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A Preliminary Study of USDA 110 (*Bradyrhizobium diazoefficiens*) Strain Nodulation Performance and Soybean Growth Under Water Scarcity Conditions

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ABSTRACT

Nitrogen fixation is one of the key benefits of the economic and environmentally sustainable approach that legumes contribute to crop production. With the fruitful cooperation of legume-rhizobia symbiosis, soybean cultivation contributes to this sustainability while drought threatens this sustainable agricultural system. Thus, this study aimed to verify the influence of water deficit on the soybean nodulating performance concerning different inoculants, crop growth and quality. A field experiment was conducted to determine the effects of irrigated and water scarcity conditions (full: WHC 100% and deficit: WHC 50%) on soybean yield and quality and also to test the nodulation performance of two different inoculants USDA 110 (*Bradyrhizobium diazoefficiens*) and Azotek (*Rhizobium* spp.) applied to 3 different soybean cultivars (Umut-2002, Cinsoy and Altınay). For this purpose, plant height (cm), first pod height (cm), number of pods per plant, 1000 seed weight (g), seed yield (kg ha^{-1}), SPAD chlorophyll content, leaf area (cm^2), crude protein and oil content (%) traits were measured. According to the field and root observations, no nodulation history was observed in both Rhizobia strains under irrigated and water scarcity conditions. Water limitation resulted with the negative impact on soybean yield (≈35% less) and yield formation. In addition to yield reduction, water scarcity caused a significant decrease in SPAD chlorophyll content in the reproductive stages and leaf area of the plant. As a result of this preliminary study, water scarcity has irreversible effects on soybean plant physiology and yield formation in the hot climate conditions of Aydın province. Further field studies are needed to observe the nodulation performance of soybean plants in the region which has not been observed in the field studies so far.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is one of the prominent crops that ensures an important source of protein for nourishment and livestock. It enriches the chemical and biological structure of the soil through its deep root system and fixes atmospheric N by biological nitrogen fixation. It is widely used as an industrial raw material around the world due to its wide adaptation and high nutrition values (Yüzbaşı, 2021). Soybean exemplifies the most significant and cultivated food legume in crop rotation. It comes to the forefront of fixing atmospheric nitrogen into the

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soil by *Bradyrhizobium japonicum* strain leads to effective and higher usage of nitrogen in sustainable agriculture with minimal input requirements (Islam et al. 2022). Also, it has the potential as a forage crop and there is an increasing trend in adopting soybean silage for animal feeding in the last years (Sürmen and Kara, 2017).

Climate change affects crop productivity through extreme weather conditions (etc. high temperature, drought and irregular rainfall). Climate change will affect crop development timing, and the exact changes will depend on variations in agronomic properties that cause yield and quality reductions in soybean response to extreme weather conditions. According to the future precipitation climate projection based on the HadGEM2-ES (Global Circulation Model) during the period (2016-2040) there would be an increase of about 10-40% in precipitation during the winter period in the coastal regions of Aegean, Central Black Sea, and East Anatolia regions it is expected to decrease about 20% in the spring precipitation amount in a large part of Türkiye. In terms of precipitation, it is predicted that the amount of precipitation tends to increase in the winter season in both RCP4.5 and RCP8.5 scenarios, will not be in the form of heavy snow and precipitation, and thus will not contribute to the water budget of the summer season (Demircan et al. 2017).

A challenging area in the field of soybean crop development is that it is produced in many arid, semi-arid and sub-humid regions where water resources are limited, and spring precipitation has preliminary importance for water storage of the soil. Although soybean production areas have increased, restricted agro-climatic conditions are not ideal for widespread soybean cultivation, especially in Europe. Long periods of high temperatures and extremely heat in semi-arid regions reveal the scarcity of water that is more common in Mediterranean climate conditions. (Koca et al. 2015). Soybean can cope with drought conditions that occur in the early vegetative stages without considerable yield reduction, but irrigation is vital for soybean plants in the reproductive stages (beginning of flowering to pod development) and until maturity. These reproductive stages are mainly observed during the summer period when stress conditions (high temperature and water deficiency) have significant reverse impacts on the crop development of soybean (Matoša Kočar et al. 2023).

Stress factors not only reduce agricultural productivity but also limit and prevent land use for agricultural purposes. Abiotic stress conditions such as light, temperature, water (drought) salt, and heavy metal stress cause physiological and metabolic changes in soybean, negatively affecting plant growth and development as well as result in quality reductions (Korkmaz & Durmaz, 2017). Drought stress can be very effective, especially during flowering, pod formation, and seed-filling periods. The stress experienced during the grain filling period causes a decrease in grain size and low yield. Decreases in grain size are attributed to shorter seed-filling periods (Brevedan and Egli, 2003). It has been stated that high temperature and drought stress also significantly reduce plant growth and development and thus grain yield, particularly the number of pods in the plant, which is one of the main yield components (Hu & Wiatrale, 2012). Water scarcity particularly occurs during the generative phases (the flowering and pod development stages) and is the most significant stress factor affecting seed yield because it reduces the flowering rate and, as a result, formation of the number of pods in the plant (He et al. 2017). Water stress adversely reduces the synthesis and breakdown of metabolites that contribute to yield energy and inhibits the function of the structure serving as primary support for photosynthetic metabolism. Drought causes a reduction in photosynthesis by restricting stomatal operations. The plants exposed to water stress have also significantly lower leaf areas compared to non-stressed plants (Mangena, 2018). Drought stress reduced chlorophyll content (SPAD) and relative water content in soybean at each growth stage. As the duration of stress increases, a dramatic decrease in these parameters is observed. Severe stress duration (10 days) caused a decrease in SPAD chlorophyll content (from 46.20 to 36.22) compared to adequate water supply conditions and the lowest SPAD values observed both seedling and seed-filling severe drought conditions (Dong et al. 2019).

Symbiotic nitrogen fixation has an indispensable property for the global nitrogen cycle and agricultural practices. To understand the mechanisms of symbiosis on plant physiology, ecology, and genetics of rhizobia have been studied to achieve better knowledge about rhizobia. In this context *Bradyrhizobium diazoefficiens* strain USDA 110 was originally isolated from a soybean nodule in Florida, USA in 1957 has been widely used for molecular genetics, plant physiology and ecology (Kaneko, 2002). The rhizobia inoculation of soybean is a sustainable practice to promote nitrogen fixation and subsequently improve crop productivity and soil fertility. Different environmental factors such as temperature, pH, salinity, genotype, and soil nitrogen content affect the legume, rhizobia symbiosis and nodulation performance of soybean (Yuan et al. 2020). Commercial inoculants of *Bradyrhizobium japonicum* strain are used in Türkiye however even after no legume history and only a small part of nodules was observed in Aydın province according to the conducted previous field experiments. This situation may be linked to heat and drought stress conditions occur during soybean growth period in the area has typical Mediterranean climate conditions. We aimed to observe the performance of USDA 110 strain in deficit and irrigated conditions representative of these climatic conditions. Within the scope of the present study, it is aimed to investigate soybean growth (yield, chlorophyll and quality) and nodulation performance under water scarcity conditions in hot climate conditions.

2. Material and Method

The study was conducted in Aydın/Türkiye ecological conditions located about 33 m (37°45´22´´N 27°45´36´´E) above sea level with Mediterranean climate conditions during the 2019 growing season. The climate is described as temperate, dry and hot summer (Csa) according to Köppen-Geiger climate classification. Three soybean cultivars (Cinsoy, Altınay, Umut-2002) described growth habit as middle-early maturing cultivars were used as genetic material obtained from Aegean Agricultural Research Institute, İzmir. As follows soybean nodulating Rhizobia applications: the commercial ~~soybean~~ inoculant (*Rhizobium spp.*-Azotek) obtained from Soil, Fertilizer, Water Resources Central Research Institute, Ankara and USDA 110 containing *Bradyrhizobium diazoefficiens* obtained from Germany were used for inoculation of soybean seeds. Full (Water Holding Capacity: 100%: FI) and deficit (Water Holding Capacity 50%: WS) irrigation doses were calculated based on cumulative evaporation amount from the class A evaporation container (US Weather Bureau Class A Pan) by different coefficients (Kanber, 1984) and drip irrigation was applied every week according to the equation given below;

I=Kpc.Ep.P.A [I: amount of irrigation to be applied to the plot, Kpc: evaporation container coefficient 100%, Ep: cumulative evaporation amount (mm), P: plant cover (%), A: Plot area (m^2)]

Irrigation was applied when the first open flower (flowering: BBCH 51) is observed on the main stem regardless of where the flower is located (Munger et al. 1997). A total of 242 mm of irrigation water was applied to the 50% irrigation dose during the growth and development period, while a total of 485 mm was given to the 100% full irrigated plots (Figure 1). The experimental layout was set up as a split-split plot design with three replications. There was 5.0 m separation between each plot to ensure minimal water movement among treatments. Each experiment plot was 5m x 2.8 m (4 rows per plot) and had a total area of 14 m^2 at sowing. The soil of the trial area categorizes as sandy loam texture with a slightly alkaline reaction. The organic matter content was low (1.7%) while phosphorus (10.7 ppm), potassium (305 ppm), calcium (1745 ppm) magnesium were high level in the experimental soil.

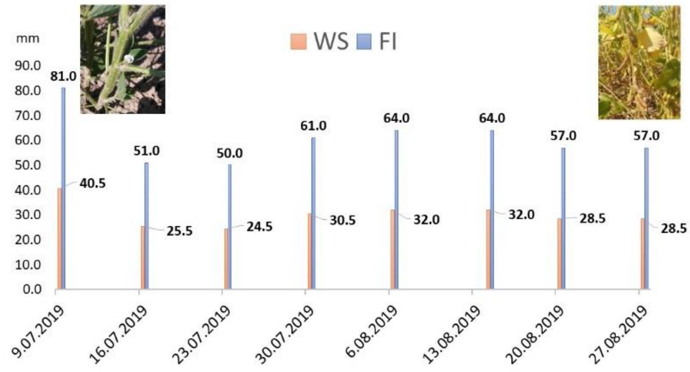

Figure 1. Irrigation amounts based on evaporation applied to plots (WS: water scarcity 50% and FI: Full irrigation 100%)

Considering the long-term climate values, mean temperature values were favorable (except in June) during soybean growth periods. There was sufficient precipitation in April before the 24th (sowing date), when the experiment was established but the precipitation amount decreased in May causing an adverse effect the on vegetative growth of soybean. The amount of precipitation required during the vegetative growth period of soybean plants was observed to shift towards July and a high amount of rainfall was observed about 97.7 mm in one month. Rainfall was almost non-existent in July and August when irrigation started due to water scarcity and full irrigation conditions, so soybean plants responded optimally to irrigation during the generative growth periods (Figure 2). Before sowing, seeds were inoculated with Rhizobium bacteria inoculants. Weed control was made by hand as a mechanical control method. Disease and pest control was performed at the required locations in addition Twospotted spider mites (*Tetranychus urticae* Koch) were controlled by Oberon Bayer® spiromesifen 240 SC (22.9%, w/w) insecticide application.

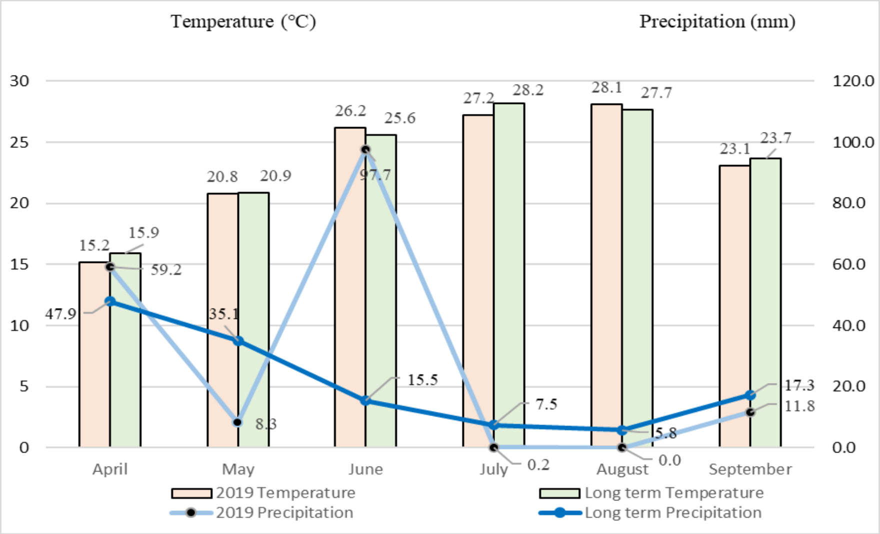

Figure 2. Monthly and long-term (1985-2019) temperature and precipitation values (Turkish State Meteorological Service, Station: Koçarlı)

For this study, agronomical, physiological and quality traits such as plant height (cm), first pod height (cm), number of pods per plant, 1000 seed weight (g), seed yield (kg ha^{-1}), SPAD chlorophyll content, leaf area (cm^2), crude protein and oil content (%) were investigated. Nodulation performance was observed from carefully dug out roots at randomly selected plants from each plot. Soil Plant Analysis Development (SPAD) chlorophyll content was measured at the last fully developed leaf in three generative growing periods; SPAD$_{FL}$: the beginning of flowering, about 10% flowers open (BBCH 61), SPAD$_{PD}$: about 50% of pods have reached final length (BBCH 75), SPAD$_{RS}$: ripening of seeds, advanced ripening (BBCH 85) using chlorophyll meter (SPAD-502 Konica Minolta, Japan). The leaf area measurement was performed by using LICOR (Lincoln, NE, USA) LI-3000C portable leaf area meter when the vegetative growth was accomplished (BBCH 49) (Figure 3). The Near Infrared Reflected Spectroscopy (NIRS) method was used to analyze crude oil and protein content of soybean seed flour using Bruker MPA^{TM} (Bruker, Ettlingen, Germany).

Figure 3. Location and general view of field experiment (© Google Earth Pro)

The analysis of variance (ANOVA) was conducted to compare the means and the LSD multiple comparison method was conducted to indicate statistically different means using TARİST software. Boxplots was created in MS Excel with the calculation of standard deviation values.

3. Results and Discussion

According to our results, irrigation caused statistically significant changes ($p \leq 0.01$) in the evaluated parameters such as plant height, first pod height, number of pods per plant, 1000 seed weight, seed yield, SPAD chlorophyll content in generative periods, and leaf area except for quality parameters (protein and oil content).

Rhizobia strains applied in the study caused no significant changes in almost all parameters and this situation can be explained because no nodule (no nodules detected in roots) history was observed in the field experiment by dug out roots.

According to the variance analysis results, the cultivar caused significant changes in all evaluated parameters except for crude oil content. The interaction between bacteria, cultivar, and irrigation (B*C*IR) showed significant differences just for plant height ($p \leq 0.05$), number of pods per plant ($p \leq 0.05$), and $SPAD_{PD}$ ($p \leq 0.05$) parameters (Table 1 and 2). Seed quality parameters did not show a clear response as there were significant differences between applications. For the mean square values of crude protein content evaluated in relation to bacteria, cultivar, and irrigation applications, only cultivar caused a statistically significant difference ($p \leq 0.05$) while no statistically differences were observed in crude oil content (Table 2).

Table 1. Analysis of variance (mean square values) for plant height, first pod height, number of pods, 1000-seed weight, and seed yield parameters.

Source	Df	Plant Height (cm)	First Pod Height (cm)	Number of pods (pods plant^{-1})	1000-seed weight (g)	Seed Yield (kg ha^{-1})
Bacteria (B)	1	476.69 ns	31.17 ns	30.61 ns	976.66*	2589.7 ns
Error-1	2	35.80	6.57	6.67	51.26	167.2
Cultivar (C)	2	1237.75**	162.04**	80.75**	414.37*	2933.9*
B*C	2	242.19 ns	25.00 ns	134.01**	307.22 ns	2333.2*
Error-2	8	57.07	17.64	6.52	74.21	428.7
Irrigation (IR)	1	5715.36**	1016.54**	529.00**	2018.55**	16571.8**
B*IR	1	5872.66**	24.83 ns	74.53**	352.37*	1587.3*
C*IR	2	268.03**	21.34 ns	102.52**	34.59 ns	917.9 ns
B*C*IR	2	120.96*	12.59 ns	17.51*	78.05 ns	437.0 ns
Combined Error	12	29.98	22.09	3.13	38.40	269.7
Corrected Total	35	477.66	55.56	40.63	193.95	1181.1
ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level						

Table 2. Analysis of variance (mean square values) for chlorophyll content (SPAD), leaf area and quality parameters.

Source	Df	SPAD_{FL}	SPAD_{PD}	SPAD_{RS}	Leaf Area (cm^2 plant^{-1})	Crude Protein (%)	Crude Oil (%)
Bacteria (B)	1	36.44*	50.17 ns	163.62 ns	49443.36*	0.63 ns	0.15 ns
Error-1	2	0.53	5.41	14.09	10532.71	0.77	0.08
Cultivar (C)	2	26.29**	31.63*	14.08**	222220.03*	27.71*	1.11 ns
B*C	2	31.58**	0.61 ns	25.75**	18234.90 ns	1.92 ns	0.13 ns
Error-2	8	1.81	4.97	1.44	29772.04	4.14	0.64
Irrigation (IR)	1	29.52**	93.83**	470.38**	2872347.04**	5.14 ns	1.42 ns
B*IR	1	26.01**	13.49*	14.50 ns	318336.68*	5.05 ns	0.01 ns
C*IR	2	7.58 ns	55.49**	24.83**	796725.53 ns	4.56 ns	0.09 ns
B*C*IR	2	2.77 ns	10.53*	2.92 ns	28742.69 ns	0.55 ns	0.20 ns
Combined Error	12	1.46	2.31	3.54	53172.37	3.29	0.62
Corrected Total	35	7.81	12.53	24.81	151052.26	4.69	0.53
ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level							

3.1. Plant height (cm)

Plant height is morphological characteristic reflects growth and development of crops and observed easily in field conditions; studying these plant characteristics help researchers to observe drought stress effectively (Dong et al. 2019). While irrigation and cultivar caused significant changes in plant height, *Rhizobia* strain applications showed non-significant changes (Table 1). According to the obtained mean values, the lowest plant height was measured in the application of USDA 110 at water scarcity conditions in Cinsoy cultivar, and the highest plant height value was obtained from the Umut-2002 cultivar with the application of USDA 110 and fully irrigated condition. Regarding cultivars, Umut-2002 had the highest plant height (88.5 cm) and Cinsoy had the lowest value (68.4 cm). Soybean plant height was interrupted by water scarcity stress and reduced plant height by approx. 27% compared to full irrigation (Table 3).

The plant height values have been found to be typical of Gaweda (2017) reported that they obtained plant height values between 73.70 cm and 135.40 cm, İlker et al. (2010) stated that soybean plant height values varied between 63.1 cm and 125.4 cm in the Mediterranean climate and Kars and Ekberli (2021) found the results varied between 88.33 cm and 127.77 cm in their studies. Drought stress caused by the lack of irrigation water for a long period leads to a reduced water supply to the upper soil layer consequently reducing the water use efficiency resulting in crops coupled with an excessive evaporation demand furthermore, drought stress can damage photosynthetic organs and reduce soybean seed germination rate, plant height, pod number and therefore yield (Wang et al. 2022).

Table 3. Average plant height (cm) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	62.5 fg	80.4 de	124.5 a	86.5 cd	88.5 A
Cinsoy	53.4 h	57.2 gh	98.0 b	65.1 f	68.4 C
Altınay	59.9 fgh	90.0 c	102.5 b	74.9 e	81.1 B
Mean Irrigation	66.7 B		91.9 A		
Mean USDA 110	82.9				
Mean Azotek	74.7				
Lsd C:7.1; Lsd IR: 3.9; Lsd B*IR: 5.6; Lsd C*IR: 6.8; Lsd B*C*IR: 9.7					

3.2. First pod height (cm)

The height of the first pod is positively related to plant height, but negatively related to seed weight, number of seeds per pod and number of pods per plant (Oz et al., 2009). According to Ramteke et al. (2012) stated in their study that the height of the first pod is positively related to plant height, number of nodes and stem diameter. However, it has been reported that high first pod height values may cause lower values in the number of pods per plant, and because the plant height is directly proportional to the first pod height, it may be negatively related to seed yield (Ghodrati, 2013). The first pod height is a genetic feature that minimizes harvest losses and the highest harvest-effective cultivars should have the traits that attach the first pod to the soil surface from a higher level (İlker et al. 2010). Irrigation and cultivar have been found statistically significant ($p \leq 0.01$) for the first pod height while bacteria applications caused non-significant differences (Table 1). As expected the results show that fully irrigated condition triggered higher plant height and higher first pod height values (21.5 cm) while water scarcity condition had the lowest first pod height value (20.9 cm).

Both Umut-2002 and Altınay cultivars had the highest first pod height values (28.5 and 28.1 cm) and Cinsoy had the lowest (22.0 cm) first pod height values as shown in Table 4.

On the other hand, there were no significant differences in the first pod height between *Rhizobia* applications and USDA 110 resulted in 27.1 cm while Azotek resulted in 25.3 cm values. Overall, applications (B*C*IR) have not been found statistically significant for the first pod height. The first pod height values ranged from 17.3 cm (Water scarcity, Cinsoy, Azotek) to 37.1 cm (Full irrigated, Altınay, USDA 110).

Table 4. Average first pod height (cm) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	21.1	26.1	34.8	32.1	28.5 A
Cinsoy	18.2	17.3	27.9	24.4	22.0 B
Altınay	23.7	18.9	37.1	32.7	28.1 A
Mean Irrigation	20.9 B		21.5 A		
Mean USDA 110	27.1				
Mean Azotek	25.3				
Lsd C:3.9; Lsd IR: 3.4					

In the study, the obtained values correlate favorably with the previous studies but higher first pod height values were obtained than the previous studies. It was stated that the first pod height values varied between 12.4 cm and 22.1 cm and yield characteristics were investigated under main crop conditions (Yetkin & Arıoğlu, 2010). Tayyar and Gül (2007) also reported in their study that the first pod height values of soybean varieties ranged from 13.1 cm to 20.6 cm.

3.3.Number of pods (pods plant^{-1})

Stress conditions at reproductive phases have irrevocable effects and hence result in severe loss of soybean productivity. The occurrence of drought and high-temperature conditions are considered to be major limiting environmental factors that affect pollen viability and increase flower abortion resulting in less productivity of soybean (Onat et al. 2017; Jumrani and Bhatia, 2018). The number of pods per plant was significantly affected by water scarcity ($p \leq 0.01$), cultivar ($p \leq 0.01$) and interaction (B*C*IR) ($p \leq 0.05$) imposed at the reproductive stages of soybean while bacteria applications had no significant effects on pod number (Table 1).

The number of pods per plant decreased as water scarcity conditions occurred (20.5 pods plant^{-1}) and as expected in irrigated condition pod number enhanced approx.. 27.0% compared to deficit irrigation with the value of 28.1 (pods plant^{-1}). Decreases in pod number appeared to be slightly higher under water deficit condition and the lowest pod number (17.2 and 16.4 pods plant^{-1}) was obtained from 50% WS, Altınay, USDA 110 and 50% WS, Umut-2002, Azotek applications, respectively. Full irrigated condition attributed to getting higher pod numbers almost for all cultivars except Cinsoy (21.0 and 22.7 pods plant^{-1}) plus 100% FI, Umut-2002 and USDA 110 application had the highest pod number value per plant. The statistical differences were observed between cultivars on the number of pods per plant. While the highest average pod number was obtained from both Umut-2002 (26.0) and Altınay (26.6) cultivars per plant, Cinsoy cultivar had the lowest pod number value (21.3) per plant (Table 5).

Rhizobia applications did not differ significantly, and mean values ranged from 23.4 to 25.8 pods plant^{-1}. Overall, water stress condition occurs in the beginning of flowering resulted with lower pod number per plant and well-watered conditions caused a significant increase in the pod number, which greatly contributes to yield. The number of pods and grain size are decreased because of the stress encountered following the onset of flowering and throughout the whole flowering period. When water stress occurs, grain yield and components suffer greatly (Korte et al., 1983). These values have been found to be typical of Boydak et al. (2018) and Yamika and Ikawoti (2012) with the values 19.46-35.80 and 42.90, respectively. Shamima and Farid (2005) investigated the effects of irrigation practices on yield parameters in soybean and reported that the number of pods varied between 35.4 and 46.9 per plant, and these values were found to be consistent with the pod number values we obtained in our study.

Table 5. Average number of pods (pods plant^{-1}) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	23.4 def	16.4 h	38.2 a	26.1 cd	26.0 A
Cinsoy	19.3 gh	22.3 efg	21.0 fg	22.7 ef	21.3 B
Altınay	17.2 h	24.3 de	32.4 b	28.5 c	25.6 A
Mean Irrigation	20.5 B		28.1 A		
Mean USDA 110	25.8				
Mean Azotek	23.4				
Lsd C:2.4; Lsd B*C: 3.4; Lsd IR: 1.2; Lsd B*IR: 1.8; Lsd C*IR: 2.2; Lsd B*C*IR: 3.1					

3.4.1000 seed weight (g)

Drought stress causes irreversible effects especially during flowering, seed formation and seed filling periods. The stress experienced during the seed-filling period causes a decrease in grain size and yield (Desclaux et al., 2000). Decreases in seed size are attributed to shorter seed-filling periods and earlier onset of ripening.

The ANOVA results of thousand seed weights of soybean varieties with different irrigation doses and *Rhizobia* treatments are presented in Table 3. According to the results of this analysis of variance, irrigation treatments were found to be statistically significant at 0.01 level, and bacteria*cultivar and bacteria*water dose interactions were found to be statistically significant at 0.05 level. There was no statistically significant effect of cultivar and *Rhizobia* treatments on kernel weight of soybean (Table 1).

The mean values of thousand-grain weight of soybean varieties in the experiment are given in Table 10. According to this average table, thousand-grain weight values varied between 103.75 g and 147.00 g in soybean varieties. In the experiment, the lowest thousand-grain weight was observed in USDA 110 bacteria application in Cinsoy cultivar at 50% irrigation dose, while the highest thousand-grain weight value was observed in USDA 110 bacteria application in Umut cultivar at 100% irrigation dose. Significant effects of irrigation doses on thousand-grain weight were determined.

Depending on the irrigation doses, thousand-grain weight averages were obtained at 50% irrigation dose with 113.86 g and at 100% irrigation dose with 128.83 g. Cultivar mean values on thousand-grain weight were close to each other. Umut cultivar had the highest thousand-grain weight with 126.15 g, followed by Altınay cultivar with 123.09 g and Cinsoy cultivar with 114.79 g (Table 6).

The effect of *Rhizobia* treatments on thousand seed weight was found significant at 0.05 level. The mean thousand grain weight of bacterial treatments varied between 116.13 g (Azotek) and 126.55 g (USDA 110). Karakaya & Ödemiş (2019) reported that the increase in irrigation levels positively affected yield, 1000 grain weight and protein ratio. The values obtained in our study are consistent with the results of previous studies (Kobraee et al., 2011; Onat et al., 2017; Yıldırım et al., 2022).

Table 6. Average 1000 seed weight (g) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	126.80	110.60	147.00	120.20	126.15 A
Cinsoy	103.75	104.43	133.93	114.07	114.79 B
Altınay	117.26	117.30	130.58	127.21	123.09 A
Mean Irrigation	113.86 B		128.83 A		
Mean USDA 110	126,55 A				
Mean Azotek	116,13 B				
Lsd B: 10.26; Lsd C: 8.11; Lsd IR: 4.50; Lsd B*IR: 6.36					

3.5.Seed Yield (kg ha^{-1})

Water deficiency has strongly negative influences on productivity, physiological and biochemical traits of soybean plants. The occurrence of immature pod opening may be one of the dysfunctions caused by the decrease in cell turgor result in yield losses (Moura et al. 2023). Although water deficit during vegetative development can cause developmental retardation, the most sensitive periods to drought are flowering and pod-filling periods. To achieve high yield, it is important to avoid water restrictions during these periods. During flowering and early pod-filling periods, yield and quality can be negatively affected by drought. (Poudel et al. 2023). The results of the study indicate that irrigation ($p \leq 0.01$) and cultivar ($p \leq 0.05$) are statistically significant factors influencing seed yield (kg/ha). Conversely, *Rhizobia* applications did not demonstrate a significant effect because of no nodulation history in soybean plants (Table 1).

Water scarcity resulted in approximately 35% less grain yield value (2520 kg ha^{-1}) compared to fully irrigated (3880 kg ha^{-1}) condition. Delice (2017) reported that soybean grain yield values varied between 202 kg/da and 439/da kg in different irrigation doses (25%, 50%, 75%, 100%, 125%) and investigated the yield losses under deficit irrigation conditions at the same level of our study results. Karakaya & Ödemiş (2019) investigated the effects of five different irrigation dose applications (25%, 50%, 75%, 100%, 125%) on yield parameters in soybean. Their findings revealed that grain yields ranged from 198.5 to 518 kg/da, with an average of 201.54-807.12 mm of irrigation during the growing season. The yield differences observed were considerable, with the greatest yields obtained under the highest irrigation levels. As in other observed parameters (plant height, first pod height, number of pods and seed weight), Cinsoy cultivar had the lowest average value in terms of grain yield compared to other cultivars (Table 7). It is important to mention that summer crop yields (soybean, maize, cotton etc.) are highly dependent on irrigation compared to rainfed production of winter crops. It is clear that a reduction in irrigation levels will result in a notable decline in yield, particularly in the context of climate change. The combination of rising temperatures and increased evaporation results in a depletion of the soil's water budget. When this occurs concurrently with deficit irrigation, it becomes inevitable that yield reductions will occur in the future. Another explanation for the overall low yield may be the absence of nodule formation. It can be postulated that plants are unable to reach their full yield potential without effective nitrogen fixation. Conversely, with effective nodulation, it is hypothesized that there may be an increase in yield potential for the region.

Table 7. Average seed yield (kg ha^{-1}) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	3280	1940	5270	3510	3500 A
Cinsoy	2140	2380	3070	2930	2630 B
Altınay	2320	3070	4730	3760	3470 A
Mean Irrigation	2520 B		3880 A		
Mean USDA 110	3470				
Mean Azotek	2930				
Lsd C: 610; Lsd B*C: 870; Lsd IR: 370; Lsd B*IR: 530					

3.6.Leaf Area (cm^2 plant^{-1})

Leaf area construction and development is a primary factor that affects the amount of solar radiation intercepted. The development of leaf area contributes to plant growth in photosynthetic metabolism, but it can be reduced by water stress conditions as a result of the function of the number and size of leaves (Gutiérrez-Boem and Thomas, 2001). As it clear in the obtained results; the leaf area of soybean plants in water scarcity condition caused decreases in the leaf area (approx. to -59% level). The soybean plants had a chance to grow higher amount of canopy with the full irrigated water supply.

Among the cultivars, Umut-2002 had higher leaf area (1236.69 cm^2/plant) amount compared to Cinsoy and Altınay (Table 8). In the previos study conducted by Herliana et al. (2019); who investigated the effects of *Rhizobium* bacteria and different doses of nitrogen fertilizer applications on yield and growth of black soybean, the highest value (138.75 cm^2) was obtained from the combination of *Rhizobium* isolate (*R.* nepotum) and 50% supplied nitrogen fertilizer application.

Table 8. Average leaf area (cm^2 plant^{-1}) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	925.69	925.08	1782.25	1313.76	1236.69 A
Cinsoy	742.58	505.98	1509.46	1121.51	969.88 B
Altınay	817.16	915.41	1452.73	1041.78	1056.77 B
Mean Irrigation	805.32 B		1370.25 A		
Mean USDA 110	1204.98 A				
Mean Azotek	970.59 B				
Lsd B: 147.79; Lsd C: 162.50; Lsd IR: 167.55; Lsd B*IR: 236.94					

3.7.SPAD Chlorophyll Changes in Reproductive Stages

The SPAD Chlorophyll Meter is used to determine the nitrogen status of plants and provides important information on chlorophyll and photosynthetic status with its ease of use and rapid measurement under field conditions, especially under water stress conditions. (Ahmed et al., 2010; Wicharuck et al. 2024). SPAD Chlorophyll meter measurements showed a significant correlation result with yield prediction during grain filling period under high temperature and water scarcity conditions in soybean (Ergo et al., 2018). With in the light of this information, our study results showed that a notable decline in SPAD chlorophyll content values was observed under conditions of restricted irrigation also this decline was particularly pronounced during the ripening of seeds (BBCH 85; SPAD$_{RS}$) period (Figure 4).

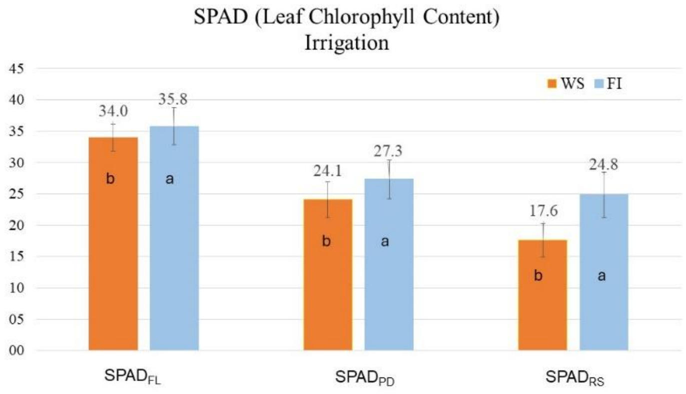

Figure 4. The changes of SPAD values observed during the flowering (SPAD$_{FL}$: BBCH 61), pod development (SPAD$_{PD}$: BBCH 75) and ripening of seeds (SPAD$_{RS}$: BBCH 85) stages under different water regimes

The highest SPAD values were observed at the beginning of flowering stage and showed a greater decline in the development and ripening of seeds stages. Plants showed a decreasing trend for SPAD values during reproductive stages, and this can be linked to senescence of plants according to maturity. Considering that water scarcity is the main factor that reduces soybean growth including yield and photosynthesis. (Felisberto et al. 2023). While both cultivars (Cinsoy and Altınay) had the lowest SPAD values in SPAD$_{RS}$ stage, Umut-2002 had the highest value. The leaf greenness and nitrogen status stayed longer in the ripening of seeds and remained more stable in Umut-2002 than the other cultivars (Figure 5). The obtained SPAD values are consistent with the previous soybean studies (Erbil and Gür, 2017; Tunçtürk et al. 2021).

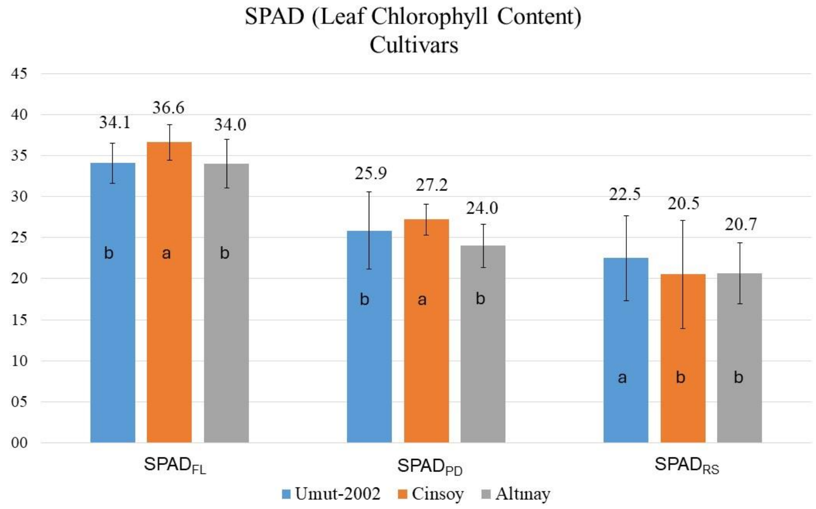

Figure 5. The changes of cultivar SPAD values observed during the flowering (SPAD$_{FL}$: BBCH 61), pod development (SPAD$_{PD}$: BBCH 75) and ripening of seeds (SPAD$_{RS}$: BBCH 85) stages

3.8.Crude Oil and Protein Content (%)

Soybean is of great interest worldwide due to its high protein content, which makes it one of the most important protein sources in the food and feed industry. Drought and water scarcity conditions not only affect yield formation of soybean, but also negatively influence the quality composition. With the reduced nitrogen fixation, the biosynthesis of protein is also affected by drought conditions (Poudel et al., 2023). However, the consistency of protein and oil content of soybean is varied in drought conditions. These quality traits are mainly controlled by genes as well as environmental constraints (Krisnawati and Adie, 2017). Considering the crude protein and oil content results, only crude protein content was affected by the cultivar in our study (Table 1). This result is supported by genetic factors that are potentially effective on the protein content of soybean (Table 9.)

Table 9. Average crude protein content (%) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	28.7	28.0	30.2	30.6	29.4 B
Cinsoy	32.4	31.8	32.6	32.8	32.4 A
Altınay	31.1	31.0	29.4	31.9	30.8 AB
Mean Irrigation	30.5		31.2		
Mean USDA 110	30.7				
Mean Azotek	31.0				
Lsd C: 1,9					

According to both quality traits, there were no statistically significant differences observed in *Rhizobia* and irrigation treatments (Table 1). Crude oil content (%) showed no statistically significant results in all treatments (Table 1).

Kırnak et al. (2010) examined the effects of different water stress conditions (0, 25, 50, 75, 100%) on soybean and reported that the highest protein value was obtained under full irrigation (100%) conditions while the highest oil yield was obtained under no irrigated (rainfed) conditions. Gök (2021) reported that the oil content of soybean grain varied between 10.76% and 22.18%, and Gaweda et al. (2017) had crude oil content results between 17.20 and 18.60% in their study. According to the previous studies, higher oil content values (19.9-21.2%) were obtained without being affected significantly by any treatments applied in our study (Table 10).

Table 10. Average crude oil content (%) mean values of treatments and cultivars

	Water Scarcity (50%)		Full Irrigated (100%)		
	USDA 110	Azotek	USDA 110	Azotek	Mean Cultivar
Umut-2002	20.7	21.0	21.2	21.0	21.0
Cinsoy	20.2	20.1	20.8	20.7	20.5
Altınay	20.6	19.9	20.6	20.5	20.4
Mean Irrigation	20.4		20.8		
Mean USDA 110	20.7				
Mean Azotek	20.6				

4. Conclusion

In this one-year experiment conducted under Mediterranean climate conditions, the effects of full irrigation and water scarcity applications on the yield and quality of soybean varieties and the nodule formation performance of *Rhizobia* bacteria applications on soybean roots were determined. In the initial study conducted in the Aydın province, the performance of the USDA 110 bacterial strain was evaluated under local ecological conditions. The objective was to identify a solution to the lack of nodule formation observed in soybean crops in the region. According to the results of the study, no nodule formation was observed in soybean plants even if in full irrigated condition and this may be linked to high ambient and soil temperature conditions in the growing season. As a result of the study, it was determined that irrigated condition (100% WHC) had positive effects on yield formation and SPAD chlorophyll content of soybean compared to water scarcity (50%, WHC) condition. Among the soybean cultivars grown in the experiment, Umut-2002 had higher yield values compared to Cinsoy and Altınay varieties used in the experiment. The experiment demonstrated that *Rhizobia* bacteria applications, which had not previously been investigated in the region under different water regimes, did not have any discernible effect on the properties examined. Introducing or developing more newly adapted inoculants may improve soybean yield potential which is important for soybean cultivation potential in Aydın province. Consequently, it was concluded that further field trials should be conducted over multiple years to observe the USDA 110 nodulation performance in the future.

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