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Research Article
Effect of Kieserite on Physiological Characteristics of Soybean Varieties under Dryland Conditions

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Abstract
Background and Objective: Chlorophyll a and b are the main pigments require magnesium for light energy and the synthesis of both. Kieserite ($MgSO_4 \cdot H_2O$) contains magnesium which plays an important role as the central atom of the porphyrin ring of green plant pigments, chlorophyll a and b. The aim of the study was to evaluate the role of magnesium in kieserite on physiological characteristics of soybean varieties. **Materials and Methods:** The study was conducted in Deli Tua, Deli Serdang Regency, Sumatera Utara from May to August, 2019. The experimental design used was a factorial randomized block design. As the first factor, soybean varieties consist of Demas, Anjasmoro, Devon-1, Dering-1. The second factor is dose of kieserite ($MgSO_4.H_2O$) applied consisting of 0, 50, 100 and 150 kg kieserite ha^{-1}. **Results:** The study result indicated that the highest of chlorophyll content was found in Anjasmoro variety compared to other varieties. The highest total chlorophyll and stomatal density was found in combination of Anjasmoro and 50 kg kieserite ha^{-1}. **Conclusion:** The treatment 50 kg kieserite ha^{-1} on Anjasmoro increased the chlorophyll content about 30% and stomatal density 15.56%.

Key words: Chlorophyll, soybean, stomatal density, magnesium, kieserite, cultivation, dryland

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

One of the legumes plants as protein sources and common use for public consumption and the food industry in Indonesia was soybean. Secondary metabolites from soybean known as isoflavones make it very important vegetable protein and functional food source for health for preventing osteoporosis, heart damage and degenerative[1-5].

Production of soybean in Indonesia is still dependent on imports, therefore, in order, it is necessary to expand soybean production to fulfill national soybean needs on marginal land including dry land as agricultural land. However, the are some problems of dryland conditions including acidic, less fertile soil, poor organic matter and, macronutrients and containing Al, Fe, Mn in high amounts[6]. Acidic dry soils such as Ultisol with low pH and Al toxicity especially at saturation of Al >25 will affect plant development[7].

Plant growth severely limits by soil acidity due to increased concentrations and toxicity of H^+, Al and Mn, decreased macro nutrient concentrations (cations), magnesium (Mg) deficiency, Ca and K, decreased P and Mo solubility and inhibition in root growth, water absorption, nutrient deficiency, drought and increased nutrient leaching. Therefore, it is necessary to role the homeostatic Mg from reactive oxygen species (ROS) in Al stress and change the photosynthate partition so that soybean productivity increases, because Mg is the only mineral that makes up chlorophyll[6,8].

Each chlorophyll molecule contains one of Mg atom so that magnesium (Mg) plays a role as the only mineral in the constituent of chlorophyll[9]. Magnesium is a macronutrient very important for stabilization of macroeconomic conformation (nucleic acid)[10], cell walls and membranes[11] and protein[12]. Magnesium also plays a role in the regulation of anion-cation balance in cells and as osmotically active ionizing cell turgor together with K[13,14].

The previous studies have reported the growth and production responses of soybean varieties under dryland conditions due to antioxidants[15], elicitors[14] and the use of technological packages[16]. Studies on the physiology characters by application Mg have not been widely reported. Therefore, based on this background, the study aims to evaluate the role of magnesium in the formation of chlorophyll and stomatal density in several soybean varieties under dryland conditions.

MATERIALS AND METHODS

Time and location: This study was conducted under dryland conditions at Deli Tua, Deli Serdang, Sumatera Utara from May-August, 2019. The soil was analysed at the Socfind's Laboratory, Medan and analysis of the content of chlorophyll and density of stomata were carried out at the Laboratory of Plant Physiology and Tissue Culture, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara. The result of soil analyses showed that a pH 4.46, C-organic 0.82%, Mg total 0.02 %, Al-exchange 0.01 me/100 g.

Materials: Materials in this study were soybean varieties, magnesium sulfate, Urea, TSP, KCl, acetone, clear nail polish, fungicides and insecticides. The study used equipment such as knapsack sprayer, scales, bucket, cuvette, spectrophotometer and microscope.

Research design: A randomized block design used in this experimental with a 4×4 factorial treatment. As the first factor is soybean varieties, namely Dering-1, Anjasmoro, Demas, Devon-1. As the second factor is kieserite ($MgSO_4$. H_2O) application of kieserite was (0, 50, 100, 150 kg ha^{-1} kieserite).

Procedures: A plot contained 50 plants/treatment. Spacing of seed planted were 40×20 cm. Fertilization is carried out according to the recommended dose for dryland such as 50 urea kg ha^{-1}, 100 TSP kg ha^{-1} and 75 KCl ha^{-1}. Urea fertilizer was given twice times at half the dose at planting date and then half dose was applied at 4 week after planting (WAP). The KCl and TSP fertilizers were given entirely at planting time. Kieserite according to the treatment dose was applied entirely at planting time. Pest and plant disease control was done by spraying insecticides and fungicides.

Chlorophyll content: The chlorophyll analysis was carried out based on a method by Hendry and Grime[17]. The chlorophyll content was determined by collecting leaves samples (0.1 g), then macerated with 10 mL of acetone using a mortar. The formula for determining chlorophyll content uses the formula as follows:

$$\text{Chlorophyll-a} = \{(12.7\times A663)-(2.69\times A645)\}/10$$

$$\text{Chlorophyll-b} = \{(22.9\times A645)-(4.68\times A663)\}/10$$

$$\text{Chlorophyll total} = \{(22.9\times A649)+(20.2\times A645)\}/10$$

Stomatal density: The density of the stomata was determined using the leaf impressed method, by applying 1×2 cm a clear nail polish to the soybean leaves of the abaxial part to make a surface impression. Observation of the stomata was carried out on the abaxial surface, because in this part the stomata were more numerous, because it minimizes water loss compared to the adaxial part which is directly exposed to the sun. After drying it was taken by using a clear tape then placed to the object glass of microscope slide. Density of stomata observation with a microscope was carried out at a magnification of 40×10. The density of stomata is expressed as unit mm^{-2}.

Statistical analysis: The data was subjected to 2 way analysis of variance (ANOVA) procedures, the SAS version 12 computer program and comparison of means were tested for significance using Duncan's multiple range test (DMRT) $p = 0.05$.

RESULTS

Chlorophyll content: Based on the Table 1, it can be seen that the treatment of kieserite, variety and interaction between variety and kieserite has significant effect on chlorophyll-a, chlorophyll-b and total chlorophyll content. The treatment of 50 kieserite kg ha^{-1} has highest of chlorophyll a, b and total chlorophyll, respectively (2.50, 1.15 and 4.99 mg g^{-1}), while the treatment of 150 kieserite kg ha^{-1} has highest of chlorophyll a, b and total chlorophyll 100 kieserite kg ha^{-1} has lowest of chlorophyll a, b and total chlorophyll. The highest of chlorophyll total was found in Anjasmoro variety compared to others varieties. The highest of chlorophyll a, b supported the highest total chlorophyll content, respectively (0.97, 0.56 and 2.04 mg g^{-1} of fresh weight).

Density of stomata: The interaction between varieties and kieserite significantly affected stomata density. The stomata density of Anjasmoro and Demas varieties is higher than that of Devon-1 and Ring-1. The highest density of stomata is in the application of 50 kg kieserite ha^{-1}). The interaction between Demas and Anjasmoro varieties and the application of 50 kg kieserite ha^{-1} produced the highest stomata density, while the Devon-1 variety with 50 kg kieserite ha^{-1} and Dering-1 with kieserite 100 kg ha^{-1} produced the lowest stomata density (Table 2).

Table 1: Effect of kieserite on the chlorophyll content of several soybean varieties under dryland conditions

		Kieserite (kg ha^{-1}) Fresh weight (mg g^{-1})				
Variable observed	Varieties	P0 (0)	P1 (50)	P2 (100)	P3 (150)	Mean
Chlorophyll-a						
	V1 (Demas)	0.43^{i}	1.37^{gh}	0.93^{i}	1.38^{i}	1.03^{b}
	V2 (Anjasmoro)	3.32^{a}	4.81^{a}	0.80^{i}	2.28^{c}	2.80^{a}
	V3 (Devon-1)	1.10h	2.02^{d}	0.56^{i}	0.84^{i}	1.13^{b}
	V4 (Dering-1)	0.79^{i}	1.78^{f}	1.57^{f}	0.56^{i}	1.18^{b}
	Mean	1.41^{b}	2.50^{a}	0.97^{d}	1.27^{c}	1.53
Chlorophyll-b	V1 (Demas)	0.25^{h}	0.76^{e}	0.54^{i}	0.66^{f}	0.55^{b}
	V2 (Anjasmoro)	1.44^{b}	2.13^{a}	0.53^{i}	1.10^{c}	1.30^{a}
	V3 (Devon-1)	0.63^{f}	0.92^{d}	0.29^{h}	0.69^{f}	0.63^{b}
	V4 (Dering-1)	0.52^{i}	0.80^{e}	0.86^{de}	0.56^{i}	0.69^{b}
	Mean	0.71^{b}	1.15^{a}	0.56^{c}	0.75^{b}	0.79
Total chlorophyll	V1 (Demas)	0.86l	2.74^{i}	1.86^{i}	2.76^{i}	2.06^{b}
	V2 (Anjasmoro)	6.64^{b}	9.62^{a}	1.60^{i}	4.56^{c}	5.61^{a}
	V3 (Devon-1)	2.20^{h}	4.04^{d}	1.12^{k}	1.68^{i}	2.26^{b}
	V4 (Dering-1)	1.58^{i}	3.56^{e}	3.56^{e}	1.12^{k}	2.46^{b}
	Mean	2.82^{b}	4.99^{a}	2.04^{b}	2.53^{b}	3.09

Numbers followed by the same letter on the same variable observed indicate no significant difference based on Duncan's multiple range test at $\alpha = 5\%$

Table 2: Effect of kieserite on stomatal density soybean varieties under dryland conditions

	Kieserite (kg ha^{-1})				
Varieties	P0 (0)	P1 (50)	P2 (100)	P3 (150)	Mean
V1 (Demas)	14.07^{d}	11.85^{h}	14.52^{c}	16.37^{a}	14.20
V2 (Anjasmoro)	13.19^{e}	16.37^{a}	12.07^{i}	15.26^{b}	14.22
V3 (Devon-1)	11.63^{i}	10.37l	11.33^{i}	11.11^{i}	11.11
V4 (Dering-1)	12.37^{f}	13.04^{e}	10.67l	12.07^{i}	12.04
Mean	12.81	12.91	12.15	13.70	12.89

Numbers followed by the same letter show no significant difference based on Duncan's multiple range test at α =5%

DISCUSSION

The difference in chlorophyll content of each variety is due to the genetic factors of plants. This is consistent with the results of previous studies regarding differences in leaf chlorophyll content associated with plant metabolic processes and related to age, morphology and plant genetic factors. Certain genes contained in chromosomes control the formation of chlorophyll. Chlorophyll accumulation in photosynthetic tissue was built by the genetic elements in plants[18-20].

The treatment of kieserite 50 kg kieserite ha^{-1} can increase chlorophyll-a, b and total chlorophyll content. The increase in kieserite dose results in an increase in chlorophyll content related to the role of Mg in the formation of chlorophyll, because Mg is a porphyrin ring on the chlorophyll molecule. Magnesium is the only mineral constituent of chlorophyll. One Mg atom is available in each chlorophyll molecule, so the absence of Mg causes plants to be unable to carry out photosynthesis. Mg collects in related processes to form chlorophyll, CO_2 fixation and assimilate translocation. The Mg deficiency continues canopy growth greater than root growth and sucrose exports to roots increase[21-22].

The highest of total chlorophyll was found in combination of Anjasmoro and 100 kg kieserite ha^{-1}. The ability of each variety in the formation of chlorophyll is different. In this study, different responses were found in the formation of chlorophyll associated with the variety and dose of kieserite given. In Demas varieties that are tolerant to acid soils, kieserite needs to be up to 100 kg kieserite ha^{-1} to increase total chlorophyll. The same thing happened to Anjasmoro and Devon-1 varieties. Whereas, in Dering-1 variety, increasing the kieserite dose to 150 kg kieserite ha^{-1} increases total chlorophyll.

The different chlorophyll content related to the variation of soybean on acidic soil pH (4.46). Previous researchers stated that acid soil pH (pH<5) has high Al-dd levels that are harmful to plants. The reduced contribution of plant Mg in soils with low pH represents plants unable to build and maintain adequate pH and increase chemical cells from the membrane gradient of root cells. In low pH soils, the predominance of H^+ in the root rhizosphere can inhibit Mg uptake increasing Mg deficient plants, chlorophyll content and increased production quality[23-24].

The difference in response to the density of each variety can occur due to genetic differences in soybean varieties. The length and width of the stomata related to the size of the stomata porus. The level of porous stomata size affects plant transpiration and water absorption by plants. Water that is absorbed by plants used for photosynthesis thus accelerating photosynthesis increases and increasing plant growth and development[25-26].

CONCLUSION

The highest of chlorophyll content was found in Anjasmoro variety compared to other varieties. The highest total chlorophyll and stomatal density was found in combination of Anjasmoro and 50 kg kieserite ha^{-1}.

SIGNIFICANT STATEMENT

This research has discovered findings that there are differences in the interaction of varieties and application of kieserite under dryland conditions on chlorophyll and stomatal density. This research will assist researchers and farmers to use varieties and application kieserite on soybean varieties under dryland conditions. Based on this research a new theory has been obtained that, the use of the application of 50 kg kieserite ha^{-1} on Anjasmoro variety can increase the chlorophyll content and stomatal density.

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