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Root Growth Response of Soybean (*Glycine max L.*) Under Water Deficit

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Abstract
Roots are plant organs that absorb water and nutrients from the rhizosphere. If the soil is dry, the roots will be affected first. This study aims to know the response of soybean roots to drought stress. This research was arranged in a randomized completely block design (RCBD) with two factors and three replications. The first factor was soil moisture content, which consisted of four levels, e.i., 100, 75, 50, and 25% field capacity. The second factor was the growth stage, which consisted of three kinds, e.i., the vegetative active, flowering time, and seed-filling period. The results showed that the soil water content below 75% field capacity decreased root length, fresh root weight, root dry weight, root volume, and increased shoot root ratio. The seed-filling period was more sensitive to water deficiency than the active vegetative and flowering time. The study findings show that soybean plants can grow well at 100% field capacity. The practical implication of planting soybeans using a soil moisture content of 100% field capacity.

Keywords: drought stress; field capacity; growth stage; root

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Introduction

Soybean is one of the world's principal crops and is rich in protein, oil, carbohydrates, and minerals (1). Soybean is a meaningful plant that requires a sufficient water supply during its growth to achieve significant yields (2). Lack of water is an environmental stress factor that significantly affects development and plant growth, reducing product quantity and quality (1). Most of the soybean crop in Indonesia is in the rice fields during the dry season. Under this condition, soybean cultivation often faces the risk of drought. The photosynthetic rate of plants will experience a sharp decrease in drought, which is lower than plants that do not experience drought (3). Soybean production will decrease when water stress increases.

Soybean was most susceptible to drought stress during the reproductive stage (4). However, if the plant was subjected to severe long-term water stress during of vegetative growth stage, it may be large enough to cause substantial yield losses. (3) added that the drought conditions at the time of flowering caused the flowers and young pods to fall, reducing the number of pods and seeds. Conversely, if the seed filling is not filled, it will cause the soybean seeds to shrink, causing production to shrink by up to 40% (5). (6), in their research, concluded that the seed formation stage was more sensitive to drought than the flowering stage, but the least susceptible stage was the vegetative phase.

Plants have developed two main mechanisms for dealing with water deficiency: stress avoidance and tolerance. Stress avoidance is achieved by forming seeds before drought conditions occur and specializing in plant architecture. Morphological adaptations and the development of unique leaf surfaces reduced transpiration rates, reduced leaf area, sunken stomata, or increased root length and density to use water more efficiently (7). The same thing was expressed by (8), that drought stress inhibits the increase in plant height and leaf area. This inhibition was increasingly evident, along with the drought stress levels, duration, and frequency rise.

Roots are the essential vegetative organs of plants that support the top of the soil, provide water, and dissolve inorganic salts necessary for plant survival. Drought conditions can alter the assimilation allocation from photosynthetic organs to heterotrophic organs (sink) (9). Roots are essential organs in plants, especially for absorbing water and nutrients in the growing medium. During drought, anatomical and physiological changes can occur in plants, especially roots (10). More plants develop root systems in response to nutrient deficiencies and drought (11). Root cells changed, among others, by increasing or decreasing the number and size in the face of drought stress.

The base of soybean plants faced a reduction in stele and xylem diameter dimensions as a plant tolerance mechanism in experiencing drought stress (12). Limited or unavailability of water will inhibit plant growth by affecting various physiological and biochemical processes. However, more information was needed on how drought affects root morphology (13).

Previous studies only stated that drought stress reduced the number of pods and seeds. Drought stress in the seed-filling phase was more sensitive than in the flowering and vegetative stages. There still needed to be more information about the effect of drought levels on the growth of soybean roots. Therefore, this study aims to know the response of soybean roots to drought stress.

Materials and Methods

Materials

This study used Alfisol soil. Polybags were used for planting media. Soybean seeds were used Grobogan variety. The inorganic fertilizers used NPK Phonska (15:15:15) and SP-36. Oven Binder FED 53–UL Forced Convection to dry the root organs of the soybean plant. Ohaus PA214 Pioneer Analytical was used to measure plant roots' fresh and dry weight.

Methods

Study area

The research was conducted in a plastic house in Demangan, Sambi, Boyolali, Central Java, Indonesia, from August to November 2020. The Department of Food Crop Agriculture, Grobogan, Central Java, Indonesia. A geographical position was between 110°22'-110° 50' East longitude and between 7°7'-7°36' South latitude with a height of 184 m above sea level (ASL). The average rainfall and temperature were 139 mm month-1 and 26-32^{o}C, respectively.

Experimental design

This study used an RCBD with two factors and three replications. The first factor was soil moisture content, which consisted of four levels, i.e., 100, 75, 50, and 25% field capacity. The second factor was the growth stage, which consisted of three phases, i.e., active vegetative, flowering time, and seed-filling period.

Research procedure

The growing media used soil and manure at a ratio of 1:1. The media was prepared and mixed. The soil media was filled in a polybag as a medium for planting the soybean seeds. Planting was done using three seeds per hole with a soil depth of 3 cm. Furthermore, the selection was made at the age of 1 week after planting, and one plant was left in a polybag. NPK Phonska and SP-36 fertilizer were used at a dose of 100 and 75 kg ha-1, respectively, and were given at planting time and age of 5 weeks after planting (WAP).

Plant maintenance was carried out by weeding weeds and controlling pests and diseases. According to the treatment, water application was in the soil moisture content of 100, 75, 50, and 25% field capacity by taking into account the growth phases, namely, the active vegetative, the flowering time, and the seed filling period. Harvesting yield was done at the age of 13 WAP.

How to determine soil moisture content:

Polybags filled with 10 kg of soil were filled with water, and the water was collected until the last drop of 1300 ml (Va). Then the soil was allowed to stand for 24 hours, and the moisture content of the soil was calculated by taking a soil sample from a polybag of as much as 10 g (A). The soil was dried in an oven at 60 0C for 24 hours and weighed (B). Soil moisture content (%) =

Soil moisture content (SM) = 32%

Soil moisture content 100% field capacity = 100% x Va- (Va x SM) = 884 ml.
Soil moisture content 75% field capacity = 75% x Va- (Va x SM) = 663 ml.
Soil moisture content 50% field capacity = 50% x Va- (Va x SM) = 442 ml.
Soil moisture content 25% field capacity = 25% x Va- (Va x SM) = 221 ml

Parameter observed

The parameters observed were the root length, fresh root weight, root dry weight, and root shoot ratio. The observations of data were made at the age of 4, 6, 8, and 10 WAP.

Statistical analysis

Observational data were analyzed using variance analysis (ANOVA) at 5% significant levels. To determine the difference between treatments tested using Duncan's new multiple range tests (DMRT) at 5% significant levels.

Results

Root length

The interaction between soil moisture and growth phase showed significant differences in the root length at 8 and 10 WAP, but 4 and 6 WAP were insignificant. The results of DMRT at the 5% significance level on the root length are shown in Table 1.

Table 1. Effect of soil moisture and growth phase on root length at 8 and 10 WAP (cm)

Soil moisture (% field capacity)	Growth stage	Observation (WAP)	
		8	10
100%	Active vegetative	49.33 a-c	49.57 a-c
	Flowering time	52.33 ab	51.67 ab
	Seed filling period	62.00 a	56.33 a
75%	Active vegetative	47.33 a-c	52.33 ab
	Flowering time	53.33 ab	47.00 b-d
	Seed filling period	40.67 bc	44.00 c-e
50%	Active vegetative	39.33 bc	40.33 de
	Flowering time	43.00 bc	46.00 b-d
	Seed filling period	45.33 bc	50.33 a-c
25%	Active vegetative	43.33 bc	50.00 a-c
	Flowering time	49.67 ab	44.00 c-e
	Seed filling period	34.00 c	38.33 e

Note: The numbers were followed by the same characters in the same column were not significant differences based on DMRT at 5% significant levels.

Table 1 shows that the longest root length was at 100% field capacity during when seed-filling period. The shortest root length occurs when seeds fill at 25% field capacity. There was no difference in the root length of the growth phase, but there were differences in field capacities of 100, 75, and 50%. Whereas at 25% field capacity, the root length in the active vegetative phase was not different from the flowering time, but the root length in the flowering time was different from the seeds filling period.

Fresh root weight

The interaction between soil moisture and growth phase was significantly different on fresh root weight at ages 8 and 10 WAP, but WAP was not significant at ages 4 and 6. The results of DMRT at the 5% significance levels for the average root fresh weight are shown in Table 2.

Table 2. Effect of soil moisture and growth phase on root fresh weight at age of 8 and 10 WAP (g)

Soil moisture (% field capacity)	**Growth stage**	**Observation (WAP)**	
		8	**10**
100%	Active vegetative	6.60 ab	6.58 ab
	Flowering time	6.79 a	6.59 ab
	Seed filling period	7.03 a	7.68 a
75%	Active vegetative	5.03 a	6.31 a-c
	Flowering time	4.23 a-c	4.70 d-f
	Seed filling period	5.33 a-c	5.58 b-d
50%	Active vegetative	2.44 c	3.23 g
	Flowering time	4.43 a-c	4.87 d-f
	Seed filling period	4.17 a-c	5.03 c-e
25%	Active vegetative	3.43 c	4.36 d-g
	Flowering time	3.65 bc	3.99 e-g
	Seed filling period	2.77 c	3.50 fg

Note: The numbers were followed by the same characters in the same column were not significant differences based on DMRT at 5% significant levels.

Table 2 shows that the highest root fresh weight occurs in the combination of 100% field capacity and seed filling period. The lowest value was at 25% field capacity during seed filling. At 10 WAP, the highest root fresh weight was in 100% field capacity at the seed filling period. Still, it was similar to the 100% field capacity in other growth phases and 75% in the active vegetative stage. The lowest root fresh weight occurs at 50% field capacity in the active vegetative phase..

Root dry weight

The interaction between soil moisture and growth phase was not significantly different on root dry weight at ages of 4, 6, and 8 WAP, but significantly different at the age of 10 WAP. The results of DMRT at the 5% significance level for the average root dry weight are shown in Table 3.

Table 3. Effect of soil moisture and growth phase on root dry weight at age of 10 WAP

Soil moisture (% field capacity)	**Growth stage**	**Root dry weight(g)**
100%	Active vegetative	1.773 ab
	Flowering time	1.740 ab
	Seed filling period	1.940 a
75%	Active vegetative	1.367 c-e
	Flowering time	1.587 bc
	Seed filling period	1.453 cd
50%	Active vegetative	1.323 de
	Flowering time	1.237 d-f
	Seed filling period	1.153 ef
25%	Active vegetative	1.100 ef
	Flowering time	1.107 ef
	Seed filling period	0.993 f

Note: The numbers were followed by the same characters in the same column were not significant differences based on DMRT at 5% significant levels.

Table 3 shows a significant interaction between soil moisture content and growth phase on root dry weight at the age of 10 WAP. The highest root dry weight occurred at 100% field capacity at seed filling and did not differ from the active vegetative or flowering time. The lowest root dry weight was at 25% field capacity and did not differ from the active vegetative or flowering time. It was indicated that the lower soil water content caused the less root dry weight.

Root shoot ratio

The interaction between soil moisture and growth phase was not significantly different on root shoot ratio at the age of 4 and 8 WAP, but significantly different at the age of 6 and 10 WAP. The results of DMRT at the 5% significance level for the average root shoot ratio are shown in Table 4.

Table 4. Effect of soil moisture and growth phase on root shoot ratio at age of 6 and 10 WAP

Soil moisture (% field capacity)	**Growth stage**	**Observation (WAP)**	
		6	**10**
100%	Active vegetative	0.145 b	0.234 de
	Flowering time	0.103 b	0.250 cd
	Seed filling period	0.080 b	0.295 a-d
75%	Active vegetative	0.095 b	0.298 a-d
	Flowering time	0.076 b	0.232 de
	Seed filling period	0.075 b	0.274 b-d
50%	Active vegetative	0.104 b	0.164 e
	Flowering time	0.154 b	0.284 a-d
	Seed filling period	0.125 b	0.323 a-c
25%	Active vegetative	0.154 b	0.364 a
	Flowering time	0.346 a	0.346 a
	Seed filling period	0.085 b	0.326 a-c

Note: The numbers were followed by the same characters in the same column indicate no significant difference based on DMRT at 5% significant levels.

Table 4 shows that the highest root-shoot ratio occurs at 25% field capacity in active vegetative and flowering time. The lowest root shoot ratio was 50% field capacity in the active vegetative.

Discussion

Drought stress affected the soybean root, including the root length, fresh weight, root dry weight, root volume, and shoot root ratio. The soybean root is the first organ sensitive to the soil water content decrease. The age of 10 WAP had the same pattern as that of 8 WAP, with the longest root length at 100% field capacity in the seed-filling phase. Root length at 100% field capacity did not differ at different growth phases. At 75% field capacity, the shortest root length was in the seed-filling phase and was significantly different from the active vegetative. At 50% field capacity, the root length differed from the active vegetative period. At 25% field capacity, the shortest root length was at the seed filling period, but not different from the flowering phase.

The less water content available caused, the lower the fresh root weight of the soybean plant. It was due to the disruption of transpiration and photosynthesis processes to damage amino acids, enzymes, and proteins (14). Soil water deficit significantly reduced the character morphology of soybean roots and then affected net photosynthesis. It was mainly due to stomatal limitations (4).

Apart from being affected by growth disturbances, the decrease in fresh root weight was also caused by inadequate turgidity of root cells due to low soil water content. When the soil water content was shallow, the soil water potential decreased, so the roots' water absorption power was also reduced. Water flow occurs when there is a potential difference, which moves to lower the water potential. Plant roots will still retain a lower water potential than the surrounding environment or soil so that water can be absorbed by the roots (15).

When exposed to drought stress, plants develop more root systems (11). Changes in root cells included increasing or decreasing the number and size of roots when facing drought stress. Morphological responses of soybean plants resistant to drought pressure increased the root dry weight, root length, and proline content and decreased the leaves' osmotic potential (16) of plants to absorb water (17). Similar results were revealed by (18), who concluded that water deficiency in green beans' vegetative phase could cause plant roots to become stunted. Meanwhile, soybeans were most susceptible to drought stress during the reproductive stage (4).

Shrinking of soil water content from 80% to 40% field capacity caused a reduction in the dry weight of soybean roots. This shrinkage was caused by plants facing limited root development due to limited soil water amounts (19). (20) have reported that inhibition of root development in plants facing drought stress is caused by increasing this development inhibition because plants cannot fully control their growth.

The root-shoot ratio was the ratio between the roots and the shoot's dry weight (12). The highest shoot ratio occurred at the age of 10 WAP at a field capacity of 25% in the active vegetative phase. The lowest shoot-root percentage occurs at 50% field capacity in the active vegetative phase. Drought conditions were thought to change the allocation of assimilation from photosynthetic organs (leaves) to heterotrophic organs such as roots and seeds, which were useful for increasing survival under adverse environments (9), (21). (10), drought stress significantly reduced the photosynthetic capacity of soybean leaves and harmed the shoot and root tissue.

The root shoot ratio of soybean at the age of 4 WAP did not differ at different moisture levels. Still, at the age of 8 WAP, the root shoot ratio at 25% soil moisture had the highest field capacity and was different with 100, 75, and 50% field capacity. At the age of 4 WAP, there was drought stress, and a decrease in root growth was offset by a reduction in shoot growth so that the root-shoot ratio was almost the same. At the age of 4WAP, it was still in a vegetative growth phase. Whereas at age 8 WAP with severe drought stress, namely 25% field capacity, the reduction in canopy growth was more significant than the decrease in the root growth to increase the root shoot ratio. The ratio of root shoots in the active vegetative phase at the age of 4 WAP was more significant than at the age of 8. On the other hand, the root shoots ratio in the flowering time, and seed-filling phases at 4 WAP was lower than at 8 WAP. It was in line with the results of research by (5), who examined two soybean cultivars.

Conclusion

Based on the results and discussion, it can be concluded that the soil water content below 75% field capacity decreased root length, fresh root weight, root dry weight, and increased shoot root ratio. The seed-filling period was more sensitive to water deficiency than the active vegetative and flowering time. The study findings show that soybean plants can grow well at a soil water content of 100% field capacity. The practical implication of planting soybeans using a soil moisture content of 100% field capacity.

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Author Contributions

Conceptualization,A.F.A.; Validation, A.F.A.; and P.; Writing-Original Draft Preparation, A.F.A.; and P.; Writing-Review&Editing, A.F.A.; and A.P.; Supervision, A.F.A.; and P.; Funding Acquisition, A.F.A. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors state no conflict of interest.

Data availability statement

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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