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EFFECTS OF DIFFERENT SOIL WATER CONTENT ON BIOLOGICAL NITROGEN FIXATION AT SOYBEAN

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Summary

Biological nitrogen fixation (BNF) is the process that provides organic nitrogenous compounds to the plants by using molecular nitrogen in atmosphere. Higher plants are not capable to use molecular nitrogen in atmosphere as a nitrogen source to generate essential proteins. Therefore plants either should be fertilized by adequate nitrogenous fertilizers or the microorganisms which are capable to produce nitrogenase should provide nitrogen to the plants by BNF. From among a number of factors affecting BNF, soil moisture content and ambient temperatures are considerably effective on the fixation rate. Therefore the global warming would be dramatically defective on BNF, thus effects of soil moisture as well as soil and ambient temperatures on BNF should evaluate prior rising temperature. A pot experiment was carried out to determine the effects of soil water contents on BNF. Four different soil water contents (%25, %50, %75 and %100 of water holding capacity) were adjusted either every 3 days or just after plants indicate wilting point. Non-inoculated pots were added to experiment as a control. The results revealed that BNF is affected by different level of soil water content. The mechanism of this effect would not be the direct effect of water, but the side effect of water on soil oxygen content; therefore, an aeration capability.

Key words: soil water content, biological nitrogen fixation, soybean

INTRODUCTION

Soybean is the most widespread crop and, similarly to the other crops, soybean productivity is limited by water stress [Araus et al., 2002]. Plants have a number of strategies to overcome stress factors such as increasing enzyme activity; e.g. Zakikhani et al. [2012] reported that the antioxidant enzyme activity was significantly increased by decreasing available water in soils. Nevertheless, water stress is a major factor limiting soybean production in the semi-arid and subhumid regions in the world, and seed yield declined statistically with increasing water deficit [Candogan et al., 2013]. Waters stress affects stomatal conductance, thus under well-watered conditions plants consume less water comparing to water-stressed conditions [Jaude et al., 2008]. Elevated CO_2 also have same effects on stomatal conductance, therefore, carbon dioxide reduces stomatal conductance in leaves, thence whole plant or canopy transpiration is reduced, resulting in delayed soil drying [Bunce and Nasyrov, 2012]. As another stress factor, soybean shoot, root and nodule biomass show decrease under salt stress conditions. Nitrogen fixation also decreases as a matter of salt stress; however it is more sensitive than other growth parameters [Salah et al., 2009].

Most proportion of the above mentioned literatures and mainly all others did not deal with the interactions between bacteria, soybean and water stress on biological nitrogen fixation. However, nitrogen fixation activity of soybean nodules is especially sensitive to the soil dehydration [Sall and Sinclair, 1991]. Due to the global warming announced frequently in the recent years, authors are expecting to meet water stressed condition resulting from elevated carbon dioxide. Therefore, a pot experiment was carried out to determine the influence of dual irrigation program as well as water deficiencies on biological nitrogen fixation on soybean.

MATERIAL AND METHODS

The study was carried out at greenhouse conditions at the temperature 18-20°C at night and 24-26°C at daytime in Isparta, Turkey. The experimental soil was clayey loam having pH 7.6 (1:2.5 soil to water ratio), 27% $CaCO_3$ [Caglar, 1949], 1.18% organic matter [Jackson, 1962], 81 $kg \cdot ha^{-1}$ 0.5 M $NaHCO_3$ extractable P [Olsen's method, Olsen et al., 1954], 0.03% total nitrogen [Bremner, 1965], 46 $kg \cdot ha^{-1}$ mineral nitrogen [Deutsche Einheitsverfahren, 1983; Fabig et al., 1978], and without salinization problem.

The experiment was set up in a completely randomized design with 3 replications. Factors were 2 irrigation time intervals, 4 different moisture contents and with or without *Bradyrhizobium japonicum* 110 inoculations, therefore the experiment was consisted of 48 pots. Four Sa88 soybean seeds placed to every pots, after germination 2 healthy plants selected and left-overs removed.

Soil sieved from 4 mm mesh and 3800 g soil placed to pots without any fertilization. Seeds were sown prior to soil moisture content was adjusted once at 100% for all pots. After one week, water content was adjusted to 25%, 50%, 75 and 100% of water holding capacity (WHC) according to experimental design. Half of the pots was irrigated every 3 days (constant period; CP) to maintain their water contents according to experimental design. The rest of the pots were monitored to determine their water contents and when they reach nearby the wilting point (NWP), their water content was adjusted to the desired value. A tap water was used for irrigation.

Due to the highest nitrogen fixation realized at the end of flowering stage, when flowering was completed, just before capsule formation, plants were harvested, root and shoot samples were collected. Nodules on root samples were separated. Samples were air dried, afterwards they were stored in 65°C until they reached constant weights. Afterwards biomass weight was determined using analytical balance and the plant materials were grinded to prepare analyses. All samples were analyzed to determine their total nitrogen contents by Kjeldahl method [Bremner, 1965].

The results were statistically analyzed using Mstatc program [Crop and Soil Sciences Department, Michigan State University, Version 1.2]. Differences between the means were separated by Duncan's Multiple Range Test at p=0.05 level.

RESULTS AND DISCUSSION

Analyses of variance generally demonstrated significant differences between factors tested herein. In some parameter, although the values seem to be considerably different from other, statistical analyses indicate insignificant relation at p=0.05 level due to high variation among replicates. Higher variation may be explained by the uniform water distribution in the pot related to soil physical properties.

Nodules

As *Bradyrhizobium spp.* was not introduced previously to the soils in Isparta region [Coskan et al., 2009], there was not any nodule formation in the non-inoculated variants. Thus, non-inoculated variants are excluded from the nodule-related tables. The highest nodule number (Table 1) and nodule weight (Table 2) was determined in the variant with the moisture content adjusted to 75%, when it reaches nearby to wilting point (NWP). Contrary to these findings, the lower nitrogen content was observed in same variant with 75% adjustment at NWP (Table 3). Not surprisingly, the lowest values for all three above mentioned parameters were observed at the variant with adjusted 25% water holding capacity at constant irrigation period (CP). Nodule number was lower in constant period irrigation in all nodule related findings, however, only the nodule number is statistically important. Considering mean values about total nitrogen amount of nodules, the highest values were in 75% which was statistically significant. Although there were slight differences between NWP and CP applications, these differences was not significant.

Table 1. Number of nodules per plant

	% of water holding capacity								Average	
	25		50		75		100			
Near wilting point	11.5	abc	12.3	ab	13.7	a	10.7	abc	**12.0**	**A**
Constant period	6.5	c	7.8	bc	11.7	abc	10.8	abc	**9.2**	**B**
Average	***9.0***	***ABC***	***10.1***	***A***	***12.7***	***A***	***10.8***	***A***		

Biomass weight

Root, shoot and total plant weight (including nodule weight) values are given in Tables 5, 6 and 7, respectively. According to mean values neither irrigation intervals (NWP or CP) nor bacteria inoculation factors were significantly effective on root weight values (Table 5). Among the water contents, only the 25% application showed lower values whereas no significant differences were observed on the other water contents applications. The highest and the lowest root weight values were determined for the CP & +bacteria applications at the 100% and 25% water content respectively. Root weights are much lower than the shoot weights (Table 6) therefore shoot weights is determinative on total biomass of the plants.

Table 2. Nodule weights

	% of water holding capacity								Average	
	25		50		75		100			
Near wilting point	15.8	bc	16.4	abc	25.8	a	20.2	abc	**19.5**	**A**
Constant period	11.2	c	14.6	bc	21.2	ab	20.6	abc	**16.9**	**A**
Average	***13.5***	***C***	***15.5***	***BC***	***23.5***	***A***	***20.4***	***AB***		

Table 3. Nodule nitrogen contents (% N) [*]

	% of water holding capacity				Average
	25	50	75	100	
Near wilting point	5.76	6.30	4.80	4.92	**5.44**
Constant period	5.25	5.94	6.54	3.87	**5.40**
Average	***5.50***	***6.12***	***5.67***	***4.39***	

[*] Statistical analyses were not realized due to the insufficient sample size

Table 4. Nodule nitrogen amounts (mg of nodule N)

	% of water holding capacity								Average	
	25		50		75		100			
Near wilting point	0.89	ab	1.01	ab	1.22	a	0.99	ab	**1.03**	**A**
Constant period	0.59	b	0.87	ab	1.29	a	0.77	ab	**0.88**	**A**
Average	***0.74***	***B***	***0.94***	***AB***	***1.25***	***A***	***0.88***	***AB***		

Shoot weight values (Table 6) clearly indicate that re-wetting soil just before reaching the wilting point results in higher shoot biomass value comparing to CP. However, bacteria inoculation is the most effective factor on biomass, as both in the NWP and CP applications bacteria increased shoot weight considerably. According to mean values, more than providing water higher than 50% of water holding capacity is adequate to achieve the highest shoot weight. Although the shoot weight at 100% of WHC is higher than the values observed at 75% of WHC, that difference is not significant.

Table 5. Root weight (mg)

		% of water holding capacity								**Average**	
		25		50		75		100			
Near wilting point	- bacteria	134	cde	195	a-d	167	b-e	198	a-d	174	*A*
	+ bacteria	149	b-e	188	a-e	208	abc	183	a-e	182	*A*
	Average	**142**	**BC**	**191**	**AB**	**188**	**AB**	**191**	**AB**	***178***	***A***
Constant period	- bacteria	121	de	167	b-e	217	ab	247	a	188	*A*
	+ bacteria	111	e	140	b-e	209	abc	187	a-e	162	*A*
	Average	**116**	**C**	**154**	**BC**	**213**	**A**	**217**	**A**	***175***	***A***
Mean		***129***	***B***	***172***	***A***	***200***	***A***	***204***	***A***		

Table 6. Shoot weight (mg)

		% of water holding capacity								**Average**	
		25		50		75		100			
Near wilting point	- bacteria	392	ef	449	def	568	cde	532	de	*485*	*C*
	+ bacteria	738	bc	756	bc	964	a	809	ab	*817*	*A*
	Average	**565**	**CD**	**602**	**BCD**	**766**	**A**	**671**	**ABC**	***651***	***A***
Constant period	- bacteria	310	f	416	def	589	cde	745	bc	*515*	*C*
	+ bacteria	538	de	600	cd	832	ab	818	ab	*697*	*B*
	Average	**424**	**E**	**508**	**DE**	**710**	**AB**	**782**	**A**	***606***	***A***
Mean		***495***	***B***	***555***	***B***	***738***	***A***	***726***	***A***		

Whole plant weights showed values parallel to shoot weights (Table 7). Bacteria inoculation increased biomass weight, however enhancement of biomass was much higher in the NWP. This result is closely related to aeration of soil which has both enough water and well aeration in the NWP irrigation sequence.

Biomass weight increase on bacteria inoculated variants is directly related to biological nitrogen fixation. As is well known the nitrogen is the key component for biomass production, therefore the more the bacteria-plant interaction develops, the more nitrogen is provided to the plant.

Table 7. Biomass weight (including nodules; mg)

		% of water holding capacity								Average	
		25		50		75		100			
Near wilting point	- bacteria	527	gh	643	f-h	735	d-g	730	d-g	*659*	*C*
	+ bacteria	903	b-e	960	a-d	1199	a	1013	ab	*1018*	*A*
	Average	**715**	**CD**	**802**	**BCD**	**967**	**AB**	**871**	**ABC**	***839***	***A***
Constant period	- bacteria	431	h	583	f-h	806	b-f	992	abc	*703*	*C*
	+ bacteria	661	e-h	755	c-g	1062	ab	1026	ab	*876*	*B*
	Average	**546**	**E**	**669**	**DE**	**934**	**AB**	**1009**	**A**	***790***	***A***
Mean		***630***	***B***	***735***	***B***	***950***	***A***	***940***	***A***		

Nitrogen contents

Nitrogen contents of root and shoot, and total nitrogen amount of biomass are presented in Tables 8, 9 and 10, respectively. Due to the fact that the plants are harvested at the end of flowering stage, both root and shoot have higher nitrogen contents. Nitrogen contents were varying within wide range between 1.14% and 2.34%. Contrary to the other parameters determined, the highest value was observed at the bacteria inoculated, CP and 25% variant. Similarly the highest shoot value (Table 9) also was not observed in the higher water applied plots, instead, the bacteria inoculated, CP and 50% variant showed the highest value. That does not mean that the mentioned applications are the best combination, because the biomass values are not in accordance with these values. In other word, due to the biomass production diminished depending on limited water, fixed nitrogen is concentrated in the biomass. In this case, total plant nitrogen amount values (Table 10) would be much more reliable than nitrogen concentration. According to the total nitrogen amount values the best option among the factors seems to be the bacteria inoculated and 75% WHC applied combinations. Decrement of the total nitrogen amount at lower water applied variants can be explained by side effects of water stress on plants. Water stress decreases the effectiveness of inoculants leading to decrease in nitrogen fixation [Sall and Sinclair, 1991]. On the other hand, the highest water application (100% of WHC) would reduce the oxygen contents of the soil where aeration is the important factor for aerobic microorganisms in soil.

Benefit of inoculation, based on total nitrogen amount of plant

The values obtained by subtraction of total nitrogen contents in non-inoculated variant from inoculated ones are presented in Table 11. Those values would be the best indicator to determine the effect of bacterial inoculations on nitrogen fixation. According to the mean values, the 75% WHC application showed the highest value. There was no statistical difference between the NWP and CP applications although the NWC application was slightly higher.

Table 8. Root nitrogen contents (%)

		% of water holding capacity								Average	
		25		50		75		100			
Near wilting point	- bacteria	1.55	bc	1.14	c	1.76	abc	1.54	bc	*1.50*	*B*
	+ bacteria	1.88	ab	1.87	ab	2.10	ab	2.00	ab	*1.96*	*A*
	Average	**1.72**	**AB**	**1.51**	**B**	**1.93**	**AB**	**1.77**	**AB**	***1.73***	***A***
Constant period	- bacteria	1.95	ab	1.49	bc	1.51	bc	1.80	abc	*1.68*	*AB*
	+ bacteria	2.34	a	1.74	abc	1.95	ab	2.00	ab	*2.01*	*A*
	Average	**2.14**	**A**	**1.61**	**B**	**1.73**	**AB**	**1.90**	**AB**	***1.85***	***A***
Mean		***1.93***	***A***	***1.56***	***B***	***1.83***	***AB***	***1.83***	***AB***		

Table 9. Shoot nitrogen contents (%)

		% of water holding capacity								Average	
		25		50		75		100			
Near wilting point	- bacteria	1.95	bc	1.76	bc	1.75	bc	2.27	abc	*1.93*	*B*
	+ bacteria	2.56	ab	2.61	ab	2.31	abc	1.94	bc	*2.35*	*AB*
	Average	**2.26**	**AB**	**2.18**	**AB**	**2.03**	**B**	**2.11**	**AB**	***2.14***	***A***
Constant period	- bacteria	2.00	bc	2.33	abc	1.89	bc	1.56	c	*1.94*	*B*
	+ bacteria	2.35	abc	3.22	a	2.70	ab	2.53	abc	*2.70*	*A*
	Average	**2.18**	**AB**	**2.77**	**A**	**2.29**	**AB**	**2.04**	**B**	***2.32***	***A***
Mean		***2.22***	***A***	***2.48***	***A***	***2.16***	***A***	***2.08***	***A***		

Table 10. Total nitrogen amount of whole plant (mg N)

		% of water holding capacity								Average	
		25		50		75		100			
Near wilting point	- bacteria	9.6	fg	10.2	efg	12.9	efg	14.7	ef	*11.8*	*B*
	+ bacteria	22.6	abc	24.2	abc	27.1	ab	20.4	cd	*23.6*	*A*
	Average	**16.1**	**B**	**17.2**	**AB**	**20.0**	**AB**	**17.6**	**AB**	*17.7*	*A*
Constant period	- bacteria	8.2	g	12.3	efg	13.9	efg	15.6	de	*12.5*	*B*
	+ bacteria	15.5	de	22.0	bc	27.9	a	25.1	abc	*22.6*	*A*
	Average	**11.9**	**C**	**17.2**	**AB**	**20.9**	**A**	**20.3**	**A**	*17.6*	*A*
Mean		***14.0***	***C***	***17.2***	***B***	***20.4***	***A***	***18.9***	***AB***		

Table 11. Benefit of inoculation based of nitrogen amount

	% of water holding capacity								Average	
	25		50		75		100			
Near wilting point	13.0	a	14.0	a	14.3	a	5.8	a	**11.8**	**A**
Constant period	7.3	a	9.7	a	14.0	a	9.5	a	**10.1**	**A**
Average	***10.1***	***AB***	***11.9***	***AB***	***14.1***	***A***	***7.6***	***B***		

Water consumptions

The water amount given to the pots to adjust water content according to the experimental design was recorded for individual pot. Total given water amount is presented in Table 12.

Table 12. Total given water amount (mL)

		% of water holding capacity				Average
		25	50	75	100	
Near wilting point	- bacteria	2237	2140	2217	2437	2258
	+ bacteria	2713	2213	2257	2483	2417
	Average	**2475**	**2177**	**2237**	**2460**	**2337**
Constant period	- bacteria	1497	1773	1823	2197	1823
	+ bacteria	1727	1927	1933	2407	1999
	Average	**1612**	**1850**	**1878**	**2302**	**1911**
Mean		***2044***	***2013***	***2058***	***2381***	

Water consumption in the NWC application seems to be lower comparing to CP, however biomass weights were also lower in CP. Therefore water consumption was related to the total plant biomass development. Similarly, rhizobium inoculation increased water consumption as related to developing more biomass. In the NWC application the 50% and 75% variant results indicate lower water consumption than 25% and 100%. However, the 75% variant results are the highest total biomass weight from among mean NWC values. Therefore, it can be said that adjustment of the 75% WHC when the soils reach nearby, caused lower water consumption to produce the highest biomass weight. Total nitrogen amount values are in accordance with this finding. Moreover, the results indicate that less water than 100% corresponds to high water-use efficiency until the 50% adjustment in NWC. On the other hand, in the CP application, the more water provided, the more plant consumed. However, in general scope of view, plant used less water when the moisture in soils sustained constant.

CONCLUSION

This study has demonstrated that the possible water stress that we may meet in the future will considerably affect the biological nitrogen fixation. Providing low water amount to the soil results in lower biomass development and, the worse, lower biological nitrogen fixation. Irrigation at the time when the soils reach nearby the wilting point stimulate water usage, however it provides more biomass development which was not statistically important according to our findings. In overall evaluation based on biological nitrogen fixation and biomass values, the best irrigation combination seems to be adjusting the 75% WHC in certain period. Additionally, adaptation of more resistant bacteria and soybean genotypes in advance would be beneficial for future.

REFERENCES

Araus, J.L., Slafer, G.A., Reynolds, M.P., Royo, C. *Plant breeding and drought in C3 cereals: what should we breed for?* Annals of Botany, 89, 2002, 925:940.

Bremner, J.M. *Inorganic forms of nitrogen*. In C.A. Black et al. (ed.) Methods of soil analysis. Part 1. Agron. Monogr. 9. ASA, Madison, WI., 1965, 93-149.

Bunce, J. and Nasyrow, M. *A new method of applying a controlled soil water stress, and its effect on the growth of cotton and soybean seedlings at ambient and elevated carbon dioxide.* Environmental and Experimental Botany. 77, 2012, 165-169.

Caglar, K.O. *Toprak Bilgisi*. Ankara Universitesi. Ziraat Fakultesi Yayinlari, Yayin No: 10, 1949, 230, pp. (In Turkish)

Candogan, B.N., Sincik, M., Buyukcangaz, H., Demirtas, C., Goksoy, A.T.G., Yazgan, S. *Yield, quality and crop water stress index relationships for deficit-irrigated soybean [Glycine Max (L.) Merr.] in sub-humid climatic conditions.* Agricultural Water Management 118, 2013, 113-121.

Coskan, A., Isler, E., Kucukyumuk, Z., Erdal, I. *Isparta kosullarında soyada bakteri asılamasının nodulasyona ve dane verimine etkisi.* Journal of the Faculty of Agriculture. University of Suleyman Demirel. ISSN 1304 9984. 4(2), 2009, 17-27. (In Turkish)

Deutsche Einheitsverfahren Zur Wasser-Abwasser Und Schlammuntersuchungen. *Fachgruppe Wasserchemie in der Gesellschaft Deutscher Chemiker (ed.) Verlag Chemie, Weinheim / Bergstrasse (BRD).* 1983,

Fabig, W., Ottow, J.C.G., Muller, F. *Mineralization von ^{14}C-Markiertem Benzoat mit Nitrat als Wasserstoff-Akseptor Unter Vollstaendig anaeroben Bedingungen sowie bei Verminderten Saurstoffpartialdruck*. Landwitsch. Forsch. 35, 1978, 441-453.

Jackson, M. L. *Soil chemical analysis*, Prentice-Hall, Inc., Englewood Cliffs, N.S., 1962, 151-185.

Jaude, B.J., Katerji, N., Mastrorilli, M., Rana, G. *Analyses of the effect of ozone on soybean in the Mediterranean region I. The consequences on crop-water status.* European Journal of Agronomy 28, 2008, 508-518.

Olsen, C.R., Cole, C.V., Watanabe, E.S., Dean, L.A. *Estimation of Available Phosphorus in Soils by Extraction with Sodium Bicarbonate*. USDA Circ. 939, U.S. Government Printing Office, Washington DC., 1954, 1-19.

Salah, I.B., Albacete, A., Andujar, C.M., Haouala, R., Labidi, N., Zribi, F., Martinez, V., Perez-Alfocea, F., Abdelly, C. *Response of Nitrogen fixation in relation to nodule carbohydrate metabolism in Medicago ciliaris lines subjected to salt stress.* Journal of Plant Physiology. 166, 2009, 477-488.

Sall, K., Sinclair, T.R. *Soybean genotypic differences in sensitivity of symbiotic nitrogen fixation to soil dehydration*. Plant Soil, 133, 1991, 31-37.

Zakikhani, H., Ardakani, M.R., Rejali, F., Gholamhoseini, M., Joghan, A.K., Dolatabadian, A. *Influence of diazotrophic bacteria on antioxidant enzymes and some biochemical characteristic of soybean subjected to water stress.* Journal of Integrative Agriculture. 11, 2012, 1828-1835.

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