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Response of Soybean Root Morphology to Drought Stress

Achmad Fatchul Aziez[1*], Paiman[2]

[1]Agrotechnology Department, Agriculture Faculty, Tunas Pembangunan University, Surakarta, Central Java, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Universitas PGRI Yogyakarta, Indonesia
*Corresponding author: achmad.aziez@yahoo.com

Abstract. Roots are plant organs that function to 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 root morphology to drought stress. This research pattern used a completely randomized block design (RBD) with two factors and three replications. The first factor is soil moisture content, which consisted of four levels, namely 100, 75, 50, and 25% field capacity. The second factor is the growth stage,wich consisted of three kinds, namely the vegetative active, flowering time, and seed filling period. The results showed that the soil water content in below 75% field capacity decreased root length, root fresh weight, root dry weight, root volume, and increased shoot root ratio. The seed filling period is more sensitive to water deficiency than the active vegetative and flowering time. This study concluded that soybean plants could grow well at a soil water content of 100% field capacity. The practical implication of planting soybeans should use a soil moisture content of 100% field capacity.

Keywords: Drought stress, root morphology,growth stage, field capacity

Running title: Response of soybean root to drought stress

1 Introduction

Soybean is one of the world's main crops, and it is rich in protein, oil, carbohydrates, and minerals (Bellaloui et al., 2015). Soybean is a meaningful plant that requires a sufficient water supply during its growth process to achieve large yields (Buezo et al., 2019). Lack of water is an environmental stress factor that significantly affects development and plant growth, consequently reducing the quantity and quality of yields (Bellaloui et al., 2015). Most of the soybean crop in Indonesia is in rice fields during of 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, and it was lower than plants that did not experience drought (Liu et al., 2004). Soybean production will decrease when water stress increases

Soybeans are most susceptible to drought stress during the reproductive stage (Chathurika Wijewardana et al., 2019). However, if the plant is subjected to severe long-term water stress during of vegetative growth stage, it may be large enough to cause substantial yield losses. Sacita et al. (2018) added that the drought conditions at the time of flowering caused the flowers and young pods to fall so that the number of pods and seeds was reduced. Conversely, if the seed filling is not fully filled, it will cause the soybean seeds to shrink, causing production to shrink by up to 40% (C Wijewardana et al., 2017). Pejić et al. (2011), in their research, concluded that the seed formation stage is more sensitive to drought than the flowering stage, but the least susceptible stage is 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 specialize in plant architecture. Morphological adaptations, for example, development of special leaf surfaces to reduce transpiration rates, reduce leaf area, sunken stomata or increase root length and density to use water more efficiently(Ramanjulu & Bartels, 2002). The same thing was expressed by Dong et al. (2019) that drought stress inhibits the increase in plant height and leaf area. This inhibition is increasingly evident along with the increase in the level, duration, and frequency of drought stress.

Roots are the essential vegetative organs of plants that support the top of the soil and provide water and dissolved inorganic salts necessary for plant survival. Drought conditions can alter the assimilation allocation from photosynthetic organs to heterotrophic organs (sink)(Xu et al. 2015).Roots are important organs in plants, especially for absorbing water and nutrients in the growing medium. During drought, anatomical and physiological changes can occur in plants, especially in the roots (Kunert et al., 2016). More plants develop root systems in response to nutrient deficiencies and drought (Lynch & Brown, 2012). Root cells change, among others, by increasing or decreasing the number and size in the face of drought stress. The base of soybean plants faces a reduction in stele and xylem diameter dimensions as a plant tolerance mechanism in experiencing drought stress (Makbul et al., 2011).

Limited or unavailability of water will inhibit plant growth by affecting various physiological and biochemical processes. However, relatively little information is available on how drought affects root morphology (Ku et al., 2013).

Previous studies only stated that drought stress reduced the number of pods and the number of soybean seeds. Drought stress in the seed filling phase was more sensitive than the flowering and vegetative phases. There is still little information about the effect of drought and drought level on the growth of soybean root morphology. Therefore, this study wanted to know the response of soybean root morphologyto drought stress.

2 Materials and methods

2.1 Materials

Alvisol soil, polybag, soybean seeds NPK Phonska, SP-36 fertilizers, Binder FED 53–UL Forced Convection , Ohaus PA214 Pioneer Analytical.

2.2 Methods

2.2.1 Study area

The research conducted the research in a plastic house in Demangan, Sambi, Boyolali, Central Java, Indonesia, from August to November 2020 with alfisol soil. The Department of Food Crop Agriculture, Grobogan, Central Java, Indonesia, provided the Grobogan variant of the soybean seeds. 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), and the average rainfall is 139 mm month^{-1} and the average temperature 26-32°C.

2.2.2Experiment design

This study used a completely randomized block design with two factors and was repeated three times. 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 kinds, i.e.,active vegetative, flowering time, and pods filling period.

2.2.3 Research procedure

Planting was carried out using three seeds, then two seeds, selected for 14 days, and the remaining one plant. NPK Phonska and SP36 fertilizer at a dose of 100 and 75 kg/ha, respectively, were given at planting time and 5 WAP. The media used was regosol soil and manure at a dose of 1:1, then the media that had been prepared and mixed was filled in a polybag as a medium and soybean seeds.

In planting with a depth of 3 cm, one polybag planted four soybean seeds. Thinning is done 1 WAP, leaving one plant per polybag. Plant maintenance is carried out by weeding weeds and controlling pests and diseases. According to the treatment, water application is the soil moisture content of 100, 75, 50, and 25% of field capacity by taking into account the growth phases, namely the active vegetative phase, the flowering phase, and the pod filling period. Harvesting was done at 90 DAP.

2.2.4 Parameter observed

The parameters observed were the length of root, fresh weight of root, dry weight of root, and root shoot ratio. Observations were made in 4, 6, 8, and 10 WAP.

2.2.5 Statistical analysis

Statistical was performed using the standard ANOVA SAS 9.1 program. If there is a significant difference between treatments, then proceed with the Duncan new multiple range test (DMRT) at the 5% significance level.

3 Results and discussion

3.1 Root length

The interaction between soil moisture and growth phase showed significant differences in root length at 8 and 10 WAP, but 4 and 6 WAP non significant differences. The longest root length is at 100% soil moisture content of field capacity when filling seeds. The shortest root length occurred at soil moisture content reached 25% of field capacity when filling the seeds. There was no difference in root length at the growth phase, but there were differences in field capacities of 100, 75, and 50%. The results of DMRT at the 5% significance level on the root length are shown in Table1.

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 indicate no significantly different based on DMRT at 5% significant levels.

Whereas at soil moisture content 25% of field capacity, the root length in the active vegetative phase was not different from the flowering phase, but the root length in the flowering phase was different from the seed filling period. The shortest root length is at the moisture content of 25% field capacity in the seed filling period.

3.2Root fresh weight

The interaction between soil moisture and growth phase was significantly different on root fresh weight at 8 and 10 WAP, but 4 and 6 WAP non significant differences. At 8 WAP, the highest root fresh weight was the interaction of 100% soil moisture content during seed filling, and the lowest value was at 25% field capacity during seed filling. The results of DMRT at the 5% significance level for the mean root fresh weight are shown in Table 2.

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

Soil moisture (% field capacity)	Growth stage	Observation (WAP) 8	10
	Active vegetative	6.60 ab	6.58 ab

100%	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 indicate no significantly different based on DMRT at 5% significant levels.

At 10 WAP, the highest root fresh weight was in the soil water content of 100% field capacity during the seed filling period. Still, it did not differ in the soil water content of 100% field capacity in other growth phases and soil moisture content of 75% field capacity in the active vegetative phase. The lowest root fresh weight was at 50% water content of field capacity in the active vegetative phase.

3.3 Root dry weight

The interaction between soil moisture and growth phase was not significantly different on root dry weight at 4, 6 and 8 WAP, but significance different at 10 WAP.The root dry weight at 10 WAP shows an interaction between soil moisture content and the soybean growth phase. The highest root dry weight at soil moisture was 100% of the field capacity at seed filling but did not differ from the active vegetative phase and the flowering phase. The lowest root dry weight at soil moisture was 25% field capacity and did not differ in the active vegetative and flowering phases. It is indicated that the lower the soil water content, the less root dry weight.

The results of DMRT at the 5% significance level for the mean root dry weight are shown in Tables 3 and 4.

Table 3: Effect of soil moisture and growth stage on root dry weight at 6 and 8 WAP (cm)

	Observation (WAP)	
	6	**8**
Soil moisture (% field capacity)		
100	0.59 a	1.00 a
75	0.65 a	0.90 ab
50	0.60 a	0.74 b
25	0.70 a	0.72 b
Growth Stage		
Active vegetatif	36.17 p	30.08 p
Flowering time	42.00 p	31.33 p
Seed filling periode	38.25 p	31.92 p

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

Table 4: Effect of soil moisture and growth phase on root dry weight at 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 indicate no significantly different based on DMRT at 5% significant levels.

3.4 Root shoot ratio

The interaction between soil moisture and growth phase was not significantly different on root dry weight at 4 and 8 WAP, but significance different at 6 and 10 WAP.The root shoot ratio at 6 WAP and 10 WAP shows an interaction between soil water content and the growth phase. The results of DMRT at the 5% significance level for the mean root shoot ratio are shown in Tables 5 and 6.

Table 5: Effect of soil moisture and growth stage on the root shoot ratio at4 and 8 WAP

	Observation (WAP)	
	4	**8**
Soil moisture (% field capacity)		
100	0.291 a	0.299 b
75	0.271 a	0.314 b
50	0.333 a	0.308 b
25	0.340 a	0.528 a
Growth Stage		
Active vegetatif	2.00 p	2.03 p
Flowering time	1.99 p	1.94 p
Seed filling periode	2.42 p	1.83 p

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

Table 6: Effect of soil moisture and growth phase on root shoot ratio at 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 significantly different based on DMRT at 5% significant levels.

At 6 WAP, the highest root/shoot ratio at soil moisture was 25% field capacity at flowering, different from others. The lowest root/shoot ratio was 75% of field capacity in the seed filling period. Still, it was not different from the others except for soil moisture, 25% of field capacity at flowering.

4 Discussion

Whereas the 10 WAP had the same pattern as 8 WAP, the longest root length at soil moisture content 100% field capacity in the seed filling phase. Root length at 100% moisture content did not differ at different growth phases. At a moisture content of 75% field capacity, the shortest root length was in the seed filling phase and was significantly different from the active vegetative phase. At a moisture content of 50% field capacity, root length is longer and different from the active vegetative period. At a moisture content 25% field capacity, the shortest root length at the seed-filling period, but not different from the flowering phase

The less water content available, the lower the root fresh weight of the soybean plant. It is due to disruption of transpiration and photosynthesis processes due to damage to amino acids, enzymes, and proteins that play a role in these processes(Laghari et al. 2016). Soil water deficit significantly reduced the morphological character of soybean roots. At 20% soil moisture, Asgrow cultivar experienced a decrease in root length by 20, 41, and Progeny cultivar 21 and 33% at 18 and 30 DAS, respectively. In 30 DAS, Asgrow soybean cultivar experienced a decrease in root surface area, root diameter, and volume respectively 41, 21, and 38%, while Progeny cultivar 33, 14, and 30% compared to the control treatment (Chathurika Wijewardana et al., 2019).

Apart from being affected by growth disturbances, the decrease in root fresh weight is also caused by inadequate turgidity of root cells due to low soil water content. When the groundwater content is shallow, the groundwater potential will decrease so that the roots' water absorption poweris 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 (Steudle, 2000).

When exposed to drought stress, plants develop more root systems(Lynch & Brown, 2012). Changes in root cells include increasing or decreasing their number and size when facing drought stress. Physiological and morphological responses of soybean plants resistant to drought stress increase root dry weight and root length, increase proline content and decrease the leaves' osmotic potential(Sepanlo et al., 2014) of plants to absorb water (Vasellati et al., 2001). Similar results were revealed by(Komariah et al. 2007), who concluded that water deficiency in green beans' vegetative phase could cause plant roots to become stunted. Meanwhile, soybeans are most susceptible to drought stress during the reproductive stage (Chathurika Wijewardana et al., 2019).

Shrinking of groundwater content from 80% field capacity to 40% field capacity causes a reduction in the dry weight of soybean roots. This shrinkage is caused by plants facing limited root development due to limited groundwater amounts (Nazirah et al., 2018). Basu et al. (2016) have reported inhibition of root development in plants facing drought stress, increasing this development inhibition because plants cannot fully control their growth.

The root shoot ratio is the ratio between the roots and the crown. With drought stress, changes in root cells include increasing or decreasing the number and size when faced with drought. Soybean roots will experience a decrease in stele and xylem diameter as a plant tolerance mechanism in overcoming drought stress (Makbul et al., 2011).

The highest shoot ratio occurred at 10 WAP with a field capacity of 25% in the active vegetative phase. The lowest shoot-root ratio was at 50% field capacity and the active vegetative phase. Drought conditions are thought to change the allocation of assimilation from photosynthetic organs (leaves) to heterotrophic organs such as roots and seeds which are useful for increasing survival under adverse environments (Rich & Watt, 2013; Xu et al., 2015). Kunert et al. (2016), drought stress significantly reduced the photosynthetic capacity of soybean leaves and harmedthe shoot and root tissue.

The rootshoot ratio of soybean at 4 WAP did not differ at different moisture levels, but at 8 WAP, the root/shoot ratio at 25% soil moisture had the highest field capacity and differed from 100, 75, and 50% of soil moisture content.

At 4 WAP, there was drought stress, a decrease in root growth was offset by a decrease in crown growth so that the root/shoot ratio was almost the same. At the age of 4WAP, it was still a vegetative growth phase. Whereas at 8 WAP with severe drought stress, namely 25% soil water content, the reduction in canopy growth was greater than the decrease in root growth to increase the root ratio. The ratio of root shoots in the active vegetative phase at 4 WAP was more significant than 8 WAP. On the other hand, the root shoots ratio in the flowering and seed filling phases at 4 WAP was lower than that of 8 WAP. It is in line with the results of research by Wijewardana et al. (2019), who examined two soybean cultivars

5 Conclusion

Based on the results and discussion, it can be concluded that drought harms soybean root morphology, including length, fresh weight, dry weight, and increased root shoot ratio, especially 8 WAP at the soil moisture content of 25% field capacity. The generative growth stage is more sensitive to water deficiency than vegetative growth. The practical implication is planting soybeans should preferably with the soil moisture content of 100% field capacity

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