Morphological characterization of soybean (*Glycine max* L. Merril) in drought stress condition and P fertilizer application

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Abstract. Drought stress in plants can occur due to an imbalance between the availability of water with the water needs of plants. Drought stress can cause morphological changes. This study aims to identify changes in the morphological character of soybean plants in drought stress conditions and P fertilizer application. This research was carried out at the screen house and laboratory of tissue culture of the Faculty of Agriculture, University of Sumatera Utara, Medan. This study uses a Completely Randomized Design (CRD) with 3 treatment factors. The first factor is the variety consists of 2 types, namely Devon 1 and Dering. The second factor is the field capacity of water content which consists of 3 levels, namely 80, 60 and 40% field capacity. The third factor is the application of P fertilizer consists of 5 levels, namely without P application, 1 recommended dosage of Rock phosphate, ½ recommended dosage of Rock phosphate, 1 recommended dosage of TSP and ½ recommended dosage of TSP. The results showed that drought stress treatment significantly reduced total leaf area (18.99%), root length (23.43%), root dry weight (16.67%) and crown dry weight (30.91%) when the available soil water content was reduced from 80% to 40% KL. Application of 1 dose of TSP resulted in the highest increase in root length, root dry weight and shoot dry weight compared to other treatments.

1. Introduction

The low productivity of soybeans in Indonesia is partly due to natural factors, biotics, cultivation techniques and plant physiology. One of the opportunities for increasing food crop production to support National Food Security is the use of dry land [1]. The potential for dryland development on the island of Sumatra, especially North Sumatra, is 0.429 million ha [2]. Planting soybean varieties that are tolerant on dry land, is one alternative in the development and improvement of soybean cultivation and planting. The amount of soybean yield losses due to drought stress is determined by the variety, the duration of stress and the growing stage [3].

Drought stress in plants can occur due to an imbalance between water availability and water needs in plants [4]. A water deficit can result in stunted growth and decreased production because the plants are experiencing drought stress. Drought stress can affect the metabolic processes, growth and production of plants and can even result in crop failure [5]. The effect of drought stress on vegetative stages can reduce the rate of leaf widening and LAI at the next developmental level. Severe water stress can cause stomatal closure, which reduces carbon dioxide uptake and dry weight production. [6]Further states that during drought stress there is a decrease in the rate of photosynthesis caused by stomatal closure and a decrease in electron transport and phosphorylation capacity in leaf chloroplasts.
Besides the availability of water, fertilizer is also needed to increase crop production. Fertilization is one of the cultivation techniques that are necessary to obtain multiple or optimal results and improve the quality of the results [7]. One of the factors that influence soybean growth is the need for phosphorus (P). The largest period of use of P begins at the time of pod formation until about 10 days before full seed development [8].

Potential land for developing soybeans is quite extensive but faces obstacles in supplying water needs for plants, resulting in low soybean production. The use of drought-tolerant and efficient varieties in water management is a potential effort or approach to reduce the decline in soybean production [9]. Besides, the application of phosphorus to legume plants serves to stimulate root and shoot growth (vegetative) and seed formation (generative), where phosphorus is needed by plants in large amounts, but the amount of P in plants is less than nitrogen and potassium. Therefore, research is needed to identify soybean varieties that are tolerant to drought stress through soybean morphology by applying P fertilizer.

2. Materials and Method
This research was conducted at the screen house of the Faculty of Agriculture, Universitas Sumatera Utara, Medan in October 2019. The ingredients used in this study were soybean seeds of Devon 1 and Dering variety, an insecticide with active ingredient Santot 0.5 cc/liter, fungicide with active ingredient Dithane M-451 cc/liter, Urea fertilizer, TSP, KCl and rock phosphate. This study uses a Completely Randomized Design (CRD) with 3 treatment factors. The first factor is the variety consisting of 2 types, namely Devon 1 and Dering. The second factor is the field capacity of water content which consists of 3 levels, namely, 80, 60 and 40% KL. The third factor is the application of P fertilizer consisting of 5 levels, namely without the application of P, 1 recommended dose of rock phosphate, ½ dose recommended rock phosphate, 1 recommended dose of TSP and ½ recommended dose of TSP.

The research carried out included: 1) planting media preparation, where before planting was carried out, the planting media was doused with water in conditions of field capacity; 2) planting, carried out with torch with 2 seeds per polybag, when the plant is 1 MST. Every planting hole is left by 1 plant that grows well; and 3) determination of field capacity, where Irrigation is provided by determining the amount of water supply based on optimal water (soybean water requirements range from 300-350 ml per planting season). Measurement of soil water content is determined by taking soil samples, which are then weighed wet weight. The soil sample is then dried in an oven to a constant weight [10]. Gravimetric method was used to maintain and calculate the field capacity water content using gravimetric methods. Plant maintenance is based on soybean cultivation recommendations. Observation parameters included leaf area, root length, root dry weight and shoot dry weight, where the roots and shoots are cleaned of dirt and then roasted for 48 hours at 80ºC.

3. Results and Discussion

3.1. Leaf area
Results of analysis of variance showed that variety treatment and drought stress had a significant effect on leaf area. Interaction between treatments did not significantly influence the leaf area. Soybean leaf area with drought stress treatment and P fertilizer application can be seen in Table 1. The leaf area of Devon 1 variety is 6.67% wider than Dering variety. Moisture content of 80% KL produces the highest soybean leaf area. When compared with leaf area in other water content treatments, leaf area decreased by 8.66% (60% KL) and 18.98% (40% KL).
Table 1. Soybean leaf area treated with drought stress and fertilizer P application

| Varieties   | Drought stress (% KL) | Application of P fertilizer | Mean |
|-------------|-----------------------|-----------------------------|------|
|             |                       | P<sub>1</sub> | P<sub>2</sub> | P<sub>3</sub> | P<sub>4</sub> | P<sub>5</sub> |      |
|             |                       | cm<sup>2</sup>             |      |              |              |              |      |
| Devon 1     | 80 (K<sub>1</sub>)   | 23.31               | 24.77 | 24.57       | 25.26       | 25.11       | 24.60 |
|             | 60 (K<sub>2</sub>)   | 20.37               | 25.11 | 23.13       | 24.37       | 25.11       | 23.62 |
|             | 40 (K<sub>3</sub>)   | 16.14               | 19.12 | 17.70       | 24.63       | 21.13       | 19.74 |
| Dering      | 80 (K<sub>1</sub>)   | 25.11               | 26.00 | 23.62       | 23.75       | 19.73       | 23.64 |
|             | 60 (K<sub>2</sub>)   | 18.33               | 20.06 | 20.04       | 19.58       | 24.25       | 20.45 |
|             | 40 (K<sub>3</sub>)   | 20.33               | 19.39 | 19.14       | 19.75       | 18.07       | 19.34 |
| Devon 1     | 80 (K<sub>1</sub>)   | 19.94               | 23.00 | 21.80       | 24.75       | 23.78       | 22.65a|
|             | 60 (K<sub>2</sub>)   | 21.26               | 21.82 | 20.93       | 21.02       | 20.68       | 21.14b|
|             | 40 (K<sub>3</sub>)   | 18.23               | 19.26 | 18.42       | 22.19       | 19.60       | 19.54c|
| Mean        | 20.60                | 22.41               | 21.37 | 22.89       | 22.23       |              |      |

Note: the numbers followed by different letters show significantly different according to Duncan’s Multiple Range Test at the 5% level. P<sub>1</sub> = without P application; P<sub>2</sub> = 1 recommended dosage of Rockphosphate; P<sub>3</sub> = ½ recommended dosage of Rockphosphate; P<sub>4</sub> = 1 recommended dosage of TSP; P<sub>5</sub> = ½ recommended dosage of TSP; KL = field capacity

Figure 1. The effect of water content in the field capacity on soybean leaf area

Leaf area is a quantitative character that is influenced by plant genotypes, where genotypes play an important role in their response to drought stress. In Table 1, it can be seen that the two varieties show different responses to drought. The Devon 1 variety has a leaf area of 6.67% wider than the Dering variety, so it can be said that the Devon 1 variety is more tolerant of drought. This is in accordance with [11], which states that for the selection of drought tolerant genotypes, it can be predicted by the large leaf area owned by the strains that are consistently high during the period when the plants are experiencing drought stress. Thus plants that are able to maintain large leaf area under dry conditions or experience relatively small leaf area reduction in dry conditions are genotypes that are relatively tolerant of drought, so that these plants are able to maintain high production in dry conditions. With the high leaf area remaining in drought conditions, the function of photosynthesis can still run well. But in genotypes that are not able to maintain leaf area, the function of photosynthesis will be disrupted because the stress of dryness in soybean leaves the leaves to age rapidly which is marked by decreasing N content and leaf chlorophyll.
The soil moisture condition significantly affects the decrease in leaf area, where the lowest leaf area is in the soil moisture condition of 40% KL. Decrease in soybean leaf area for varieties of Devon 1 and Dering in the treatment of drought 40% respectively by 19.76% and 18.19% from the condition of 80% KL. The decrease in leaf area due to drought stress can be seen in Figure 1.

This occurs because low levels of soil water inhibit cell division, thereby suppressing the expansion of plant leaves in an effort to prevent excess water loss in the plant's body through the process of transpiration. According to [12], the reduction in leaf area due to drought stress was initially caused by reduced cell growth, especially in terms of enlargement of leaf epidermal cells. This reduction in leaf area further leads to reduced water loss through transpiration and decreased plant growth. In addition, a decrease in leaf area occurs in response to plants in order to survive in conditions of low water availability. According to [13], decreasing leaf area is a morphological adaptation strategy of plants to drought stress, which aims to reduce transpiration and increase the efficiency of the root system in absorbing water. Both of these are related to the efficiency of water use. Furthermore [14], decreased leaf area occurs due to loss of cell turgidity due to decreased relative water content, thus inhibiting cell division and development.

3.2. Root length

Results of analysis of variance showed that variety treatment, drought stress and P fertilizer application had a significant effect on root length, but the interaction between treatments did not significantly influence root length. The length of soybean roots with the treatment of drought stress and application of P fertilizer can be seen in Table 2.

Table 2. Length of soybean roots treated with drought stress and application of P fertilizer

| Varieties | Drought stress (% KL) | Application of P fertilizer | Mean |
|-----------|-----------------------|-----------------------------|------|
|           |                       | P₁  | P₂  | P₃  | P₄  | P₅  |      |
|           |                       | cm  |     |     |     |     |      |
| Devon 1 (V₁) | 80 (K₁)   | 11.93 | 14.73 | 13.07 | 16.90 | 13.97 | 14.12 |
|           | 60 (K₂)   | 9.80  | 12.97 | 11.80 | 14.58 | 12.25 | 12.28 |
|           | 40 (K₃)   | 7.77  | 11.83 | 10.40 | 12.43 | 10.85 | 10.66 |
| Dering (V₂) | 80 (K₁)   | 10.57 | 13.60 | 11.40 | 15.97 | 11.87 | 12.68 |
|           | 60 (K₂)   | 9.08  | 10.80 | 10.02 | 14.00 | 11.87 | 11.15 |
|           | 40 (K₃)   | 6.53  | 11.42 | 9.65  | 11.83 | 9.92  | 9.87  |
| Devon 1   | 9.83      | 13.18 | 11.76 | 14.64 | 12.36 | 12.35a| 9.87  |
| Dering    | 8.73      | 11.94 | 10.36 | 13.93 | 11.22 | 11.23b| 9.87  |
|           | 80 (K₁)   | 11.25 | 14.17 | 12.23 | 16.43 | 12.92 | 13.40a|
|           | 60 (K₂)   | 9.44  | 11.88 | 10.91 | 14.29 | 12.06 | 11.72b|
|           | 40 (K₃)   | 7.15  | 11.63 | 10.03 | 12.13 | 10.38 | 10.26c|
| Mean      | 9.28d     | 12.56b| 11.06c| 14.29a| 11.79c|      |

Note: the numbers followed by different letters show significantly different according to Duncan's Multiple Range Test at the 5% level. P₁ = without P application; P₂ = 1 recommended dosage of Rockphosphate; P₃ = ½ recommended dosage of Rockphosphate; P₄ = 1 recommended dosage of TSP; P₅ = ½ recommended dosage of TSP; KL = field capacity

The root length of soybean plants in Devon 1 variety is 9.06% longer than the Dering variety. 80% KL water content produced the highest root length compared to other water content treatments. Root length decreased when the level of drought stress was increased from 80% KL to 60% KL by 12.53% and 23.43% at 40% KL. Likewise with the treatment of P fertilizer, giving 1 recommended dose of TSP produces the highest root length compared to other doses. The root length of the Devon variety 1 is 1.12 cm longer than the Dering variety (Table 2).
This shows that root length is an indicator of water shortages in plants, where the growth of soybean roots in Devon 1 variety is better in drought conditions than in Dering varieties. According to [15], root length is related to plant resistance during drought. The lengthening of roots to deeper soil layers when water shortages occur indicates that the plant is tolerant and can be used as a potential root morphological character [16]. Root length in both soybean varieties decreased with increasing water stress level, where the decrease in root length from the available water conditions at 80% KL to 40% KL reached 23.43%. The decrease in root length due to drought stress can be seen in Figure 2.

Figure 2. The effect of water content in the field capacity on soybean root length

This is because drought stress inhibits the process of division and enlargement of cells in the roots, consequently the growth of the root length becomes inhibited. According to [17], inhibition of root development is caused not only by the inhibition of cell activity but also by areas of root penetration in a dry state (low soil moisture) so that newly formed roots cannot penetrate and eventually the root tips die. In addition, [18] explained that when stress occurs the root system will experience changes and increase in structure, namely the increase of root branches to support the function of roots in water absorption. The physiological changes are because the roots expand in volume to expand the area of water absorption. The increase in soybean root length in both varieties was influenced by the application of P fertilizer. The treatment of P fertilizer dosage which showed the highest root length was the application of 1 recommended dose of TSP. The increase in soybean root length can be seen in Figure 3.

Figure 3. The effect of P application on the length of soybean roots under drought stress
This increase occurs because the provision of P nutrients can meet the needs of plants for the growth process. According to [19], soybeans need relatively large amounts of P because P is needed throughout growth. Soybean requires more P to form seeds compared to other legumes. Furthermore [20] state that P fertilization results in an increase in P uptake due to higher P concentrations in the medium or due to root extension or both. Phosphorus is actively absorbed by the roots of the soil solution and stored in plant bodies in high concentrations.

3.3. Root dry weight

Results of analysis of variance showed that variety treatment, drought stress and P nutrient had a significant effect on root dry weight, but the interaction between treatments did not significantly influence root dry weight. The dry weight of soybean roots with the treatment of drought stress and the application of P fertilizer can be seen in Table 3.

Table 3. Dry weight of soybean roots with drought stress treatment and P fertilizer application

| Varieties | Drought stress (% KL) | Application of P fertilizer | Mean |
|-----------|-----------------------|-----------------------------|------|
|           |                       | P1  | P2  | P3  | P4  | P5  |      |
| Devon 1 (V1) | 80 (K1) | 0.20 | 0.25 | 0.27 | 0.34 | 0.25 | 0.26 |
|            | 60 (K2) | 0.21 | 0.21 | 0.15 | 0.29 | 0.23 | 0.22 |
|            | 40 (K3) | 0.15 | 0.23 | 0.14 | 0.28 | 0.19 | 0.20 |
| Dering (V2) | 80 (K1) | 0.19 | 0.23 | 0.20 | 0.31 | 0.19 | 0.22 |
|            | 60 (K2) | 0.16 | 0.21 | 0.17 | 0.28 | 0.21 | 0.21 |
|            | 40 (K3) | 0.16 | 0.22 | 0.17 | 0.24 | 0.18 | 0.19 |
| Devon 1    | 0.19 | 0.23 | 0.19 | 0.30 | 0.22 | 0.23 |
| Dering     | 0.17 | 0.22 | 0.18 | 0.28 | 0.19 | 0.21 |
|            | 80 (K1) | 0.19 | 0.24 | 0.24 | 0.33 | 0.22 | 0.24 |
|            | 60 (K2) | 0.19 | 0.21 | 0.16 | 0.29 | 0.22 | 0.21 |
|            | 40 (K3) | 0.15 | 0.23 | 0.16 | 0.26 | 0.19 | 0.20 |
| Mean       | 0.18 | 0.23 | 0.19 | 0.29 | 0.21 | 0.21 |

Note: the numbers followed by different letters show significantly different according to Duncan's Multiple Range Test at the 5% level. P1 = without P application; P2 = 1 recommended dosage of Rockphosphate; P3 = ½ recommended dosage of Rockphosphate; P4 = 1 recommended dosage of TSP; P5 = ½ recommended dosage of TSP; KL = field capacity

Dry weight of soybean root of Devon variety 1 is 8.69% higher than Dering. The highest root dry weights were 80% KL drought stress and continued to decrease at 60% and 40% KL, where the decreases were 12.50 (60% KL) and 16.67% (40% KL). Giving 1 recommended dosage of TSP increased root dry weight by 37.93% compared to that without P (control) fertilizer. The dry weight of soybean roots of Devon 1 variety was 8.69% higher than that of Dering, indicating that the root growth of Devon 1 variety was more adaptive to the water deficit condition than the Dering variety. This shows that the root dry weight is influenced by plant genotypes. According to [21], root dry weight indicates the ability of a plant to absorb water, because plants that have a high root dry weight have a higher rate and have a higher tolerance level to dryness compared to plants that have low of root dry weight. The level of water availability significantly influences the root dry weight. Reduction of root dry weight is in line with the lower availability of water. The decrease in root dry weight due to drought stress can be seen in Figure 4.
Figure 4. The effect of water content in the field capacity on soybean root dry weight

This decrease is thought to be related to a decrease in the rate of photosynthesis during drought stress. The results of the study of [22], reported that Willis experienced the lowest root dry weight reduction in drought stress, where the slow growth of roots is caused by the plant being unable to regulate its growth perfectly due to lack of organic matter produced, so that it can directly reduce dry weight root. Besides being affected by growth disturbance, the decrease in wet weight is also due to the root cell turgidity which is not optimal due to the low water content in the soil. When the water content in the soil is very low, the groundwater potential will decrease, so that the process of absorption of water by the roots also decreases. The existence of water flow occurs when there is a potential difference, which moves to a lower water potential, so that the roots of the plant will still maintain the potential for lower water conditions than the surrounding environmental conditions, in this case the soil, so that water can be absorbed by the roots [23]. Application of P fertilizer with various doses significantly affects the increase in root dry weight and dry weight of soybean shoot plants. The highest increase in root dry weight and canopy occurred in the treatment of P nutrient administration at a dose of 1 recommended TSP (P4). The increase in soybean root dry weight can be seen in Figure 5.

Figure 5. The effect of P application on the dry weight of soybean roots under drought stress
3.4. Shoot dry weight

Results of analysis of variance showed that variety treatment, drought stress and P fertilizer application had a significant effect on shoot dry weight, but the interaction between treatments did not significantly influence the canopy dry weight. Shoot dry weight of soybean by treatment of drought stress and application of P fertilizer can be seen in Table 4.

| Varieties | Drought stress (% KL) | Application of P fertilizer | Mean |
|-----------|-----------------------|-----------------------------|------|
|           |                       | P1  | P2  | P3  | P4  | P5  |      |
| Devon 1 (V1) | 80 (K1) | 5.57 | 6.52 | 5.43 | 6.68 | 5.00 | 5.84 |
|           | 60 (K2) | 4.03 | 5.67 | 3.80 | 5.40 | 4.72 | 4.72 |
|           | 40 (K3) | 3.17 | 4.32 | 3.52 | 4.62 | 3.90 | 3.90 |
| Dering (V2) | 80 (K1) | 4.25 | 5.63 | 4.65 | 5.85 | 5.40 | 5.16 |
|           | 60 (K2) | 3.73 | 5.47 | 3.68 | 5.40 | 5.25 | 4.71 |
|           | 40 (K3) | 3.20 | 3.48 | 3.58 | 4.37 | 3.85 | 3.70 |
| Devon 1   | 80 (K1) | 4.26 | 5.50 | 4.25 | 5.57 | 4.54 | 4.82a |
|           | 60 (K2) | 3.73 | 4.86 | 3.97 | 5.21 | 4.83 | 4.52b |
|           | 40 (K3) | 3.20 | 3.48 | 3.58 | 4.37 | 3.85 | 3.70 |
| Mean      |            | 3.99c | 5.18ab | 4.11bc | 5.39a | 4.69b |

Note: the numbers followed by different letters show significantly different according to Duncan's Multiple Range Test at the 5% level. P1 = without P application; P2 = 1 recommended dosage of Rockphosphate; P3 = ½ recommended dosage of Rockphosphate; P4 = 1 recommended dosage of TSP; P5 = ½ recommended dosage of TSP; KL = field capacity

Figure 6. The effect of water content in the field capacity on soybean shoot dry weight

The highest shoot dry weight was found in Devon 1 variety, which was 4.82 g (6.22% higher than the Dering). Shoot dry weight decreased with increasing drought stress from 80% KL to 60% KL by 0.78 g, which shows the same response at 40% KL in the form of a decrease in shoot dry weight by 1.7 g. The same result was obtained with root dry weight, giving 1 recommended dose of TSP increased shoot dry weight 25.97% compared to control. Soybean varieties Devon 1 and Dering show a significant difference in the decrease in shoot dry weight due to drought stress. The Devon 1 variety
has a shoot dry weight of 0.3 g heavier than the Dering variety (Table 4). This is possible because the two varieties have different adaptability capabilities to withstand water stress conditions. [25] suggested that plants that are resistant to drought stress conditions will reduce the weight and number of leaves they have to reduce the amount of water loss from plants. The decrease in root dry weight due to drought stress can be seen in Figure 6.

The difference in the availability of water content has a significant influence on the dry weight of plant shoot. The lower the water content available, the lower the shoot dry weight of the soybean. This is one way of soybeans to be able to withstand drought stress. In accordance with [26], drought stress can inhibit the growth of soybean shoot, even greater than inhibition of root growth due to plant efforts in maintaining water balance by reducing shoot surface area to reduce evaporation rate and maintain root development so that water demand can be solved. Furthermore [27] stated that water is a compound that is needed by plants in large quantities, more than 80% of wet cell weight and plant tissue consists of water. The reduced amount of water available in the soil can affect the vegetative growth of soybean plants, so that canopy growth will be inhibited. Shoot dry weight decreased also due to a decrease in photosynthetic activity due to the closure of stomata to avoid excessive release of water [28]. In addition, the low water content causes the nutrient content in the soil is difficult to be absorbed by plant roots. Nutrients and minerals are constituents of plant organic matter. The reduced supply of nutrients that dissolve with water often causes the formation of plant organic material decreases, so that the dry weight of the shoot directly decreases [29].

4. Conclusions
The treatment of drought stress decreases the total leaf area (18.99%), root length (23.43%), root dry weight (16.67%), and shoot dry weight (30.91%) when available soil water content is reduced from 80% to 40% KL. While application of 1 dose of TSP resulted in the highest increase in root length, root dry weight and shoot dry weight compared to other treatments.

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