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Examining Use of Sonic Bloom Technology on the Stomata Opening of Drought-Stressed Soybean

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***Sonic bloom* is a technology that combines high frequency sound waves and organic nutrients, intended for better plant growth to increase itsproductivity.This study aimed to determine the effect of sound wave frequency and drought stress on stomatal opening, nutrient uptake efficiency through leaf, and soybean yield. We designed the research as a split plot experiment.The main plot was sound wave frequency consisting of four levels (no frequency imposed, frequencies 2, 4and 6 kHz. The sub-plot wasthree soil moisture contents (50,75, and 100% field capacity). We found that the interaction of frequency and soil moisture content affected the width of stomata at the age of 30, 40 and 50 days after planting (dap), the efficiency of nitrogen uptake, phosphorusuptake and potassiumuptake and the protein content of seeds. The width of stomatal opening at a frequency of 4 kHz in soil moisture 100% FC showed the highest value and was not significantly different from soil moisture 75% FC.There was a positive correlation between exposure to plants with a frequency of 4 kHz with stomatal opening, nutrient uptake and increased yield of soybean crops.The use of *sonic bloom* technology with plant exposure at a frequency of 4 kHz could increase drought tolerance to 75% soil moisture content. Soybean seed yield increased by 40.89% and seed protein content increased by 10.3%.**

Keywords: *sonic bloom*, frequency, soil moisture content, nutrient uptake efficiency, soybean yield.

Soybeans are one of the important agricultural commodities in Indonesia after rice and corn. At present the consumption of soybeans for the people of Indonesia is 2.2 million tons per year and only 30% of domestic soybean products while 70% are still imported. Therefore, efforts are needed to increase soybean productivity. Sonic bloom is a technology that combines the exposure of certainfrequency sound waves and organic nutrients to make plants grow better so as to increase their productivity. This technology utilizes natural sound waves with high frequencies that are able to stimulate the leaf mouth (stomata) wide open so as to increase the rate and efficiency of absorption of leaf fertilizer which is beneficial for plants. In other words, this technology is a way to improve photosynthetic efficiency and end photosynthesis results in order to increase the

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amount of production with good quality (Irianiet *al,* 2005; Widyawati*et al.,* 2011).

One of the obstacles in soybean cultivation is the availability of water, because the soil moisture was low, it resulted stunted growth, high loss of flowers, and pods. In accordance with photosynthesis, water is the main raw photosynthetic material in which it is needed in the photolysis process, water, then, will be decomposed and produces electrons which are then used in dark reactions. Water also affects the opening of the stomata. Plants that grow in low soil moisture conditions or drought stress,its stomata's opening is relatively smaller, so CO_2 absorption is low, consequently its photosynthesis is not optimal and plant growth is inhibited (Khan *et al*., 2016).

Water shortages in plants occurs because there is not enough water in the media and excessive transpiration or a combination of these two factors. In the field, even though it is sufficient in the homeland, plants can experience stress (lack of water). This happens if the absorption speed is not balanced with the water-lossduring the transpiration process. In general, plants will show a certain response if they experience drought stress. The response of plants to water stress is largely determined by the level of stress and the phase of plants growth when they experiencing stress. There are two kinds of responses that can improve the status of water when plants experience dry conditions, namely; (1) plants change the distribution of new assimilates to support root growth at the expense of canopy, so as to increase root capacity to absorb water and inhibit leaf expansion to reduce transpiration; (2) plants will regulate the degree of opening of the stomata to inhibit water loss through transpiration (Brittlate, 2007).

Leaf fertilization practice has several advantages, including low cost, fast response by plants, only needs a small amount of fertilizer, can be combined with the application of other chemical compounds, and becomes very important when soil conditions become a problem and root growth is inadequate (Oosterhuis, 2009). On the other hand, the leaffertilization practice has disadvantageswhere leaves might get burnt, causesolubility problems, and only a small amount of nutrients can be applied at one time. Wojcik (2004) added that some problems in leaf fertilization practice include: low absorption, especially in leaves whichhave thick cuticles, slipped fertilizer solution from the surface of hydropobic leaves, the solution tends to get easily dried from the leaf surface, and leaf fertilization cause damage to the leaves. Responses of varying results in application of leaf fertilization have been widely reported. This may be related to the wrong time of application, improper use of fertilizer ingredients, and unavailability of nutrients in the soil, as well as environmental conditions. The efficiency of leaf fertilization can be influenced by the type of fertilizer, concentration and pH of the solution, application time, surfactant use and compatibility with other chemical compounds (Oosterhuis, 2009).

Dobermann (2007) provides several approaches to calculate fertilizer efficiency, including: absorption efficiency, physiological efficiency, and agronomic efficiency. Absorption efficiency is a comparison between nutrients absorbed from fertilizer and the amount of fertilizer given, stated in percentagescales. Absorption efficiency figures are useful as a correction factor in fertilization recommendations. Physiological efficiency is useful for assessing plant responses in optimizing nutrients derived from fertilizers to produce products. While the agronomic efficiency is useful to assess how much increase in production is achieved from each amount of fertilizer added. The efficiency of nutrient use can be expressed in several ways. Mosier (2004) describes 4 agronomic indices which are generally used to explain nutrient use efficiency, namely: partial factor productivity (PFP, states kg yield for each kg of fertilizer used); agronomic efficiency (AE, kg increase in crop yield for each kg of fertilizer applied); apparentrecovery efficiency (RE, kg of fertilizer absorbed for each kg of fertilizer given) and physiological efficiency (PE, kg increase in yield for each kg of fertilizer absorbed). The term crop removal efficiency, which is the nutrient percentages used to produce products from nutrients that are applied, is also often used (Roberts, 2008).

Information about the use of sonic bloom technology in accordance with stomatal opening at drought stress conditions has not been widely studied. If the stomata can widely open followed by leaves fertilizationprocess, the efficiency of the nutrient uptake can be further enhanced. In relation to the soybean's stomata opening in drought stress conditions, it is necessary to assess the amount of soil fertilizer given through sonic technology can be tolerated so as to produce optimal quantity and quality of soybeans.

MATERIALS AND METHODS

Experimental place

The study was conducted from February to June 2018 in the green house of the Faculty of Agriculture, University of Islam Malang, and Laboratory of Physiology, Faculty of Medicine, University of Islam Malang, Dinoyo-Lowokwaru Malang, East Java province of Indonesia with an altitude of about 540 meters above sea level. The research location was located at $07^0$59'South Latitude and $112^0$36' East Longitude. The average temperature is 24^0C in the morning, 31^0C in the afternoon and 25^0C in the afternoon, with humidity of 57% - 83%. The average rainfall is 1.883 mm/ year with solar radiation intensity ranging from 7.036 lux to 696.4 x 102 lux. Clay texture with a ratio of 11% sand: 34% dust: 55% clay. N_{Total} 0.46%, P_{Bray} 296.95 mg.kg^{-1} and K 1.98 me.100g^{-1}. Water content at pF 2 = 0.55 g.g^{-1} and pF 4.2 = 0.29 g.g^{-1}

Experimental design

The study usedSplit Plot Design, with main plot isgiven frequency of sound waves in four levels: F_0= no frequency, F_2=frequency 2 kHz, F_4 =frequency 4 kHz and F_6 =frequency 6 kHz. While the sub plotconsisted of soil moisture in 3 levels: L_{50} = 50% Field Capacity (FC), L_{75} = 75% FC, and L_{100} = 100% FC. The exposure of sound waves is carried out from 08:00a.m. for about 20 minutes.

Soybean plants were exposed to frequencyof sound waves from the age of 20 days up to 70 days after planting (*dap*) and treated in 10 days interval. The exposure was carried out for 20 minutes. After that,the soybean plants were sprayed with 2 g/liters of liquid fertilizer (*Growmore*).The observation of the width of the stomatal opening using the molding method (replica) with fixation using a clear nail.The observed yield variables werefresh weight and dry weight of seeds, protein content of seeds. The supporting variables observed were nutrient uptake efficiency.

Statistical analysis

The statistical analysis usedwas the analysis of variance (*ANOVA*) testwith split plot design, and the mean difference was tested further by using Honestly Significant Difference (*HSD*) test at the 5% level.

Table 1. Average Stomata Width in the Interaction of Frequency and Soil Moisture Content

Frequency (kHz)	Soil Moisture Content (% FC)	Stomata Width (µm)		
		30 dap	40 dap	50 dap
No frequency	50	8,57 a	6,80 ab	6,50 a
	75	9,73 ab	7,87 b	6,53 a
	100	10,00 ab	8,00 b	6,47 a
2	50	8,67 a	6,17 a	6,83 ab
	75	10,80 b	8,73 bc	8,57 b
	100	11,00 bc	8,60 bc	9,53 b
4	50	8,67 a	5,93 a	5,40 a
	75	12,30 bc	9,90 c	9,23 b
	100	12,63 bc	10,10 c	10,03 b
6	50	9,20 ab	6,47 ab	6,03 a
	75	9,43 ab	8,60 bc	9,60 b
	100	9,47 ab	8,77 bc	9,67 b
	HSD 5%	1,78	1,66	1,87

Notes: - The numbers accompanied by the same letter in the same column show no significant difference in the 5% HSD (Honest Significan Difference) test - dap: days after planting

RESULTS AND DISCUSSION

Stomata width

The interaction of sound wave frequency and soil moisture content significantly affected the average of stomata width at the age of 30, 40 and 50 days after planting (*dap*).

The width of stomatal opening at 4 kHz in soil moisture 100% FC showed the highest value and was not significantly different from soil moisture 75% FC. In soil moisture 50% FC shows the lowest average width of stomatal opening for all levels of frequency of sound wave exposure.

Efficiency of nutrient uptake through leaves

The nutrient uptake efficiency of nitrogen, phosphorus and potassium was very significantly affected by the interaction of frequency and soil moisture content.

The highest efficiency of nitrogen uptake was achieved at 4 kHz frequency treatment at 100% soil moisture content, and it was not significantly different from the 6 kHz frequency at 100% soil

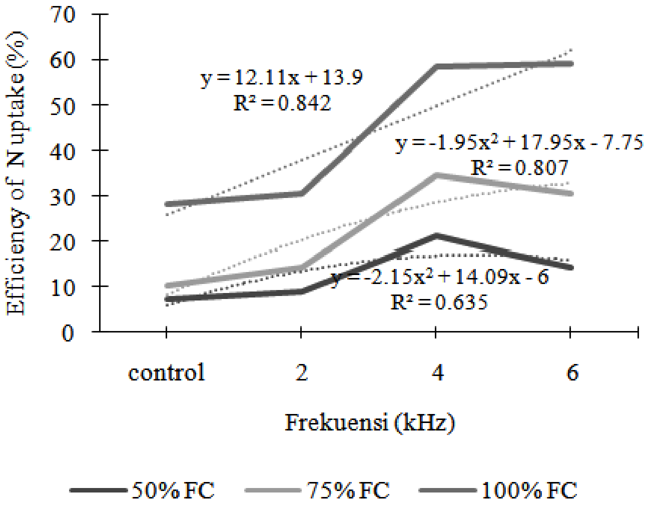

Fig. 1. Efficiency of N uptake on the interaction of frequency and moisture content

Table 2. Average Efficiency of Nutrient Uptake on Interaction of Frequency and Soil Moisture Content

Frequency (kHz)	Soil Moisture Content (%FC)	Efficiency of Nuptake (%)	Efficiency of Puptake (%)	Efficiency of K uptake (%)
	50	7,42 a	13,53 c	2,09 c
No frequency	75	10,26 a	13,13 c	2,05 c
	100	28,24 d	26,52 f	4,08 f
	50	9,21 a	5,54 a	0,91 a
2	75	14,18 b	9,69 b	1,56 b
	100	30,70 de	25,10 ef 3,91 ef	
	50	21,32 c	15,04 c	2,35 c
4	75	34,69 e	22,98 e	3,56 e
	100	58,46 f	45,92 h	7,05 h
	50	14,51 b	4,28 a	0,75 a
6	75	30,77 de	18,70 d	2,95 d
	100	59,29 f	38,98 g	6,02 g
	HSD 5%	4,19	2,27	0,35

Note: The numbers accompanied by the same letter in the same column show no significant difference in the 5% HSD test moisture content. Meanwhile, the lowest nitrogen uptake efficiency was resulted in non-frequency treatment with the moisture content of 50% FCand it was not different fromwithout frequency of 75% FC and frequency of 2 kHz at moisture content of 50% FC (Figure 1).

The highest efficiency of phosphorus uptake was obtained at 4 kHz frequency interaction with 100% moisture content. Meanwhile at each frequency level, 50% soil moisture content produced the lowest nutrient uptake efficiency (Figure 2).

Potassium uptake had the same pattern as phosphorus uptake, where the highest absorption efficiency was obtained at a frequency of 6 kHz with 100% soil moisture content. The lowest elemental potassium uptake at each frequency level occurred in soil moisture content of 50% FC (Figure 3).

Fresh Weight and Dry Weight of Seeds

The average fresh weight and dry weight of seeds was not influenced by the interaction of frequency and soil moisture content. Separately, the effects of both treatments are presented in Table 3.

The average high fresh and dry weight of seeds achieved at 4 kHz was not significantly different from 6 kHz, with an increase in yields of 40.89% and 30.67% respectively. While at moisture content of 100% field capacity, yield fresh weight and dry weight of seeds were not significantly

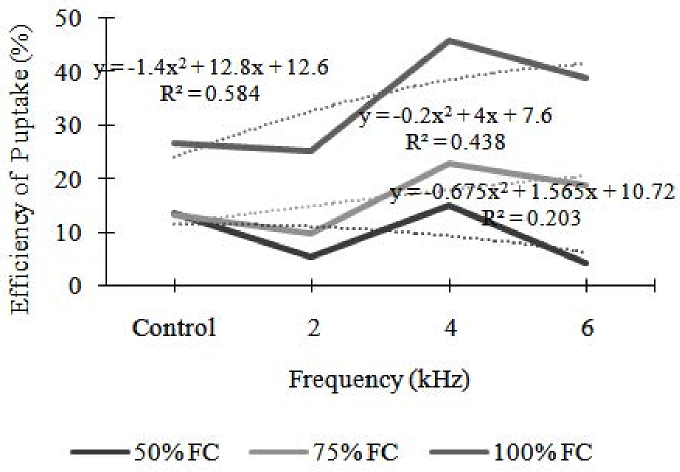

Fig. 2. Efficiency of P uptake on the interaction of frequency and moisture content

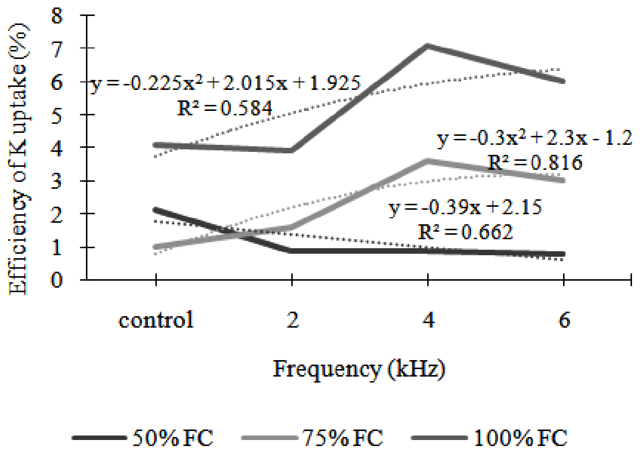

Fig. 3. Efficiency of K uptake on the interaction of frequency and soil moisture content

different from moisture content of 75% field capacity with increasing yields of 35.56% and 28.89% respectively.

Seed Protein Content

Seed protein content was significantly affected by the interaction of the frequency and moisture content of the soil. The average protein content in seeds is presented in Table 4.

The highest seed protein content was produced at the exposure treatment at a frequency of 6 kHz at 100% soil capacity, increasing by 10.3% from the description of the variety of 41.78%. The lowest seed protein in the treatment without exposure to sound waves with soil moisture content of 50% field capacity (Figure 4).

To see the relationship between the width of the stomatal opening and the nutrient uptake efficiency given through the leaves, soybean growth and yield are presented in Table 5 below.

There was a positive correlation between the width of the stomatal opening and the efficiency of N, P and K nutrient uptakeand growth (which is represented by the relative growth rate variable) and the fresh weight and dry weight of the seeds.

Table 3. Average Fresh Weight and Dry Weight of Seeds at Frequency and Soil Moisture Content

Treatment	Fresh Weight of seeds per Hectare (t.ha^{-1})	Dry Weight of seeds per Hectare (t.ha^{-1})
Frequency (kHz)		
No frequency	2,39 a	1,89 a
2	2,32 a	1,92 a
4	3,17 b	2,66 b
6	2,94 b	2,51 b
HSD 5%	0,34	0,34
Soil Moisture Content (% FC)		
50	2,16 a	1,71 a
75	2,90 b	2,43 b
100	3,05 b	2,60 b
HSD 5%	0,39	0,30

Note: The numbers accompanied by the same letter in the same column show no significant difference in the 5% HSD test (Anjasmoro variety production potential: 2.25-2.30 t.ha^{-1})

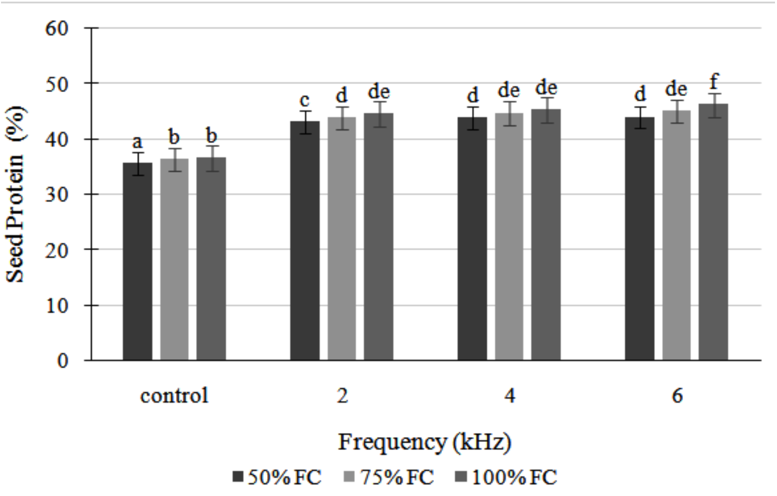

Fig. 4. Seed protein content in the interaction of frequency and soil moisture content

DISCUSSIONS

In general, at all frequency levels, at 100% soil moisture content, the field capacity resulted in a wide opening of the stomata which was not significantly different from the soil moisture content of 75% field capacity. This shows that the turgidity of guard cells can still be maintained at a decrease in soil moisture content by 25%. Misra*et al.*, (2015) suggested that stomatal opening and closing is determined by increasing and decreasing turgor pressure in guard cells in response to environmental conditions and biological systems. Marschner (2012) suggested that potassium ions (K +) along with other anions such as Ca2 +, H + have a very important role in regulating the pressure of guard cells (guard cells) during stomatal opening. Increasing the concentration of K + in the guard cell causes the osmotic potential of the cell to decrease, the water will move into the guard cell and the turgidity of the guard cell increases, so the stomata open. Although the drought conditions spurred the closure of the stomata, in the condition of soil moisture 75% the field capacity of stomata guard cells can still maintain their turgidity. The results of research by Budisantoso and Proklamasiningsih (2003) concluded that the use of sonic bloom technology can eliminate the effects of drought to 75% of the soil moisture content available.

The interaction between the frequency and level of soil moisture significantly affected the efficiency of fertilizer absorption given through the leaves and the protein content of seeds. The highest efficiency of elemental absorption of nitrogen, phosphorus and potassium at all frequency levels at 100% soil moisture content, was not significantly different from that produced at 75% soil moisture content. This related to the optimal opening of stomata to the level of soil moisture content of 75% field capacity, thus it also supportedthe optimal nutrient uptake through stomata. The results of the correlation analysis showed a positive relationship between the width of the stomatal opening and the nutrient uptake efficiency, growth and yield of soybean plants. Wide opening stomata supports the entry of nutrients given through the leaves (Oosterhuis, 2009) so as to produce photosynthetic products which are reflected in the increase in plant growth and yield. The yield component which was influenced by the interaction between the frequency and level of soil moisture was the seed protein content, where the highest seed protein was obtained at a frequency treatment of 6 kHz with soil moisture content of 100% field capacity which

Table 4. Average Soybean Seed Protein Content in the Interaction of Frequency and Soil Moisture Content

Frequency (kHz)	Seed Protein Content (%) Soil Moisture Content 50%	75%	100%
No Frequency	35,53 a	36,23 b	36,50 b
2	43,00 c	43,70 d	44,47 de
4	43,70 d	44,57 de	45,23 de
6	43,97 d	45,00 de	46,07 f
HSD 5%	0,69		

Note: The numbers accompanied by the same letter in the same column show no significant difference in the 5% HSD test

Table 5. Correlation Coefficients between Stomata Width, Nutrient Uptake Efficiency, Relative Growth Rate (RGR), Fresh Weight of Seeds (FWS) and Dry Weight of Seeds (DWS)

	Stomata Width	Uptake of N	Uptake of P	Uptake of K	RGR	FWS	DWS
Stomata Width	1.000**	0.574*	0.618*	0.616*	0.611**	0.775**	0.124
Uptake of N	0.574*	1.000**	0.937**	0.930**	0.024	0.877**	0.900**
Uptake of P	0.618*	0.937**	1.000**	0.974**	0.013	0.818**	0.827**
Uptake of K	0.616*	0.930**	0.974**	1.000**	0.019	0.813**	0.822**
RGR	0.611*	0.024	0.013	0.019	1.000**	0.013	0.015
FWS	0.775**	0.877**	0.818**	0.813**	0.013	1.000**	0.991**
DWS	0.124	0.900**	0.827**	0.822**	0.015	0.991**	1.000**

Note: ** very significantly different at p <0.01, significantly different at p <0.05

was 46.07%. At a frequency of 4 kHz the protein content of seeds produced on soil moisture content of 100% field capacity was 45.23% not significantly different from soil moisture 75% field capacity of 44.57%. At a frequency of 2 kHz, the highest seed protein content was produced in soil moisture content of 100% field capacity of 44.47% while in the treatment without frequency the protein content of seeds on soil moisture content of 100% field capacity was only 36.50%, and it was not different from 75% field capacity which was 36.23%. There was an increasing content in soybean protein up to 10.3% in plants which were given sound waves exposure compared to the its varieties description of 41.78 - 42.05%. Chowdhury*et al.* (2014) explained that there is a very close relationship between sound waves and plant growth. Sound waves with certain frequencies have a significant effect on biological, biochemical, physiological and gene expression activities in plants. Further Yiyao*et al.* (2002) explained that the division and growth of plant tissue is strongly influenced by dissolved proteins in the tissues. Accumulation of dissolved protein content affects cell division, enzyme content and level of metabolism. Zhao *et al.* (2003) added that sound waves at specific frequencies increase dissolved protein and sugar content in the *Dendranthemamorifolium callus cytoplasm*. The optimum frequency of sound waves stimulates an increase in soluble protein content and callus growth in *Chrysanthemum calli* (Bochu*et al.*, 2001; Yiyao*et al.,* 2002; Yi *et al.,* 2003). Sound waves exposure at frequency of 5 kHz for 4 weeks increases the amount of alanine and glycine in the grain endosperm tissue compared to control plants (Measures and Weinberger, 1973).

Separately the frequency of sound waves of 4 kHz with 6 kHz produced insignificant fresh weight and dry weight. This is in line with the research conducted by Martens *et al.* (1982)that only a certain frequency range could stimulate plant growth and yield. Furthermore Chowdhury*et al.* (2014) added that sound with a certain frequency and intensity that has a positive influence on plant biological activities such as seed germination, root extension, plant height, callus growth, enzyme and hormone activity and gene expression. Meanwhile the soil moisture content treatment of 100% field capacity increased the fresh weight of the seed by 35.56% from its potential production of 2.25 t/ha. This result was not significantly different from soil moisture treatment 75% field capacity with a production increase of 28.89%. This is in line with the result of research conducted by Budisantoso and Proklamasiningsih (2003) where the use of sonic bloom technology can eliminate the effects of drought to 75% soil moisture content available. Soil moisture is an environmental factor that limits plant growth and yield. The response of plants to water shortages can be identified from its metabolic activity, morphology, and physiology. Chlorophyll content of the plant showed the lowest yield in soil moisture treatment 50% field capacity. The results of the research by Song Ai and Banyo (2011) stated that the decreasing chlorophyll content is one of the plant's physiological responses to water shortages. The decreasing chlorophyll content relates to photosynthetic device activity, and it decreases plant photosynthesis rate. Ashraf and Harris (2013) added that chlorophyll as a photosynthetic pigment would be damaged in the presence of drought stress resulting in a decrease in the efficiency of light absorption in both photosystem-I and photosystem-II, so the photosynthetic capacity is reduced. Light energy is absorbed by chlorophyll and converted to chlorophyll fluorescence, despite the fact that the level of chlorophyll fluorescence is not more than 1-2% of the total absorbed light.

CONCLUSION

Sonic bloom technology was able to increase the wide opening tolerance of soybean stomata to drought stress conditions of 75% field capacity. Nutrient uptake efficiency increased to 52.4% for nitrogen, 31.9% for phosphorus, and 32.2% for potassium than without using sonic bloom. The increasingnutrient uptake efficiency led to the increasing seed production to 40.89% and soybean seed protein content increased to 10.3%.

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