight of seed, dry weight of seed, and harvest index (HI) were also observed and computed (HI = (dry seed weight/total dry weight of the plant) x 100%)) (Sitompul and Guritno, 1995).

Statistical Analysis

Statistical analysis was conducted by using analysis of variance (ANOVA test) and means separation were tested by using the Least Significant Difference test at p = 5%. The analysis of comparison between control and each treatment was performed by a t-test at p = 5% and p = 1%.

RESULTS AND DISCUSSION

The Effect of Harmonic Frequency and Sound Intensity on the Width of Stomata Opening

The ANOVA was performed at frequencies of 4, 8, and 12 kHz, and sound intensities of 50, 80, and 110 dB which affect the stomata opening at each DAP. The results also indicated there was no significant effect of sound frequency and intensity on the measured responses. However, there was highly significant effect between the plants exposed to sound waves at a frequency of 4-12 kHz and at intensity of 50-110 dB with controlled plants as shown in Table 1.

Table 1
The average width of stomata opening (μm) on harmonic frequency and sound intensity at different age observations

Treatment	Mean of stomata opening width (μm) at different plant age					
	20	30	40	50	60	70
Frequency (kHz)						
4	10.38a	11.32a**	11.39a**	9.27a	9.47a	11.70a**
8	10.45a*	11.35a**	10.45a*	8.85a	9.29a	11.32a**
12	11.46a*	10.66a	11.42a**	9.38a	9.28a	10.31a*
Control	9.07	8.65	7.50	7.50	7.59	6.88
LSD 5%	2.56	2.41	1.69	1.76	2.29	2.25
Sound Intensity (dB)						
50	10.94a	11.49a*	10.21a*	8.85a	9.64a	11.56a*
80	10.83a	11.63a	11.77a**	9.58a	9.04a	11.88a**
110	10.83a	10.21a	12.19a*	9.38a	9.73a	11.67a
Control	9.07	8.65	7.50	7.50	7.59	6.88
LSD 5%	2.56	2.41	1.69	1.76	2.29	2.25

Notes: Means followed with same letter within a column of each main effect are not significantly different at p=0.05 by LSD test
* and ** are different at p=5% and 1%, respectively by t-test for comparison between control and each level of frequency and sound intensity
DAP: days after planting

Previous works (Pujiwati & Djuhari, 2011) showed that the width of stomata opening was different when the plant's stomata opening occurred at 4 to 5 kHz. The result of this experiment (Table 1) showed the width of stomata opening was not different when exposed by sound wave at 4, 8 and 12 kHz, but it was different to that of the control.

The width of stomata openings were similar among those frequencies possibly due to resonance effect to the stomata opening caused by sound waves. In a physical system, when a system affected by a specific frequency from external stimulus, for example sound wave, the system will have a similar response to the harmonic frequency of the stimulus signal. The same mechanism can also exist in the stomata opening affected by sound wave.

The Effect of Harmonic Frequency and Sound Intensity on Growth

The results showed the frequency affected plant height at 41 and 48 days after planting (DAP) (Figure 1), leaf area at 48 and 62 DAP (Figure 3), and relative growth rate at an interval of 49 to 56 DAP (Figure 5). Additionally, the intensity of the sound affected plant height at 41 DAP (Figure 2) and leaf areas at 48 DAP (Figure 4).

At 41 DAP control plants without frequency produced the highest plant height while at 48 DAP plants exposed to the frequency of 8 kHz had similar height as the control plants (Figure 1). Meanwhile, at 41 days, the highest plant height was at 80 dB and the shortest plant height was at 110 dB among all intensity treatments excluding control (Figure 2).

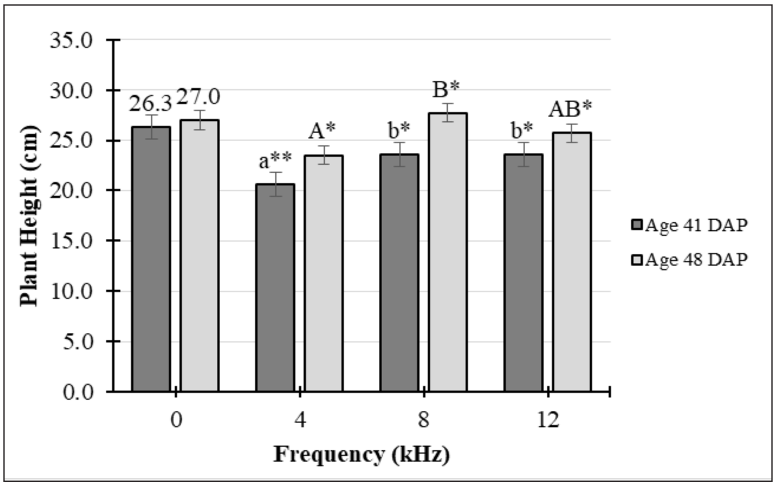

Figure 1. The effect of the frequency on plant height at 41 and 48 days after planting. Means followed by same letter among 4, 8, and 12 kHz are not different based on LSD at p=5% (lower and upper cases are for 41 and 48 DAP plants, respectively). The * and ** indicate significant difference between control (0 kHz) and each level of frequency by t-test at p=5% and 1% respectively.

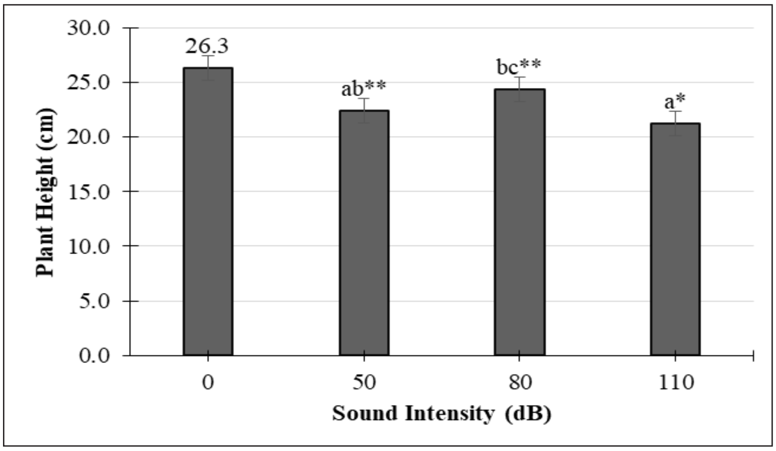

Figure 2. The effect of the sound intensity on plant height at 41 days after planting. Means followed by same letter among 50, 80, and 110 dB are not different based on LSD at p=5%. The *and ** indicate significant difference between control (0 dB) and each level of sound intensity by t-test at p=5% and 1% respectively.

The frequency of 8 kHz and 12 kHz produced a better leaf area than the frequency of 4 kHz on the growth of leaf area at 48 DAP entering pod phase formation, whereas at 62 DAP (charging phase and ripening seeds), many leaves turned yellow and fell off the plant (Figure 3).

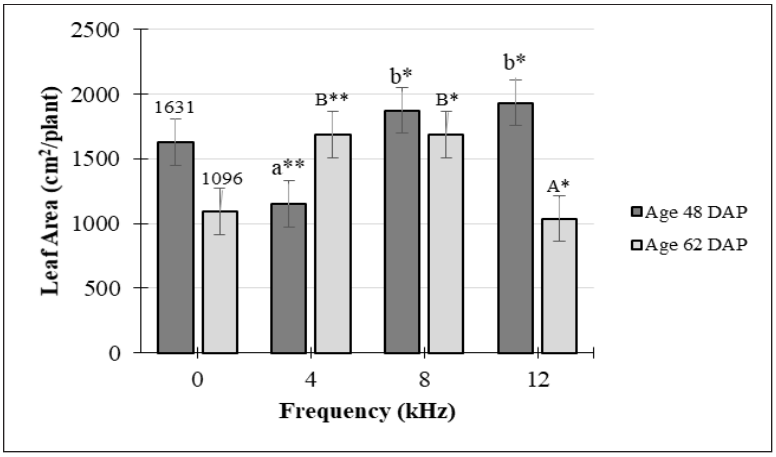

Figure 3. The effect of the frequency on leaf area at 48 and 62 days after planting. Means followed by same letter among 4, 8, and 12 kHz are not different based on LSD at p=5% (lower and upper cases are for 48 and 62 DAP plants, respectively). The * and ** indicate significant difference between control (0 kHz) and each level of frequency by t-test at p=5% and 1% respectively.

At 48 DAP, the leaf area decreased with the increasing of sound intensity and the best leaf area was produced at the 50 dB intensity (Figure 4).

Figure 5 shows that at the interval age of 49 to 56 DAP with the exposure of soybean crop at 4 and 12 kHz, produced a relatively higher relative growth rate than that of the 8 kHz level.

The soybean crop that was exposed to harmonic frequency and sound intensity of 50 to 110 dB showed different plant height (at 41 and 48 DAP), leaf area development (at 48 and 62 DAP), and relative growth rate (at 49 to 56 DAP). This finding is in line with that of Hassanien et al. (2014) as every plant has different responses toward sound frequency and sound intensity. Furthermore,

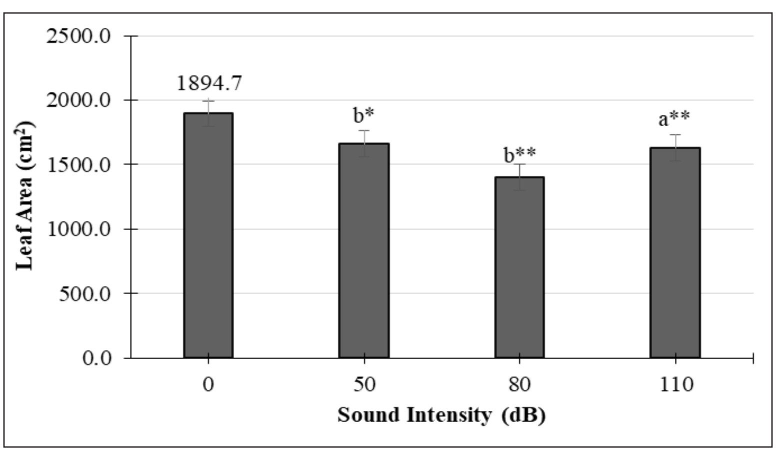

Figure 4. The effect of the sound intensity on leaf area at 48 days after planting. Means followed by same letter among 50, 80, and 110 dB are not different based on LSD at p=5%. The *and ** indicate significant difference between control (0 dB) and each level of sound intensity by t-test at p=5% and 1% respectively.

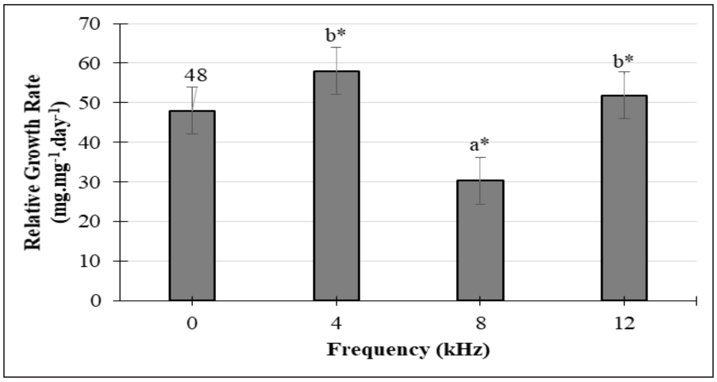

Figure 5. The effect of frequency on the relative growth rate at 49-56 days after planting. Means followed by same letter at 4, 8, and 12 kHz are not different based on LSD at p=5%. The * indicate significant difference between control (0 kHz) and each level of frequency by t-test at p=5%.

this finding was supported by Chowdhury et al. (2016) who reported that sound was an external factor that had a great impact on the biological index of plant and could either promote or suppress growth.

The Effect of Harmonic Frequency and Sound Intensity on the Yield

The harmonic frequency and the sound intensity showed a significant result on the fresh and dry weight of seed, and the harvest index.

The frequency of 4 kHz produced the highest fresh weight of seed that was 2.84 t.ha^{-1}, an increase of 26.2% compared with the potential production by 2.25 - 2.30 t.ha^{-1}. Similarly, for dry weight of seed, 4 kHz frequency also gave the highest yield (Figure 6).

The sound intensity of 50 dB produced the highest fresh weight and dry weight of the seed (Figure 7).

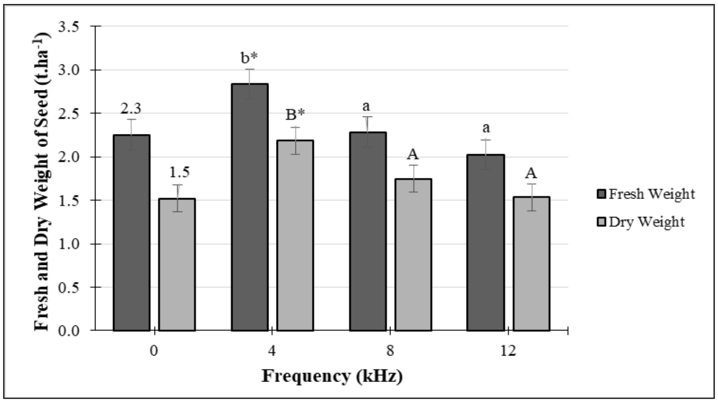

Figure 6. The effect of the frequency on fresh weight and dry weight of seed. Means followed by same letter among 4, 8, and 12 kHz are not different based on LSD at p=5% (lower and upper cases are for fresh weight and dry weight, respectively). The * indicate significant difference between control (0 kHz) and each level of frequency by t-test at p=5%.

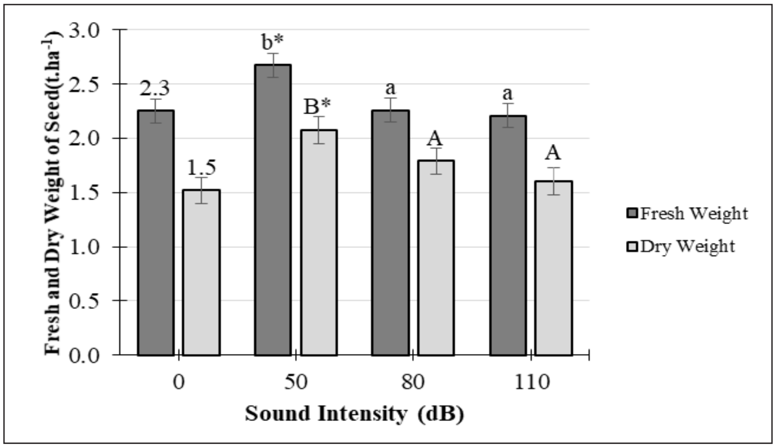

Figure 7. The effect of the sound intensity on fresh weight and dry weight of seed. Means followed by same letter among 50, 80, and 110 dB are not different based on LSD at p=5% (lower and upper cases are for fresh weight and dry weight, respectively). The * indicate significant difference between control (0 dB) and each level of sound intensity by t-test at p=5%.

The average of harvest index at the frequency of 4 kHz produced the highest result, 42.65%. The harvest indexes among frequencies of 8 kHz and 12 kHz, and without frequency were not significantly different (Figure 8). Additionally, the highest harvest index was generated at a sound intensity of 50 dB with 41.22%. Meanwhile the harvest indexes were not different without sound intensity and intensities of 80 dB and 110 dB (Figure 9).

The best results of the average of fresh and dry seed weight per hectare and harvest index were obtained when the plants were exposed to the sound wave frequency of 4 kHz. An increase in grain yield amounted

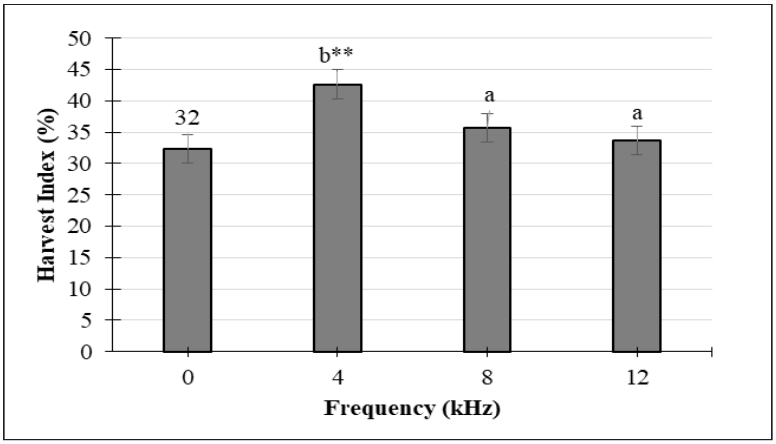

Figure 8. The effect of the frequency on harvest index. Means followed by same letter among 4, 8, and 12 kHz are not different based on LSD at p=5%. The ** indicate significant difference between control (0 kHz) and each level of frequency by t-test at p= 1%.

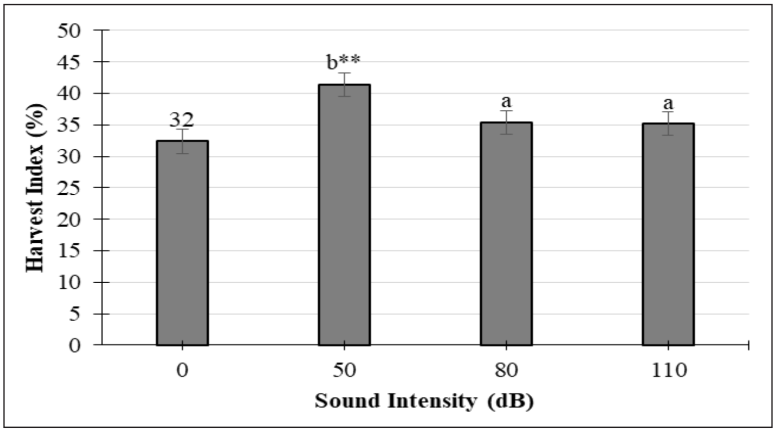

Figure 9. The effect of the sound intensity on harvest index. Means followed by same letter among 50, 80, and 110 dB are not different based on LSD at p=5%. The ** indicate significant difference between control (0 dB) and each level of sound intensity by t-test at p=1%.

to 26.2% was achieved when compared to the potential production of the Anjasmoro soybean variety (2.25 to 2.30 $t.ha^{-1}$). The result was supported by previous work (Pujiwati & Djuhari, 2011) which showed that the stomata opening increased at sound frequency from 4-5 kHz. However, if the frequency reached 6-7 kHz, the opening of the stomata decreased. This finding was in line with the experimental result of Martens et al. (1982) which found that only the optimal frequency range could stimulate growth and yield. Furthermore, Chowdhury et al. (2014) reported that sound waves with specific frequencies and intensities could have positive effects on various plant biological indices, including seed germination, root elongation, plant height, callus growth, cell cycling, signalling transduction systems, enzymatic and hormonal activities and gene expression.

The fresh weight and dry weight of the seed, harvest index decreased as the sound intensity increased. The high sound intensity, could be in a level of intensity with energy that affected the biomass by photosynthesis mechanism (principally through affecting stomata opening). It means that the sound level intensity should be optimised to achieve the best biomass. Other studies on microorganism (*E. coli*) showed a negative effect of high sound intensity to bacteria growth (Gu et al., 2016).

CONCLUSION

This study concluded the stomata opening mechanism was due to the resonance of events indicated by their higher stomata opening than the value at the control treatment (0 kHz and 0 dB). The phase of vegetative growth of soybean plants had different patterns of response to the frequency of sound waves. The yield of soybean crop exposed to the sound wave frequency of 4 kHz showed an increase of 26.2%. The highest result of the leaf area, fresh weight, dry weight of seeds and harvest index was also found at 50 dB sound intensity.

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