Chlorophyll content and stomatal density of soybean varieties on technological packages application under dry land conditions

Y Hasanah*, H Hanum and A S Hidayat

Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia-20155

E-mail: *yaya@usu.ac.id

Abstract. The development of dry land as agricultural land for increasing soybean production is faced with a number of problems. Therefore, a technological package is needed for soybean cultivation under dry land condition and also need the varieties that are tolerant under dry land conditions. The study aimed to identify the chlorophyll content and stomatal density of soybean by applying several packages of soybean cultivation technology under dryland conditions. This research was conducted under dry land conditions at Tanjung Jati, Langkat (Indonesia). This research was conducted using a factorial randomized block design with 2 factors and 3 replications. The first factor was soybean varieties (Demas, Anjasmoro, Dering, Devon) and the second factor is technology packages (P₁, P₂, and P₃). The result showed that each variety had different chlorophyll-a, chlorophyll-b, and total chlorophyll content. Dering variety has the highest total chlorophyll content, while Anjasmoro variety has the lowest total chlorophyll. Each variety had a significantly different stomatal density. Devon variety has the highest stomatal density, while Demas variety has the lowest stomatal density. The technology package with the addition of dolomite lime 2,000 kg/ha on Dering variety had the highest of chlorophyll content and stomatal density.

1. Introduction
Soybean includes the Leguminosae family that is used by the people in Indonesia as food and protein sources. Soybean is also a source of functional food because it contains secondary metabolites which are beneficial for human health namely isoflavones. Isoflavones in soybeans are effective in preventing heart damage and osteoporosis, anti-tumor, prevent atherosclerosis, diabetes mellitus, colon, and prostate cancer and reducing menopausal syndrome [1-3].

Soybean production in Indonesia is currently insufficient, so it still depends on import. Until now, the soybean production has not reached the expectations. The main problems in soybean production are not extensive planting areas, only 550-661 thousand ha (2010-2014) and low productivity (1.35-1.55 t/ha). The use of paddy fields as soybean production area is around 8.1 million ha because it is necessary another land to increase soybean production through expansion of planting land to suboptimal land such as dry land [4].

The development of dry land as agricultural land for increasing soybean production is faced with a number of problems such as infertile soil, acidic reaction, high Al, Fe and Mn content, poor organic matter and macronutrients such as N, P and K. Therefore, a technological package is needed for...
soybean cultivation under dry land conditions and also need the varieties that are tolerant under dryland conditions.

The application of several packages of soybean cultivation technology under dry land condition can be affected on physiological of soybeans such as chlorophyll content and stomatal density. Therefore, based on this background, the research aimed to identify the soybean chlorophyll content and density of stomata by applying several packages of soybean cultivation technology under dry land conditions.

2. Materials and Methods

2.1. Research area and materials

This research was conducted under dry land condition at Tanjung Jati, Langkat (Indonesia). The dry land has climate characteristic such as the average of rainfall is 170.67 mm/month, temperature minimum monthly is 21.6 °C and temperature maximum monthly is 32.5 °C, temperature average monthly is 27.6 °C. The soil characteristic was content of P total 0.13%, K totals 0.13% N 0.19%, and pH 5.12.

The materials and equipment used in the research are soybean varieties (Devon-1, Demas, Dering-1, and Anjasmoro), fertilizers such as Urea, TSP and KCl, dolomite lime, insecticides, fungicides, ascorbic acid, aceton, kutex, hoe, knapsack sprayer, microscope, and spectrophotometer.

2.2. Design of experiment and management of crop

This research was conducted using a factorial randomized block design with 2 factors and 3 replications. The first factor was soybean varieties (V1 = Demas, V2 = Anjasmoro, V3 = Dering-1 and V4 = Devon-1). The second factor was the application of technological packages of soybean under dryland conditions as shown in Table 1.

Table 1. Technological packages of soybean cultivation under dry land conditions

| Technology package of soybean cultivation | Input |
|------------------------------------------|-------|
| P1 = Package 1                           | Fertilizer (Urea 25 kg/ha), inoculant *Bradyrhizobium japonicum* 200 g/40 kg seed, KCl 50 kg/ha, SP-36 100 kg/ha, spacing 40 cm x 20 cm, dolomite 500 kg/ha, farmyard manure 2 ton/ha, maximum tillage, antioxidant ascorbic acid 100 ppm. |
| P2 = Package 2                           | Fertilizer (Urea 25 kg/ha), inoculant *B. japonicum* 200 g/40 kg of seed, KCl 100 kg/ha, SP-36 150 kg/ha, dolomite 1.000 kg/ha, spacing 40 cm x 20 cm, farmyard manure 2 ton/ha, maximum tillage, antioxidant ascorbic acid 200 ppm |
| P3 = Package 3                           | Fertilizer (Urea 25 kg/ha), inoculant *B. japonicum* 200 g/40 kg of seed, KCl 150 kg/ha, SP36 250 kg/ha, spacing 40 cm x 20 cm, dolomite 2.000 kg/ha, farmyard manure 5 ton/ha, maximum tillage, antioxidant ascorbic acid 300 ppm |

Planting soybean seeds is carried out using a spacing of 40 cm x 20 cm. Before planting, soybean seeds are inoculated first with *Bradyrhizobium japonicum* (20 g/40 kg of seeds). Fertilizers used such as Urea, SP-36 and KCl are applied based on the treatment of technology package cultivation (Table 1). Half the dose of Urea is given during planting, and the rest is given 4 weeks after planting (WAP). Plant maintenance includes weeding and controlling plant disease and pest. Weed control is carried out at 3 and 7 WAP, while pest control is done by spraying insecticides with a dose of 2 cc/litre. The variables observed included chlorophyll-a content, chlorophyll-b content, total chlorophyll content and density of stomata.
Determination of chlorophyll content is done by collecting samples of fully expanded leaflets (0.1 g) for each treatment and macerated it in a mortar with 10 mL of 80% aqueous acetone (v/v). Measurement of chlorophyll-a, chlorophyll-b and total chlorophyll using the formula refers as reported by Henry and Grime [5].

\[
\text{Chlorophyll} - a = \frac{(12.7 \times A_{663}) - (2.69 \times A_{645})}{10}
\]

\[
\text{Chlorophyll} - b = \frac{(22.9 \times A_{645}) - (4.68 \times A_{663})}{10}
\]

\[
\text{Total of Chlorophyll} = \frac{(8.02 \times A_{663}) + (20.2 \times A_{645})}{10}
\]

Determination of stomatal density used leaf impression method. The clear nail polish is used to make an impression of the leaf surface, then the cast is removed with sticky tape and placed on a microscope slide. The stomatal density expressed as a unit. mm\(^{-2}\).

2.3. Data analysis

Data were analysed by analysis of variance, if there were significant differences then proceed with Duncan’s Multiple Range Test at the level of \(\alpha = 5\%\).

3. Results and Discussions

3.1. The content of chlorophyll-a, chlorophyll-b, and total chlorophyll

Table 2. The content of chlorophyll-a, chlorophyll-b, and total chlorophyll of soybean varieties with the application of technological packages under dry land conditions

| Variable observed          | Variety  | Technology package | Mean |
|---------------------------|----------|--------------------|------|
|                           |          | \(P_1\)           | \(P_2\) | \(P_3\) |
| Chlorophyll-a              | \(V_1\) (Demas) | 0.97               | 0.72  | 0.87  | 0.85  |
|                           | \(V_2\) (Anjasmor) | 0.70               | 0.87  | 0.92  | 0.83  |
|                           | \(V_3\) (Dering-1) | 1.12               | 1.40  | 1.23  | 1.25  |
|                           | \(V_4\) (Devon-1) | 1.43               | 0.79  | 1.34  | 1.19  |
|                           | Mean     | 1.06               | 0.94  | 1.09  |      |
| Chlorophyll-b              | \(V_1\) (Demas) | 1.38               | 0.48  | 0.53  | 0.80  |
|                           | \(V_2\) (Anjasmor) | 0.45               | 0.37  | 0.62  | 0.48  |
|                           | \(V_3\) (Dering-1) | 0.94               | 1.26  | 0.96  | 1.05  |
|                           | \(V_4\) (Devon-1) | 1.21               | 0.50  | 1.07  | 0.93  |
|                           | Mean     | 0.99               | 0.65  | 0.79  |      |
| Total of chlorophyll       | \(V_1\) (Demas) | 2.35               | 1.20  | 1.41  | 1.65  |
|                           | \(V_2\) (Anjasmor) | 1.15               | 1.24  | 1.54  | 1.31  |
|                           | \(V_3\) (Dering-1) | 2.06               | 2.66  | 2.19  | 2.44  |
|                           | \(V_4\) (Devon-1) | 2.64               | 1.29  | 2.41  | 2.11  |
|                           | Mean     | 2.05               | 1.60  | 1.99  |      |

Each variety has different chlorophyll-a, chlorophyll-b, and total chlorophyll. In this research, Dering variety has the highest chlorophyll content. The difference in chlorophyll content of each variety showed that each variety has different abilities in forming chlorophyll (Table 2). One factor that
influences the chlorophyll content in plants is genes. Chlorophyll formation is determined by certain genes contained in chromosomes. Several previous studies have reported on the genetic elements ability to control the accumulation of chlorophyll in photosynthetic tissues [6-9].

In the process of photosynthetic, chlorophyll-a also plays a role in photosystem II, while chlorophyll-b in photosystem I. Ohmiya [10] reported that there are three phases in the chlorophyll metabolic pathway, namely chlorophyll-synthesis of glutamate, interconversion between chlorophyll-a and b (chlorophyll cycle) and chlorophyll-a degradation into a non-florescent chlorophyll catabolic.

The application of the P1 technological package increased the content of chlorophyll-a, chlorophyll-b, and total chlorophyll. Each variety has different compatibility with the technological package tested. In Devon-1 variety, the application technological package P1 and P3 produced the highest chlorophyll-a and total chlorophyll, while the highest chlorophyll b contained in the P1 technological package. In Anjasmoro variety, the application of P2 and P3 technological packages decreased total chlorophyll by 7% and 33% respectively.

In this research, the addition of Ca$^{2+}$ in the form of dolomite lime increased the content of chlorophyll of Anjasmoro variety. The previous study of P. vulgaris by Patrick and Ndakidemi [10] reported that stabilizing calcium supply apoprotein from chlorophyll-a/b protein complex of photosystem II and finally improved the leaves chlorophyll content. This research is similar to our study. As reported by Barry et al [11] that Calcium plays a role in the oxidation of water during the photosynthetic process. Low level of Ca$^{2+}$ associated with reduced photosynthesis in plants and efficiency of photosynthetic in rice leaves were improved significantly with Ca$^{2+}$ supply.

3.2. Stomatal density

The results showed that each variety had a significantly different stomatal density. Devon variety has the highest stomatal density, while Demas variety has the lowest stomatal density. The application of the P1 technology package increases stomatal density compared to P2 and P3 technology packages. Each variety has different compatibility with the technology package tested. For Devon varieties, P1 and P2 technology packages produce the highest stomatal density. For Anjasmore varieties, P2 technology package produces the highest stomatal density, whereas, for Anjasmore varieties, P3 technology package produces the highest stomatal density. The P1 technology package on the Demas variety produces the lowest stomatal density.

The difference in stomatal density of each variety indicated that the formation of stomata is strongly influenced by genetic factors. It is supported by Bozoglu and Karayel [12] that the variation of stomatal density in genotypes affect the ability in gas exchange and the ability to adapt. Stomatal density affects the ability in gas exchange.

Table 3. The stomatal density of soybean varieties with the application of technological packages under dry land conditions

| Variety     | Technology package | Mean    |
|-------------|--------------------|---------|
|             | P1                 | P2      | P3      |
|             | Mean               |         |         |
| V1 (Demas)  | 118.23ab           | 97.10c  | 81.77d  | 99.03d  |
| V2 (Anjasmore) | 102.53c       | 121.43a | 100.57c | 108.18c |
| V3 (Dering-1) | 105.87bc        | 104.10bc | 122.03a | 110.67b |
| V4 (Devon-1)  | 120.57a           | 120.47a | 107.43abc | 116.16a |
| Mean         | 111.80a           | 110.78b | 102.95c |

The same letter behind the numbers indicated not significantly different according to Duncan’s Multiple Range Test at the level of $\alpha=5\%$. 


The high stomatal density in P3 treatment on Dering varieties showed that Calcium in the form of dolomite lime plays a role in stomatal formation. High calcium application plays a role in increasing stomatal density, due to calcium plays a role in opening and closing stomata. Stomata play a very important role to regulate carbon dioxide influx and loss of water vapour. Cytosolic-free Ca$^{2+}$ is presumably to be involved in linking of signal transduction under environmental conditions to stomatal movements [13].

4. Conclusion
Each variety had different chlorophyll-a, chlorophyll-b, and total chlorophyll content. Dering variety has the highest total chlorophyll content, whereas Anjasmoro variety has the lowest total chlorophyll. Each variety had a significantly different stomatal density. Devon variety has the highest stomatal density, while Demas variety has the lowest stomatal density. The technology package with the addition of calcium 2,000 kg/ha on Dering variety had the highest chlorophyll content and stomatal density.

References
[1] Garcia-Lafuente A, Guilamon E, Villares A, Rostagno M A and Matinez J A 2009 Flavonoids as anti-inflammatory agents: Implications in cancer and cardiovascular disease Inflamm Res 58 pp 537-52
[2] Anthony M S, Clarkson T B and Williams J K 1998 Effect of soy isoflavones on atherosclerosis: potential mechanism Am J Clin Nutr 68 pp 1390S-3S
[3] Sacks F M, Lichtenstein A, Van Horn L, Harris W, Kris-Etherton P and Winston M 2006 Soy protein, isoflavones and cardiovascular health An American Heart Association Science Advisory for Professionals from the Nutrition Committee Circulation 113 pp 1034-44
[4] Subandi 2016 Info teknologi: Teknologi budidaya kedelai pada lahan kering beriklim kering [Info technology: Soybean cultivation technology in dry climate dry fields] (Malang: Balai Penelitian Aneka Kacang dan Umbi)
[5] Henry G A and Grime J P 1993 Methods in comparative plant ecology (A Laboratory Manual: Chapman and Hall)
[6] Aftab T, Masroor M, Khan A, Idrees M, Naeem M and Mo'inuddin 2010 Salicylic acid as potent enhancer of growth, photosynthesis and artemisinin production in Artemesia Annua L J Crop Sci Biotech 13 pp 183-88
[7] Hortensteiner S 2013 Update on the biochemistry of chlorophyll breakdown Plant Mol Biol 82 pp 505-17
[8] Hasanah Y and Sembiring M 2018 Role of Elicitors in chlorophyll content and stomatal density of soybean cultivars by foliar application J Agron 17 pp 112-17
[9] Hasanah Y, Mawarni L and Irmansyah T 2017 Production and Physiological Characters of Soybean under Drought Stress with Foliar Application of Exogenous Antioxidant Proc. of the Conference on International Research on Food Security, Natural Resource Management and Rural Development
[10] Ohmiya A, Hirashima M, Yagi M, Tanase K and Yamamizo C 2014 Identification of genes associated with chlorophyll accumulation in flower petals PloS One 9 e113738
[11] Bambara S and Ndakidemi P A 2009 Effect of Rhizobium inoculation, lime, and molybdenum on photosynthesis and chlorophyll content of Phaseolus vulgaris Afr J Microbiol 89 pp 393-401
[12] Barry B A, Hicks C, De Riso A and Jenson D L 2005 Calcium ligation in photosystem II under inhibiting conditions Biophys J 89 pp 393-401
[13] Bozoglu H and Karayel R 2006 Investigation of Stomata Densities in Pea (Lisumsativum L.) Online Journal of Biological Sciences 6 pp 56-61
[14] Ridolfi M, Roupsard O, Garrec J P and Dreyer E 1996 Effect of calcium deficiency on stomatal conductance and photosynthetic activity of Quercus robur seedlings grown on nutrient solution Annales de sciences forestieres INRA/EDP Sciences 53 pp 325-35
Acknowledgment

The authors gratefully thank Research Institution-Universitas Sumatera Utara for funding this research in accordance with Research Contract TALENTA Universitas Sumatera Utara, Fiscal Year 2018. Number: 2590/UN5.1.R/PPM/2018 dated March 16, 2018.