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Increasing Soybean (*Glycine max* L) Drought Resistance With *Osmolit Sorbitol*

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Abstract

Efforts to increase soybean production has been pursued for years in Indonesia through the process of intensification and extensification. Increased production through intensification of increasing grain yield per hectare, among others includes the improvement of cultivation system such as the use of cultivars that have superior resistance to drought. Increased soybean production has been through the expansion of planting areas utilizing available idle dry land. However, one of the constraints faced in dryland agriculture was the limited water supply due to low intensity of rainfall that leads to low crop production. In order to ensure that soybeans are cultivated on dry land remains capable of high production, it is necessary to physiologically engineered the soybean with an open stomata. The study was conducted in the greenhouse of *Balai Penelitian Tanaman Serealia* (*BALITSEREAL*) Maros, Sulawesi, Indonesia with a completely randomized block design *h* factorial pattern. The first factor was the water stress stadia while the second was the amount of *sorbitol osmolit* concentration application. Results indicated that there was an interaction between the plant height growth and number of leaves between the water clamping time and concentration of the *osmolit sorbitol*. The vegetative stage especially during flowering and pod formation was inhibited when the water was clamped, but by spraying *osmolit sorbitol, soybean* growth interms of its height and number of leaves was enhanced. This study implies that the application of *osmolit sorbitol* may enhance the drought resistance of soybean growth. Future research suggested that more work should be done on the application of *osmolit sorbital* to other agriculture crops to increase their drought resistance in the drylands.

Keywords: drought, *osmolit sorbitol*, soybean, stomata, engineered physiology

1. Introduction

The main cause of low soybean production in Sulawesi, Indonesia is due to 65% of the soybean is grown in the dryland areas while the balance of 35% of the crop is grown in the cultivated and irrigated rice fields during the dry season. Part of the problems arises in dryland agriculture systems is the limited water supply which is very dependent on the amount and intensity of rainfall that leads to the relatively low crop production. For that reason, soybeans grown and cultivated in dry land must be able to withstand the drought and remain capable of producing high yield soybeans through the physiological engineered and regulated stomatal opening process. It has been well understood that drought stress crops may affect and lead to changes in molecular biochemistry, physiology and morphology of plants. Biochemical changes that occur may include the accumaulation of *osmolit sorbitol* applied to various crops and specific proteins involved in stress tolerance (Shinozaki & Shinozaki, 2007). Soybeans experiencing drought will close their stomata in order to reduce the process of transpiration. However, over time the stomatal closure will result in impaired growth that leads to low crop yield production. In order to prevent the stomatal closure continues over time, it is pertinent to minimize the role of abscisic acid (ABA) in the leaves by using a local compound known as *osmolit sorbitol.* Plants experiencing drought will trigger the abscisic acid (ABA) biosynthesis. The process occurs due to water shortage conditions in the protoplasm where oxidation process takes place continuously (Bray, 1988). Arifin (2001) further supported that the green bean plants usually experience water shortage stress during the vegetative stage and throughout their growth cycle where abscisic acid content (ABA) increased three-folds compared to plants that were not frozen. ABA is a hormone that were synthesized in the roots and translocated to the leaves, and may also be synthesized by the guard cells themselves (Assmann & Shimazaki, 1999). ABA contained in the leaves especially on guard cells normally control the stomatal closure (Turner, 1998). Therefore, the objective of this study was to assess the effects of applying *osmolit sorbitol* in increasing the soybean's resistance to drought through the stomatal physiological engineering.

2. Methods and Materials

Experiments were conducted in the Greenhouse Crops Research Institute for Maize and Cereals (BALITSEREAL) Maros, Sulawesi, Indonesia. The analysis was carried out in the Plant Crop Laboratory, Bogor Agricultural University (IPB), Bogor, Indonesia. The study was conducted in May to December 2012. The experiments used a designed randomized block design (RBD) in the two-factor factorial with three replicates. The time factor (Factor No. 1) was Water clamping (W), which consists of six standards namely, W1 = not gripped over the life of the plant, W2 = gripped throughout life (0-85 days), W3 = gripped from the beginning of growth (0-15 days), W4 = seized during active vegetative stadia (16-30 days), W5 = gripped the flowering-pod filling stadia (at the age 31-65 days), W6 = gripped the stadia seed maturity (age of 66-85 days). Meanwhile, the second factor (Factor No. 2) used a local compound concentration (*osmolit sorbitol (S)*, which consists of four levels, namely S0 = No *osmolit sorbitol* sprayed, S1 = 10 ml/l, S2 = 15 ml/l, and S3 = 20 ml/l of *osmolit sorbitol* sprayed. Of the two factors, a total of 24 combined treatments were obtained and each was repeated three times to obtain 72 units of the experiments. The 24 combined treatments were:

W1S0	W2S0	W3S0	W4S0	W5S0	W6S0
W1S1	W2S1	W3S1	W4S1	W5S1	W6S1
W1S2	W2S2	W3S2	W4S2	W5S2	W6S2
W1S3	W2S3	W3S3	W4S3	W5S2	W6S3

Determination of the amount of water provision (clamping) is based on 50% of the normal water demand and its distribution was based on the normal water requirements for the growth of soybeans in each period. The growth media was prepared with a mixture of soil and manure in the 4:1 ratio, which was filled in a polybag with a diameter of 20 cm and a height of 40 cm with an average weight of 8 kg each. Once the growth media was ready, it is filled up with water until it reached the field capacity limit. The soybean seeds were then planted as many as four points per hole. Once the soybeans reached the age of seven years old, they were then thinned untill two plants per polybag remained. With some phospohorous (P) fertilization and urea at 15 Days After Planting (DAP), the dosage of urea was given at 50 kg/ha (0.66 g urea/polybag) and SP-36 at a dose of 90 kg/ha (1.19 SP-36/polybag) at planting time. According to Fagi and Tangkuman (1995), the average amount of water requirement of soybean (variety *Wilis*) during the growing season was 325 mm/season. Using a water clamp control system, as much as 50% water was injected into the polybag to cater for the soybeans normal water requirement. The water clamping control time applied to the treatments are shown in Table 1.

Table 1. Water clamping time and *osmolit sorbitol* spraying of soybean

Treatment	Implementation of water clamping time at the age (days)															
	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80	85	Frequency
W1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
W2	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	16
W3	X	X	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
W4	0	0	X	X	X	0	0	0	0	0	0	0	0	0	0	0	3
W5	0	0	0	0	0	X	X	X	X	X	X	0	0	0	0	0	6
W6	0	0	0	0	0	0	0	0	0	0	0	X	X	X	X	X	5

Description: 0 = not gripped by water; X: gripped Water

* = Osmolit spraying frequency adjusted with treatment

O*smolit sorbitol* was sprayed to the 10-85 days old soybeans at the lower leaf surface once in every five days with a concentration in accordance to the frequency of spraying treatment of 2-16 times. *Osmolit* s*orbotil* was sprayed between 1000-1200H once in every five days, particularly at 0815-0945H, 1000-1200H and 1200-1400H, despite no significant differences were observed by Ariffin (1997). The distribution and amount of water in each soybean polybags averaged at 94 ml.

3. Results

3.1 Effects of Water Clamping Time and Osmolit Sorbitol on Soybean Growth Parameters

The effects of water clamping time and *osmolit sorbitol* on soybean plant height, number of leaves, leave surface area, root dry weight, leaf area, dry weight of root, relative growth rate, net assimilation rate, chlorophyll content and stomatal density can be shown in Tables 2-10, respectively.

Table 2. Effect of time, water clamping and *osmolit sorbital* on soybean plant height growth

Water clamping time	*Osmolit sorbitol* concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	79.83 bcy	76.00 cz	88.00 bx	99.10 ax	10.64
(W2) gripped 0-85 days	67.60 bx	92.85 axy	82.83 by	95.50 ax	
(W3) gripped 0-15 days	76.67 cy	86.83 byz	86.59 bx	103.67 ax	
(W4) gripped 16-30 days	87.67 ax	88.00 axyz	89.65 ax	89.67 ay	
(W5) gripped 31-65 days	75.83 by	98.33 ax	77.83 by	92.50 ay	
(W6) gripped 66-85 days	78.33 bx	78.67 bz	9500 ax	89.83 ay	
HSD 0.05	10				

*HSD = Honestly significant difference

Table 3. Effect of time, water clamping and *osmolit sorbital* on soybean number of leaves growth

Water clamping time	Osmolit Sorbitol concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	27.00 cy	30.50 bcy	36.50 axy	33.00 abz	5.75
(W2) gripped 0-85 days	28.50 cy	36.00 bx	37.50 abxy	42.50 axy	
(W3) gripped 0-15 days	34.50 bx	35.83 bx	32.50 by	43.17 ax	
(W4) gripped 16-30 days	34.50 ax	33.00 ax	35.50 axy	38.00 axyz	
(W5) gripped 31-65 days	28.50 cy	30.00 bcy	35.00 abxy	39.00 axy	
(W6) gripped 66-85 days	30.00 cxy	35.50 bcx	40.00 ax	37.00 abyz	
HSD 0.05	5.41				

*HSD = Honestly significant difference

Table 4. Effect of time, water clamping and *osmolit sorbital* on soybean leaf area (cm^2)

Water clamping time	Osmolit Sorbitol concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	351.15 bxy	274.95 by	555.97 ax	660.45 axy	201.01
(W2) gripped 0-85 days	227.81 bx	497.45 ax	389.19abx	392.29 abz	
(W3) gripped 0-15 days	312.56 ax	332.73axy	492.21 ax	747.84ax	
(W4) gripped 16-30 days	288.72 xy	284.87 by	286.09 by	557.40 ay	
(W5) gripped 31-65 days	246.69 bx	364.47 bx	508.82abx	666.90 axy	
(W6) gripped 66-85 days	375.47 bx	438.43 by	785.22 ax	491.55 byz	
HSD 0.05	180.34				

*HSD = Honestly significant difference

Table 5. Effect of time, water clamping and *osmolit sorbital* on soybean root dry weight

Water clamping time	Sorbitol Osmolit concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	1103.00 dcz	1490.00cyz	1790.00 by	2185.00 ax	239.95
(W2) gripped 0-85 days	987.00 bz	1070.00 abz	1236.00 az	1263.00 az	
(W3) gripped 0-15 days	1583.00 cy	2178.00 bx	2081.00 bx	2865.00 av	
(W4) gripped 16-30 days	1496.00 by	1923.00 ax	1821.00 ay	1487.00 by	
(W5) gripped 31-65 days	1725.00 bx	1975.00 ax	1185.00 dz	1403.00 cyz	
(W6) gripped 66-85 days	1657.00 cy	1876.00 bxy	2087.00 ax	2204.00 aw	
HSD 0.05	214.74				

*HSD = Honestly significant difference

Table 6. The Effect of time, water clamping and *osmolit sorbital* on soybean on plant dryweight

Water clamping time	Sorbitol Osmolit concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	15.72 by	16.00 by	19.70 aby	24.60 ax	2.95
(W2) gripped 0-85 days	12.23 bz	13.09 byz	16.12 ayz	16.17 az	
(W3) gripped 0-15 days	15.26 by	18.47 ax	17.01 by	22.12 axy	
(W4) gripped 16-30 days	19.29 ax	19.56 ax	18.56 ay	19.87 ay	
(W5) gripped 31-65 days	14.47 by	14.60 bz	18.47 ay	19.60 ay	
(W6) gripped 66-85 days	17.25 bx	19.30 bx	27.27 ax	24.45 ax	
HSD 0.05	2.64				

*HSD = Honestly significant difference

Table 7. Effect of time, water clamping and *osmolit sorbital* on soybean relative growth rate ($mg.mg^{-1}.days$)

Water clamping time	*Osmolit Sorbitol* concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	26.55 bx	25.68 bxy	31.33 bx	48.11 ax	15.31
(W2) gripped 0-85 days	14.29 ay	14.93 ay	20.57 ay	22.52 ay	
(W3) gripped 0-15 days	22.18 axy	22.62 axy	23.76 ay	30.93 ay	
(W4) gripped 16-30 days	12.11 ay	19.04 ay	12.96 ayz	26.55ay	
(W5) gripped 31-65 days	13.45 by	14.78 by	28.36 aby	37.88 ax	
(W6) gripped 66-85 days	29.40 bxy	30.58 bx	40.18 abx	47.82 ax	
HSD 0.05	13.73				

*HSD = Honestly significant difference

Table 8. Effect of time, water clamping and *osmolit sorbital* on soybean net assimilation rate ($mg.cm^{-2}days^{-1}$)

Water clamping time	*Osmolit Sorbitol* concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	0.64 ax	1.35 ax	0.89 az	1.31 ay	0.77
(W2) gripped 0-85 days	0.04 by	0.39 ay	0.39 ax	0.44 ayz	
(W3) gripped 0-15 days	0.70 ax	0.84 ax	0.93 ax	1.14 ayz	
(W4) gripped 16-30 days	0.39 bcx	0.14 cy	0.98 abx	1.20 ay	
(W5) gripped 31-65 days	0.42 bx	0.46 by	0.45 bx	1.88 axy	
(W6) gripped 66-85 days	0.56 bx	0.87 bxy	1.10 bx	2.17 ax	
HSD 0.05	0.69				

*HSD = Honestly significant difference

Table 9. Effect of time, water clamping and *osmolit sorbital* on soybean chlorophyll content (g/g)

Water clamping time	*Osmolit Sorbitol* concentration (ml/l of water)				HSD 0.05
	0 (S0)	10 (S1)	15 (S2)	20 (S3)	
W1 without stress	3.00 cxy	3.54 bx	3.66 bx	4.83 ax	0.43
W2 gripped 0-85 days	2.33 cz	4.36 bw	3.68 cx	3.21 az	
W3 gripped 0-15 days	3.35 awx	3.40 axy	3.67 ax	3.47 ayz	
W4 gripped 16-30 days	2.72 cy	2.94 cyz	3.59 bxy	4.67 ax	
W5 gripped 31-65 days	2.82 ay	3.05 ay	3.06 ay	3.81 ay	
W6 gripped 66-85 days	3.68 cw	2.60 cz	3.19 by	3.60 ayz	
HSD 0.05	0.39				

*HSD = Honestly significant difference

Table 10. Effect of time, water clamping and *osmolit sorbital* on soybean stomatal density

Water clamping time	*Osmolit Sorbitol* concentration (ml/l of water)				HSD 0.05
	0 (S0)	10 (S1)	15 (S2)	20 (S3)	
W1 without stress	42.12 ax	44.15 axy	48.10 ax	52.75 ax	8.06
W2 gripped 0-85 days	22.17 bz	27.96 aby	29.87 abz	32.75 ay	
W3 gripped 0-15 days	27.25 byz	24.15 bz	25.00 bz	38.92 ay	
W4 gripped16-30 days	26.75 az	21.83 az	22.09 az	34.69 ay	
W5 gripped31-65 days	31.17 bxy	33.78 aby	35.78 aby	40.42 ay	
W6gripped 66-85 days	33.85 bxy	48.19 ax	51.00 ax	49.50 ax	
HSD 0.05	7.58				

Description: The average value followed by the letters a, b, c significantly different in each row and x, y, z in each column

3.2 Effects of Water Clamping Time and Osmolit Sorbitol on the Content of Abscisic Acid (ABA) and Number of Pods, Pod Weight and Seed Weight per Plant and Weight of 100 Seeds

ABA is a plant hormone that acts as a natural barrier formed by itself due to the lack of water and environmental stress. Based on the analysis of clamping turning times, there was an effect of water on the ABA content in the leaves of green soybean plants, whereas spraying *osmolit sorbitol* provided no significant interactions as demonstrated in Tables 11-13. Results indicated that the time of water clamping had very significant effects on the weight of 100 seeds while spraying *osmolit sorbitol* had a no significant interaction with water clamping time as shown in Table 13.

Table 11. Effect of water clamping time on ABA content (ug/g)

Water clamping time	Average content of ABA (ug/g)			
	35 DAP	50 DAP	65 DAP	80 DAP
W1 without stress	0.09 b	0.11 b	0.12 bc	0.13 c
W2 gripped 0-85 days	0.25 a	0.36 a	0.50 a	0.62 a
W3 gripped 0-15 days	0.09 b	0.10 b	0.10 c	0.12 c
W4 gripped 16-30 days	0.08 b	0.08 b	0.10 c	0.12 c
W5 gripped 31-65 days	0.21 a	0.34 a	0.48 a	0.10 c
W6 gripped 66-85 days	0.09 b	0.10 b	0.25 b	0.32 b
HSD 0.05	0.05	0.06	0.14	0.12

*(DAP) =Days After Planting, *HSD = Honestly significant difference

Table 12. Effect of water clamping time and osmolit sorbitol on soybean total pods, pod weight and weight seeds

Number of soybean pods					
Water clamping time	*Osmolit sorbitol* concentration (ml/l of water)				HSD 0.05
	S_o (0)	S_1 (10 ml)	S_2 (15 ml)	S_3 (20 ml)	
(W1) without stress	21.17 bxy	23.32 bwx	24.50 by	37.50 ax	3.97
(W2) gripped 0-85 days	15.50 bz	17.17 abz	20.84 ay	21.83 az	
(W3) gripped 0-15 days	22.17 bxy	23.49 abx	22.50 by	26.50 ay	
(W4) gripped 16-30 days	19.34 ayz	21.16 axy	21.67 ay	26.52 ay	
(W5) gripped 31-65 days	19.00 ayz	18.51 abyz	14.83 bz	18.00 abz	
(W6) gripped 66-85 days	24.33 cx	26.17 cw	31.50 ax	28.82 ay	
HSD 0,05	3.74				
Observation Pods Weight (g) Soybean Plants					
(W1) without stress	5.64 bxy	7.82 ax	7.78 ax	11.28 ax	2.25
(W2) gripped 0-85 days	3.95 by	4.15 bz	4.85 abyz	5.21 az	
(W3) gripped 0-15 days	6.70 ax	7.05 ax	7.00 axy	6.98 ayz	
(W4) gripped 16-30 days	5.05 axy	6.68 axy	5.74 axyz	6.58 ayz	
(W5) gripped 31-65 days	4.92 axy	4.60 ayz	4.46 az	6.66 ay	
(W6) gripped 66-85 days	6.79 bx	7.20 bx	7.55 bx	8.26 ay	
HSD 0,05	2.12				
Observations Weight Seeds / Crops (g) Soybean					
(W1) without stress	3.84 bxy	5.15 bx	5.45 bxy	8.19 ax	1.99
(W2) gripped 0-85 days	2.28 ay	2.70 ay	2.85 az	3.87 ayz	
(W3) gripped 0-15 days	4.77 ax	5.11 ax	5.00 axy	6.65 ax	
(W4) gripped 16-30 days	3.30 axy	4.60 axy	3.72 ay	4.53 ay	
(W5) gripped 31-65 days	3.45 axy	3.16 axy	3.22 ay	4.23 ay	
(W6) gripped 66-85 days	4.74 ax	4.82 ax	5.21 axy	5.80 ay	
HSD 0.05	1.87				

*HSD = Honestly significant difference

Table 13. The effects of clamping time and *osmolit sorbitol* on the weight of 100 seeds

Water Clamping Time	Concentration of *osmolit sorbitol* (ml/l of water)				Average	HSD 0.05
	0 (S0)	10 (S1)	15 (S2)	20 (S3)		
(W1) without stress	10.35	10.61	10.45	9.81	10.31 a	1.37
(W2) gripped 0-85 days	8.83	9.14	8.93	9.3	9.05 b	
(W3) gripped 0-15 days	9.28	11.26	11.98	11.78	11.07 a	
(W4) gripped 16-30 days	10.66	10.6	9.94	9.21	10.10 a	
(W5) gripped 31-65 days	10.26	9.49	9.72	9.19	9.67 b	
(W6) gripped 66-85 days	10.63	9.93	8.65	10.8	10.00 a	

Description: Average value, followed by the letters a, b, c significantly different by HSD test at level α = 0.05

4. Discussion

The results of the study clearly showed that the growth of soybean was significantly affected by the availability of water. This is because water is deemed necessary for the metabolic activities of the plant for a paired cell growth and development. Lack of water during the vegetative stadia and flowering until pod formation showed the lowest period of growth. When compared with normal plants that obtain sufficient water, lack of water at each stadia results in a decrease of crop growth. Aminah (2007) found that soybean with a water stress at 150 mm/season (below the normal requirement) showed highly significant growth differences with soybean getting water at 300 mm/season. The most sensitive and critical growth phase to drought is during the vegetative stadia, flowering and pod formation, as evidenced by the low growth measurements of their vegetative organs. Simillar results were obtained by Boyer (1996) where he observed that the growth of soybean pod formation wash and water shortages could affect the later low seed yield obtained. Aminah (2011) revealed that a strong osmotic regulation can improve the adaptability of plants during drought where the water supply is limited. Apparently, spraying *osmolit sorbitol* in soybeans when experiencing water shortages can increase its growth resistance to drought. This has been proven with water clamping time and spraying with *osmolit sorbitol* effects both in terms of plant height (for all observation time) and number of leaves (50 observations and 65 DAP).

In the process of photosynthesis, stomatal opening is an important part of the plant where the process of gas or water vapor diffusion into and out of plant tissue occurs. The process of opening and closing of stomata is one of the obstacles that occur in the diffusion process. The process of opening and closing of stomata is usually affected by stomatal guard cell turgor where it closes under the conditions of water shortage (Turner, 1998).

The results of the analysis shows that spraying *osmolit sorbitol* to soybeans under stress experiencing water shortages can increase its resistance to water shortages in terms of plant height and total number of leaves. *Osmolit sorbitol* can also control the cell's humidity where the cell turgor can be maintained with sufficient water for higher yields.

5. Conclusion

Soybean growth (W4) especially its plant height, leaf number, leaf area, root dry weight, content chlorophyll, and variable production is heavily reliable and sensitive to the availability of water. An *osmolit sorbitol* concentration of 20 ml/l sprayed on the soybeans increased its water drought resistance by controlling its physiological stomatal activity at various stadia growth and soybean yield. This study implies that the local compound *osmolit sorbitol* can enhance the soybean growth in drought resistance dryland areas.

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