The role of Magnesium Sulphate in the formation of chlorophyll and density of stomata of soybean varieties (Glycine max (L.) Merril)

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Abstract. Magnesium is a very important nutrient for the formation of chlorophyll and affects the density of stomata. The aim of the research was to evaluate the role of magnesium in the formation of chlorophyll and stomatal density. 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, Devon-1, Anjasmoro, Dering-1. The second factor is dose of magnesium applied consisting of 0; 100; 200 and 300 kg MgSO₄/ha. The research result indicated that the highest total chlorophyll was found in Dering-1 compared to other varieties. The highest of stomatal density was found in Demas variety. The highest of chlorophyll total was found in the treatment of 100 kg MgSO₄/ha on Dering-1 variety, while the highest stomatal density was found in the control treatment (0 kg MgSO₄/ha) on Demas variety.

1. Introduction
Soybean is one legumes plant as protein sources for public consumption and the food industry. Soybean plays a role as a very important source of vegetable protein and functional food source because it contains isoflavones as the main secondary metabolite that are very beneficial for health. Isoflavones play a role in preventing osteoporosis, heart damage, and degenerative [1-5]

Soybean production in Indonesia is still dependent on imports, therefore, in order to fulfill national soybean needs, it is necessary to expand soybean production on marginal land including dry land as agricultural land. The efforts to increase soybean production under dryland condition must refer to the characteristics of dryland which has a number of problems including acidic, less fertile soil, containing Al, Fe, Mn in high amounts, poor organic matter and macro nutrients [6]. Plant development on acidic dry soils such as Ultisol is faced with low pH and Al toxicity especially at saturation of Al > 25 [7]

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

Soil acidity severely limits plant growth 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].

The previously studies have reported the growth and production responses of soybean varieties under dryland condition due to elicitors [14], use technological packages [15] and antioxidant [16]. Studies on the role of Mg in the formation of chlorophyll and stomata density 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 stomata density in several soybean varieties under dry land conditions.

2. Materials and methods

2.1. Research area and materials

The study was carried out at Deli Tua, Deli Serdang, Sumatera Utara, under dry land condition. Soil analysis was conducted at the Socfind’s Laboratory, Medan and analysis of the content of chlorophyll and density of stomata was carried out at the Laboratory of Plant Physiology and Tissue Culture, Faculty of Mathematics and Natural Science, Universitas Sumatera Utara. The soil characteristics at the study site has a pH 4.46, C-organic 0.82 %, Mg total 0.02 %, Al-exchange 0.01 me/100g.

The research used materials such as soybean varieties, Urea, TSP, KCl, magnesium sulphate, acetone, clear nail polish, fungicides and insecticides. The equipment used in the research namely knapsack sprayer, scales, bucket, cuvet, spectrophotometer and microscope.

2.2. Procedures

The experimental design used was a 4 x 4 factorial treatment design with a randomized block design. Soybean varieties as the first factor, namely Dering-1, Anjasmoro, Demas, Devon-1. As the second factor is magnesium sulphate application (0, 100, 200, 300 kg MgSO₄/ha).

Each treatment plot contained 50 plants. Seeds were planted with a spacing of 40 cm x 20 cm. Fertilization is carried out according to the recommended dose for dry land such as 50 kg Urea/ha, 100 kg TSP/ha and 75 KCl/ha. Urea fertilizer was given at half the dose at planting date, and the remaining half dose was applied at 4 weeks after planting (WAP). KCl and TSP fertilizers were given entirely at planting time. Magnesium sulphate according to the treatment dose was applied entirely at planting time. Pest and plant disease control was done by spraying insecticides and fungicides.

The chlorophyll analysis was carried out based on a method by Henry 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:

\[
\text{Chlorophyll-a} = \frac{(12.7x A663) - (2.69 x A645)}{10}
\]

\[
\text{Chlorophyll-b} = \frac{(22.9 x A645) - (4.68 x A663)}{10}
\]

\[
\text{Chlorophyll total} = \frac{(22.9 x A649) + (20.2 x A645)}{10}
\]

Observation of the stomata was carried out on the abaxial surface, because in this part the stomata was more numerous, because it minimizes water loss compared to the adaxial part which is directly exposed to the sun. The density of the stomata was determined using the leaf impressed method, by applying 1 cm x 2 cm a clear nail polish to the soybean leaves of the abaxial part to make a surface impression. 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 x 10. The density of stomata is expressed as unit. mm⁻².
3. Result and discussion

3.1. Chlorophyll content

The treatment of varieties, magnesium sulphate, and the interaction between varieties and magnesium sulphate significantly affected on the content of chlorophyll. The highest of chlorophyll total was found in Dering variety compared to Demas, Anjasmo and Devon-1. The highest content of chlorophyll total is supported by the high content of chlorophyll-a and chlorophyll-b in Dering-1 variety (Table 1). The difference in chlorophyll of each variety can affect genetic factors in the formation of chlorophyll. The genetic factors can influence the formation of chlorophyll in each variety. This is consistent with previous researchers that differences in leaf chlorophyll content are related to plant metabolism related to age, morphology and genetic factors of leaves in plants. The formation of chlorophyll is determined by certain genes contained in chromosomes. Genetic elements in these genes can control the accumulation of chlorophyll in photosynthetic tissue [18-20].

The treatment of MgSO₄ up to 200 kg MgSO₄/ha can increase the total chlorophyll and chlorophyll-a. The chlorophyll-b content in 200 kg MgSO₄/ha treatment was not significantly different with 300 kg MgSO₄/ha. The increase in chlorophyll content with increasing dose of MgSO₄ applied is related to the role of magnesium in the formation of chlorophyll, because magnesium is the only mineral as a chlorophyll constituent. Each chlorophyll molecule has one Mg atom so that the absence of Mg results in the plant being unable to photosynthesize [9]. Metabolic process carried out by Mg related to the formation of chlorophyll, CO₂ fixation, and photosynthate loading [21]. Mg deficiency inhibits leaf growth greater than root growth and increases sucrose exports to roots [22-24].

The highest total chlorophyll was found in combination of Dering-1 and 100 kg MgSO₄/ha, while the lowest chlorophyll-a and total chlorophyll were found in the treatment of Anjasmo varieties with 100 kg MgSO₄/ha (Table 1).

### Table 1. Effect of magnesium sulphate on the chlorophyll content of several soybean varieties

| Variable observed | Dose of MgSO₄ (kg/ha) | V₁ (Demas) | V₂ (Anjasmo) | V₃ (Devon-1) | V₄ (Dering-1) | Mean |
|-------------------|-----------------------|------------|-------------|-------------|-------------|------|
| Chlorophyll-a     | M₀ (0)                | 1.70i      | 0.81o       | 1.46k       | 2.58cd      | 1.64d|
|                   | M₁ (100)              | 1.56j      | 0.61n       | 1.37l       | 3.71a       | 1.81c|
|                   | M₂ (200)              | 2.74bc     | 0.96m       | 2.48e       | 2.75b       | 2.23a|
|                   | M₃ (300)              | 1.87h      | 1.94g       | 2.04f       | 2.55d       | 2.10b|
|                   | Mean                  | 1.97b      | 1.08d       | 1.84c       | 2.90a       |      |
| Chlorophyll-b     | M₀ (0)                | 0.77h      | 0.50l       | 0.75i       | 0.88f       | 0.73c|
|                   | M₁ (100)              | 0.73j      | 0.41n       | 0.70k       | 1.42a       | 0.82b|
|                   | M₂ (200)              | 1.07b      | 0.47m       | 0.78gh      | 1.03c       | 0.84a|
|                   | M₃ (300)              | 0.79g      | 0.91e       | 0.79g       | 0.92d       | 0.85a|
|                   | Mean                  | 0.84b      | 0.57d       | 0.76c       | 1.06a       |      |
| Total of Chlorophyll | M₀ (0)                | 2.48j      | 1.30o       | 2.20l       | 3.46e       | 2.36d|
|                   | M₁ (100)              | 2.29k      | 1.02p       | 2.07m       | 5.13a       | 2.63c|
|                   | M₂ (200)              | 3.81b      | 1.42n       | 3.27f       | 3.78c       | 3.07a|
|                   | M₃ (300)              | 2.66i      | 2.84g       | 2.82h       | 3.48d       | 2.95b|
|                   | Mean                  | 2.81b      | 1.65c       | 2.59b       | 3.96a       |      |

Numbers followed by the same letter on the same variable observed indicate no significant difference based on Duncan’s Multiple Range Test at α = 5%.
Each variety has a different ability to form chlorophyll. In this study, different responses were found in each soybean variety related to the formation of chlorophyll in the presence of magnesium sulphate. In Demas variety that is tolerant to acid soils, the addition of magnesium up to 200 kg/ha increased total chlorophyll. In Anjasmoro variety, the addition of MgSO₄ up to 300 kg/ha still increased chlorophyll content. In the Devon-1 variety, the addition of MgSO₄ up to 200 kg/ha increased the total chlorophyll content, whereas in the Dering-1 variety that has a drought tolerant character, the addition of MgSO₄ up to 100 kg/ha increased the total chlorophyll content.

The difference in total chlorophyll content is thought to be related to the tolerance of soybean varieties to the soil pH conditions of the study (4.46). As explained by previous researchers that acid soil (pH <5) have high Al-ld level which are harmful to plants. In this case, the reduced availability of plant magnesium at low soil pH is a consequence of inability to build and maintain an adequate pH and increase the chemical cells of the membrane gradient of root cells. Therefore, in low pH soil, it can occur increased exchangeable magnesium concentrations, however, the dominance of H⁺ in the rhizosphere can inhibit Mg absorption, resulting in Mg deficiency and decreasing the total chlorophyll, yield and quality of agricultural product [25-26].

3.2. Density of stomata
The interaction between variety and MgSO₄ significantly affect the density of stomata. Demas and Anjasmoro varieties have higher stomata density compared to Devon-1 and Dering-1. The highest stomata density was in the control treatment (0 kg MgSO₄/ha). The treatment without MgSO₄ produced the highest stomata density. The interaction between Demas and Anjasmoro varieties and without MgSO₄ treatment produced the highest stomata density, while Devon-1 varieties without MgSO₄ treatment produced the lowest density of stomata (Table 2). The stomatal density of Demas and Anjasmoro variety were higher than Devon-1 and Dering-1 (Figure 1).

| Dose of MgSO₄ (kg/ha) | Variety | Mean |
|----------------------|---------|------|
|                      | V1 (Demas) | V2 (Anjasmoro) | V3 (Devon-1) | V4 (Dering-1) |
| M₀ (0)               | 111.11a   | 109.28a     | 83.86e       | 98.53bc      | 100.69     |
| M₁ (100)             | 92.77cde  | 91.72cde    | 96.44bcd     | 94.34bcd     | 93.82      |
| M₂ (200)             | 103.25ab  | 86.48de     | 87.00de      | 88.05de      | 91.19      |
| M₃ (300)             | 90.15cde  | 102.20ab    | 95.91bcd     | 91.72cde     | 94.99      |
| Mean                 | 99.32     | 97.42       | 90.80        | 93.16        |

Numbers followed by the same letter show no significant difference based on Duncan’s Multiple Range Test at α = 5%

![Figure 1. Density of stomata of soybean varieties](image-url)
The difference in response may be due to genetic Mg efficiency of four soybean varieties are different. Previous researcher has reported that the length and width of the stomata are closely related to the size of the stomata porus, the greater the size of the stomata, the stomata porus will also be even greater. This results in a high rate of transpiration. The high transpiration causes an increase in water absorption from the soil. The nutrients absorbed will be used for photosynthesis which causes an increase in the rate of photosynthesis which will affect the growth and development of plants [27-28]. This is a reinforcement that in this study, the stomata of the Dering-1 variety were lower because the Dering-1 variety was drought tolerant.

4. Conclusion
The highest total chlorophyll content was found in Dering-1 compared to other varieties. The highest of stomatal density was found in Demas variety. The highest total chlorophyll was found in combination of Dering-1 and 100 kg MgSO₄/ha, while the highest stomatal density was found in the control treatment (0 kg MgSO₄/ha) on Demas variety.

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