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YIELD AND QUALITY RESPONSES OF SOYBEAN (*Glycine max.* L. Merr.) VARIETIES INOCULATED WITH *RHIZOBIA* STRAINS UNDER DROUGHT STRESS

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ABSTRACT

The aim of this study was to investigate the effects of nodule formation and nodulation performance of *Rhizobia* bacteria on yield and quality in soybean roots under limited water application conditions in soybean varieties depending on climate change. In this study, 4 different irrigation applications (limited vs irrigated: 25%, 50%, 75%, 100%), 2 soybean varieties (Cinsoy and Altinay) and 3 different *Rhizobia* inoculants (Control, AZOTEK-2, USDA-110) were applied for two years (2020-2021) in order to determine the yield and quality characteristics of soybean. The experiment was established according to randomized complete block split-split plots experimental design with 3 replications. In the experiment, the main factor was *Rhizobia* inoculant treatments, the first sub-factor was soybean varieties, and the second sub-factor was irrigation applications. Within the scope of the study, yield and quality parameters such as plant height (cm), first pod height (cm), number of pods plant^{-1}, number of seeds pod^{-1}, seed yield (kg ha^{-1}), 1000 seed weight (g), leaf area (cm^2 plant^{-1}), seed crude protein (%) and oil content (%) were examined. It was concluded that irrigation and inoculant applications and combinations of these factors had significant effects on yield parameters of soybean varieties. The study revealed that there was no discernible nodulation development observed in soybean roots under both irrigated and limited irrigation conditions. It was determined that under conditions of limited irrigation combined with high temperature conditions, the growth of the soybean was significantly impacted, resulting in a notable reduction in yield and leaf area but this was not observed in the quality characteristics.

Keywords: *Bradyrhizobium*, Drought, Irrigation, Nitrogen fixation, Soybean, Water stress

INTRODUCTION

Soybean (*Glycine max.* L. Merr.) is one of the most widely cultivated legumes in the world due to its valuable seed composition. Soybean is used in human and animal nutrition due to its oil (18-20%), protein (36-40%), carbohydrate (30%) and mineral substance (5%) content (Shea et al., 2024). Soybean is one of the most important oilseed crops worldwide and contains high amounts of protein for human food and animal feed (Wysokinski et al., 2024). Growing awareness of sustainable food systems has led to an increased demand for plant-based proteins, increasing the popularity of soybeans with their high protein quality (Zhang et al., 2021). Soybean is a legume plant that can form tubers, called nodules, on its roots with *Rhizobium* bacteria and bind free nitrogen from the air into the soil. *Bradyrhizobium japonicum* is the strain of bacteria that can form effective nodules in soybeans (Miransari, 2016).

Extreme weather events, such as droughts, reduce crop production, making it difficult to obtain and distribute crops worldwide. High temperatures occur during critical phenological stages, causing crop yield and quality losses (Yigit and Chmielewski, 2024). With climate change on the rise, long periods of high temperatures in semi-arid regions result in water scarcity for crop production (Parkes et al, 2022). Irrigation water is the most important input for increasing yield under arid and semi-arid conditions. Limited irrigation strategies are an important production alternative to reduce water demand in agricultural production (Miransari et al., 2022). Drought increases the stress levels in plants, shortens their development periods and decreases yield potential (Hatfield and Prueger, 2015). The most damaging stages of heat and water stress in soybean are during flowering and pod development. Water stress experienced during these stages leads to disruption of flower structure and pollen sterility. On the other hand, high temperature conditions affect assimilate accumulation in pods, leading to smaller seeds and therefore lower yields (Soba et al., 2022). Drought stress during the flowering and pod formation periods in soybean bean reduces the flowering rate, thus causing a decrease in the number of pods in the plant and leading to low yields (He et al., 2017). Restricted irrigation and drought conditions significantly affect the protein and oil content in soybean seeds, and drought can increase the protein content and decrease the oil content (Hu and Wiatrak, 2012; Ravelambola, 2022). Poeta et al. (2016) also reported that the increase in protein content was positively and linearly related to the increase in water stress and negatively related to the oil content.

It is known that biological nitrogen fixation and photosynthesis are particularly sensitive in soybean under drought stress. Drought leads to inhibition of biological nitrogen fixation and limitation of nitrogen transport, resulting in reduced grain yield (Cerezini et al., 2020). Drought stress is an important factor affecting the symbiotic relationship. Nitrogen obtained by the soybean-*Rhizobia* association is of great agricultural importance. Under stress conditions, the number and development of *Bradyrhizobium japonicum* bacteria decrease (Akinrinlola et al., 2024). Nodulation in soybean occurs between 20-30 °C. At high temperatures, the number of roots in soybean decreases and capillary and lateral root development weakens. Therefore, nodulation initiation and nitrogen fixation may not occur under high temperature conditions (Miransari et al., 2022). Lumactud et al. (2023) reported that drought stress in the early vegetative stage significantly reduced nodule formation and nitrogen fixation in soybean. Under arid conditions, survival rates of *Rhizobia* present in the soil and introduced by seed/bacteria inoculation may be reduced (Thilakarathna et al., 2021). For this reason, it is necessary to select effective breeds with high nitrogen fixation power in sowing. Effective breeds are strain that can fix more nitrogen per unit of time and per plant (Sarimurat et al., 2022).

In this context, *Bradyrhizobium diazoefficiens* bacteria belonging to the USDA 110 strain was used in the inoculation of soybean seeds in the experiment, since it can grow under the temperature conditions in the soybean growing periods in Aydin province. The specific host plant of USDA 110 (*Bradyrhizobium diazoefficiens*) bacterial strain is soybean. This effective strain is a gram-negative (rod-shaped) bacterium that can develop a healthy symbiotic relationship and fix free nitrogen from the atmosphere. It is a bacterial strain that can fix nitrogen in the soil at higher rates than other species. It was first isolated from soybean nodules in the US state of Florida and is widely used in studies.

In this study, it was aimed to investigate the nodule formation performance of *Rhizobia* bacterial species in soybean, which is one of the most important plants for sustainable agriculture, and the effects of restricted irrigation conditions on both nodule formation and nitrogen fixation and plant growth stages in soybean varieties. In the study, it was aimed to provide an effective biological nitrogen fixation by working with USDA 110 *Bradyrhizobium diazoefficiens* bacteria strain, which has not been applied in Aydin ecological conditions before, and to develop this in sustainable agricultural systems. Depending on the treatments, yield and quality characteristics of soybean were also examined. Within the scope of the study, it was aimed to investigate the yield and quality parameters and nodulation performances of soybean varieties under high temperature and water scarcity conditions.

MATERIALS AND METHOD

Field experimental area and design

The field experiment was conducted during the 2020 and 2021 growing seasons in the experimental area, situated at 37°45'N 27°45'E on the Aydin Adnan Menderes University Faculty of Agriculture Research and Application Farm (Figure 1). In order to evaluate the yield and quality characteristics of soybean in the experiment, two mid-early soybean varieties (Cinsoy and Altinay) were provided as material from Aegean Agricultural Research Institute, Izmir. In the experiment, two *Rhizobia* bacterial materials were used for inoculation to soybean seeds. These materials were AZOTEK-2 (*Rhizobium spp.*) inoculant obtained from Ankara Soil, Fertilizer and Water Resources Central Research Institute and USDA 110 inoculant containing *Bradyrhizobium diazoefficiens* obtained from Humboldt-Universität zu Berlin, Germany.

Figure 1. Location and general view of field experiment

The experiment was carried out following the split-split plots over randomized complete block design. The main factor in the experiment was *Rhizobia* applications, the first sub-factor was soybean varieties, and the second sub-factor was irrigation (limited vs irrigated) applications. The experiment had 3 replications and consisted of 2 soybean varieties (Cinsoy and Altinay), 3 *Rhizobia inoculant* strain applications (Control, AZOTEK-2 and USDA-110) and 4 different irrigation (irrigated vs limited) treatments (25%, 50%, 75% and 100%). The experiment was established with a total of 72 plots. Each experiment plot was 5 x 2.80 m (4 row plot^{-1}) and had a total area 14 m^2 plot^{-1} at sowing.

The soil characteristics of the experimental site has a sandy loam soil structure with low organic matter (1.7%). The soil pH is alkaline (7.92), lime content was high (7.93%), sodium (89 ppm), potassium (224 ppm), calcium (2481 ppm) and phosphorus (11.53 ppm) levels were moderately useful.

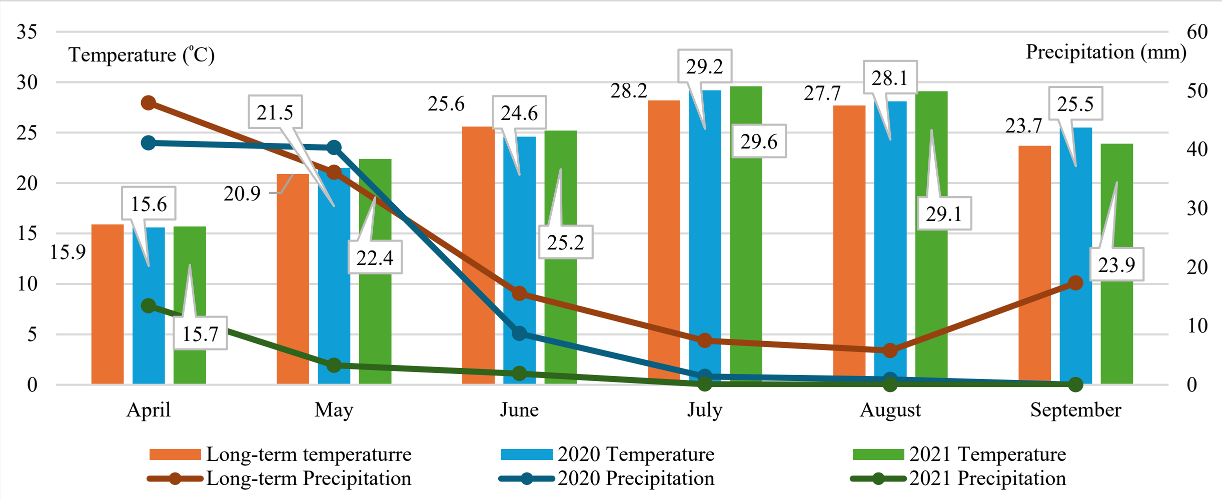

Figure 2. Monthly and long-term mean temperature and precipitation values (Turkish State Meteorological Service, Station: Kocarli/Aydin)

Figure 2 illustrates the comparison of temperature and precipitation values in Aydin province during the 2020 and 2021 soybean growing seasons, with data presented in relation to the long-term average. In both experimental years, it is observed that the total rainfall in the months of April through September is significantly below the long-term average rainfall. In general, the mean temperatures during the trial years were similar, with the exception of the July and August months for both years.

In the experiment, the irrigation water to be applied to the plots was calculated based on the cumulative evaporation amount (25%, 50%, 75% and 100%). The amount of irrigation water to be applied to the plots was calculated with different coefficients according to the evaporation amounts obtained from the Class A evaporation pan (US Weather Bureau Class A Pan) (Kanber, 1984), applications were made regularly once every 7 days using the drip irrigation system according to the equation 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)]

In both years of the experiment, irrigation of plots started when the first flowers (BBCH 61) appeared and gradually terminated according to the maturity periods. The total amount of water applied to the plots; 858.77 mm irrigation water in 100%, 644.05 mm in 75%, 399.37 mm in 50% and 199.67 mm in 25% during the growth and development period in 2020. In the 2021 trial year, 816.98 mm irrigation water was given in 100%, 612.73 mm in 75%, 376.99 mm in 50% and 169.99 mm in 25% water application.

Sowing and maintenance

Sowing was carried out in the last week of April in both years of the experiment. Seeds were sown with a pneumatic seeder machine at a soil depth of 4-5 cm, taking soil moisture into consideration. In both years of the experiment, sowings were made one day apart due to different bacteria applications. In the sowing of the seeds, the varieties in the control blocks were sown without any *Rhizobia* inoculant application. In the sowing of AZOTEK-2 (*Rhizobium*) bacteria-treated plots, the varieties were inoculated in both experimental years so that the bacteria covered the entire surface of the seeds in a shaded environment. In USDA-110 bacteria application, soybean seeds were inoculated in both experimental years with USDA-110 bacteria in liquid form and sowing was completed. In both years, if nodulation was not at the desired level, fertilization was applied twice (at the beginning of flowering (BBCH 60) and at the beginning of pod setting (BBCH 70). During the development period, chemical control was carried out with the most common pests (whitefly, green worm, thrips, Twospotted spider mite (*Tetranychus urticae* Koch), and *Spodoptera littoralis*).

Measurements and statistical analysis

In this study, agronomic, physiological and quality parameters such as plant height (cm), first pod height (cm), number of pods plant^{-1}, number of seeds pod^{-1}, 1000 seed weight (g), seed yield (kg ha^{-1}), leaf area (cm^2 plant^{-1}), crude protein content (%) and crude oil content (%) were investigated. No counts or statistical evaluations were made for nodules in the observations related to nodule formation in the experiment. Leaf area measurements were performed by sampling plants in each plot and the leaves were separated (all leaves plant^{-1}) and measured by using the LI-COR 3000 C (Lincoln, NE, USA) portable leaf area device in two times: the first at the flowering stage (BBCH 61-65, 10-50% flowering) and the second at the pod formation stage (BBCH 71-75, 10-50% pod formation). Grain protein content was determined in soybean flour (milled to 0.8 mm) samples according to the AOAC method 997.09 using Velp® Scientifica (Italy) NDA 702 DUMAS Nitrogen Analyzer at the Aydin Adnan Menderes University, Agricultural Biotechnology and Food Safety Application and Research Center (ADU-TARBIYOMER). Determination of crude oil content was made in the Solvent Extraction device in accordance with the Soxhlet method, based on the principle of extracting the oil using hexane solvent. Variance analyses of the data obtained in both years of the field trials were evaluated in the TARIST package program in accordance with the randomized complete block split-split plots trial design. The LSD test was used to compare the means (Acikgoz et al., 2004).

RESULTS AND DISCUSSION

The response of phenological development related to climate and observation of nodulation in roots

In both years of the experiment, observations were carried out on the plants rooted outside the edge rows and in the center of the plots in order to follow the nodule formations in the soybean roots. For this purpose, the soil in each plot was excavated to a depth of 0.50 m and 1 m and the root zones were examined under field conditions. In the first year of the experiment, although not in all plots, nodule formation on the roots was observed in AZOTEK-2 (*Rhizobium*) and USDA 110 (*Bradyrhizobium*) bacteria treatments. A better nodule formation was observed in both Cinsoy and Altinay varieties in USDA 110 inoculant application for 75% and 100% water treatments. However, the results of inoculation only observed in a few plants and upon examination of all plots, it was determined that stable and homogeneous nodule development was not evident. In the second year of the experiment, nodule formation in soybean roots in the plots were detected in much lower number as compared to the previous year where *Rhizobia* inoculants were applied. It is thought that the reason for this may be the high daily maximum temperature values experienced during the vegetative development periods in both years (Miransari et al., 2022). The impact of daily maximum temperature values on developmental periods is illustrated in Figure 3. In both experimental years, it was observed that daily maximum temperature values during the vegetative periods resulted in significant differences. Although the monthly temperature values during the phenological development periods were higher in the second year compared to the first year, the daily maximum temperature values did not exceed 35°C (except for a few days) in the second year in vegetative period. However, increasing daily maximum temperature values observed in flowering period of the second year. In the 2020 growing season, temperatures reached approximately 25°C at the time of sowing and remained at approximately 25-35°C range until the onset of flowering. In the 2021 experimental year, it was determined that the daily maximum temperature values exceeded 35°C between vegetation and end of flowering periods, commencing from the date of sowing. This period of elevated temperatures represents the most susceptible phase for the development of *Rhizobia* inoculation in soybean roots. It is hypothesized that this period of time coincides with the formation of signal compounds between bacteria and soybean roots (Soba et al. 2022). The daily maximum temperature values exerted a considerable influence on the flowering period (BBCH 60-69). In 2021, the daily temperature averages during the flowering period were below 35°C, indicating that the flowering period was more temperate than in 2020. In both years of the experiment, the pod setting (BBCH 70-79) periods exhibited minimal discrepancy. In the 2021 experimental year, higher daily maximum temperature values permitted the plants to reach maturity at a faster rate. Productivity under drought conditions varies depending on the intensity and duration of the stress occurred in which phenological developmental stages. The phenological development periods where drought is most effective are the period from sowing to emergence, flowering period and pod setting period (Lewandowska et al., 2020). The low yield of soybean is due to the stress conditions during the pod setting and grain filling periods rather than drought conditions during the flowering period (Staniak, et al., 2023). Drought stress at the onset of flowering results in a reduction in the number of pods. The stress experienced at the onset of flowering and at full flowering has been demonstrated to result in a reduction in the number of pods and grain size (Korte et al., 2023) Furthermore, the absence of elevated stress (heat) levels and earlier onset of the flowering period in the 2021 caused to reveal lower daily maximum temperature conditions (Figure 3) indicate that the yield was positively influenced (Table 2), aligning with the yield values obtained despite the elevated temperatures during the pod tying and grain maturity periods. Flower shedding is a consequence of drought stress during the flowering period. However, given that flowering occurs over a prolonged period (approximately 3-4 weeks) along the length of the stem, it is possible to offset the losses incurred through flower shedding. This suggests that the impact of drought stress during the pod setting period may be more pronounced than during the flowering phase (Mandić et al., 2020).

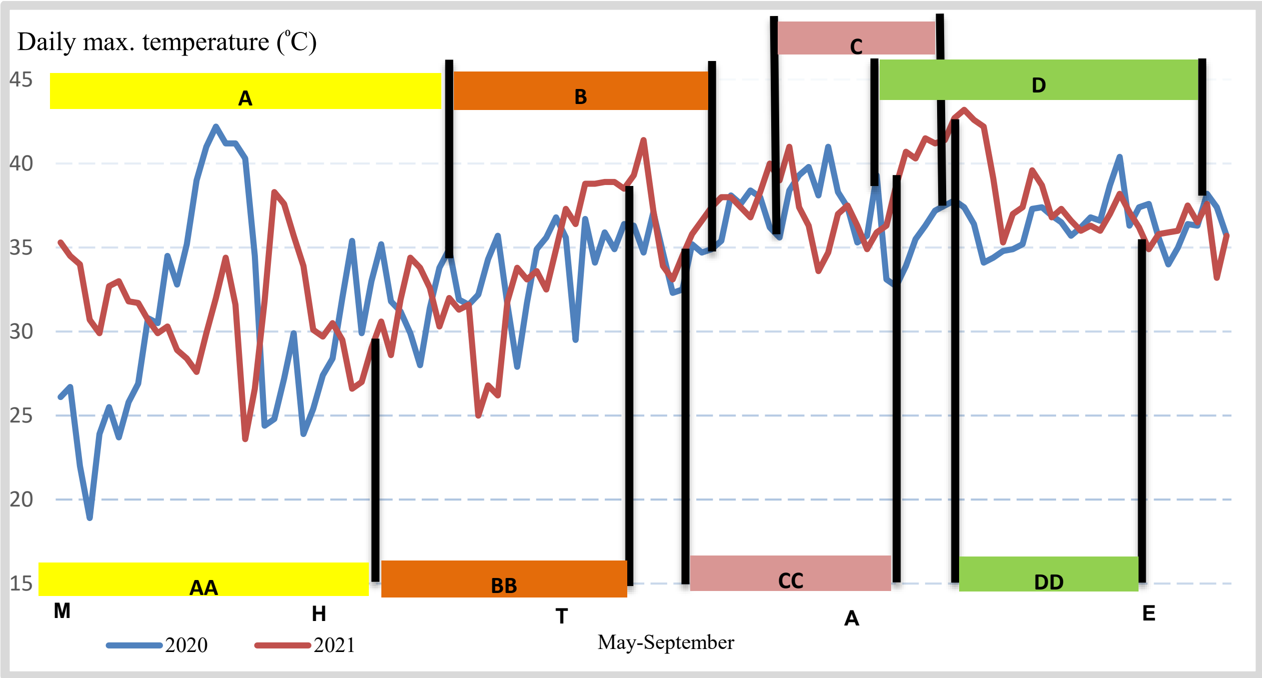

Figure 3. Daily maximum temperature values for the 2020 and 2021 soybean growing season (May-September)

A: Vegetative period 2020; AA: Vegetative period 2021; B: Flowering period 2020; BB: Flowering period 2021; C: Pod maturity period 2020; CC: Pod maturity period 2021; D: Grain maturity period 2020; DD: Grain maturity period 2021
M: May; H: June; T: July; A: August; E: September
BBCH Phenological growth stages of the soybean: 09: Cotyledons emerge, 11: First pair of true leaves unfolded, 60: Beginning of flowering, 69: End of flowering, 70: Beginning of pod development, 79: End of pod development, 80: Beginning of pod ripening, 85-89: End of pod ripening, 99: Harvest period (Munger et al., 1997)

The temperature values of July and August, when the pod-setting and grain-filling periods of soybeans coincide (BBCH 75-85), were observed to be higher in 2021 than in 2020. In the second year of the experiment, the phenological development periods in these months exhibited accelerated growth in correlation with the elevated temperatures, resulting in a reduction in the maturity periods compared to the first year of the experiment.

In this study, the effects of *Rhizobia* inoculation on some yield and quality characteristics of soybean varieties were investigated depending on different limited and irrigated water applications in order to ensure the continuity of soybean production in sustainable agriculture considering the importance of climate change. According to the variance analysis results we obtained, irrigation treatments (limited vs irrigated) were found to be statistically significant at the $p \leq 0.01$ level on yield parameters such as plant height, first pod height, number of pods plant^{-1}, number of seeds pod^{-1}, 1000 seed weight, seed yield and leaf area (Table 1). *Rhizobia* applications were found to be statistically significant in other parameters examined except protein content. In the experiment, bacteria, variety and water interaction were found to be statistically significant at the $p \leq 0.01$ in the number of pods per plant, the number of seeds per pod and seed yield, while it was found to be significant at the 0.05 level ($p \leq 0.05$) in 1000 seed weight. While water treatments were found to be statistically significant at 0.05 level ($p \leq 0.05$) on protein ratio, variety and bacteria treatments were found to be statistically significant at 0.01 level ($p \leq 0.01$) on crude oil ratio (Table 2).

Plant Height (cm)

The experiment on plant height revealed that the effects of years, *Rhizobia* applications, irrigation applications, and the year*inoculant*irrigation interaction on plant height were significant (Table 1). Upon examination of the mean values obtained, it was found that the mean plant height in the first year was 88.34 cm, while the mean plant height in the second year was 91.01 cm. It was observed that the mean plant height in the second year was greater than that observed in the first year. The data indicated a notable increase in plant height values with each incremental increase in irrigation application. The highest plant height value of 109.76 cm was obtained in the treatment group receiving 100% irrigation, as determined by the irrigation application averages. The lowest recorded plant height value was 63.28 cm, which was observed in the 25% irrigation treatment. The main reason for this is thought to be the decline in plant growth as the amount of irrigation decreases. The application of *Rhizobia* inoculant treatments did not result in a significant increase in plant height compared to control. A comparison of the plant height values obtained in the present study with those reported in previous studies revealed that Surgun Acar (2022) observed plant height values between 95.01 and 108.33 cm in soybean, while Ilker (2017) documented values between

63.10 and 94.90 cm in the first year and between 81.50 and 105.40 cm in the second year. In a study conducted in in 2021, the plant height of soybeans was found to range from 88.33 to 127.77 centimeters (Kars and Ekberli, 2021).

Table 1. Average values of yield and yield parameters in soybean for the combined trial years

Experimental Factor	Plant height (cm)	First pod height (cm)	Number of pods plant^{-1}	Number of seeds pod^{-1}	1000 seed weight (g)
Mean Year (A)					
2020	88.34b	20.68a	39.91b	2.66	147.35a
2021	91.01a	17.20b	76.73a	2.61	134.27b
Mean Bacteria (B)					
Control	93.30a	18.96b	54.41c	2.51b	136.17c
Rhizobium	88.34b	19.56a	57.88b	2.75a	141.09b
USDA 110	87.02b	18.73c	62.96a	2.65a	145.16a
Mean Variety (C)					
Cinsoy	89.09	19.41a	59.29b	2.60b	135.86b
Altinay	90.26	18.46b	60.35a	2.67a	145.76a
Mean Irrigation (D)					
%25	63.28d	16.83c	37.21d	2.56b	139.40b
%50	85.61c	20.97a	56.11c	2.68a	136.65b
%75	100.06b	19.22b	65.95b	2.72a	144.42a
%100	109.76a	18.73b	74.01a	2.59b	142.68a
LSD values					
A	4.07**	1.27**	3.86**	ns	5.65**
B	2.65**	0.46**	1.92**	0.12**	2.78**
C	ns	0.45**	2.40**	0.04**	2.19**
D	2.36**	0.64**	3.40**	0.06**	3.09**
A*B*C	4,.10**	ns	5.89**	ns	5.36**
A*B*D	5.80**	1.58**	8.34**	0.16**	7.58**
A*C*D	ns	1.29**	6.80**	0.13**	6.16*
B*C*D	ns	ns	8.34**	0.16**	7.58*
A*B*C*D	ns	ns	11.37**	ns	10.67**

ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level

Unal and Onder (2008) found plant height averages between 90.67-119.90 cm, Bateman et al. (2020) between 38.1- 142.2 cm, Cevheri and Yilmaz (2018) between 87.00-112.00 cm in a study conducted with soya bean lines. Boydak et al. (2018), in their study on the yield characteristics of 12 different soybean varieties, reported that plant height values were determined as 79.37-126.07 cm in the first year and 67.90-102.80 cm in the second year and found in agreement with our values.

First Pod Height (cm)

First pod height is an important parameter in soybean cultivation and in mechanized harvesting, it is important that the first pod is high in order to minimize harvest loss (Gizlenci et al., 2005). First pod height is positively correlated with plant height but negatively correlated with seed weight, number of seeds pod^{-1}, number of seeds plant^{-1} and number of pods plant^{-1} (Oz et al., 2009).

The effects of year, inoculant, variety, and irrigation on first pod height were found to be statistically significant ($p \leq 0.01$). Upon analysis of the mean values presented in Table 1, it was determined that the first pod height was 20.68 cm in the initial year of the experiment. In the second year, a reduction in the initial pod height was observed, with a mean value of 17.20 cm. The positive effect of irrigation applications was observed in the 50% water application group (20.97 cm), and the results obtained from the 75% and 100% water application groups were statistically similar (Table 1). The results were obtained within the expected range for the first pod height values of the varieties. A comparison of the values obtained in the experiment with those from previous studies revealed similarities. The first pod height was determined to be between 9.67 and 20.33 cm by Unal and Onder (2008), between 12.4 and 22.17 cm by Yetkin and Arıoglu (2010), and between 12.86 and 19.37 cm by Ozer (2021) in soybean varieties. The values obtained in our experiment were found to be in agreement with those reported in previous studies.

Number of pods plant^{-1}

The effects of year, variety, inoculant, irrigation, and the interaction between year, inoculant, variety and irrigation on pod number per plant were found to be statistically significant ($p \leq 0.01$). Significant differences were observed between the mean values of the experimental years. As illustrated in Table 1, the mean number of pods per plant was 76.73 in 2021, while the mean number of pods per plant in 2020 was 39.91.

This notable discrepancy is believed to be attributable to the deleterious impact of elevated temperatures experienced during the flowering phase in 2020 (Figure 2). It was observed that the application of irrigation resulted in a notable variation in the mean number of pods per plant.

The highest mean number of pods per plant was observed in the 100% water treatment (74.01 pods plant^{-1}), representing the average non-restricted water treatment. As the quantity of irrigation increased, the number of pods plant^{-1} also increased. The onset of water stress at the beginning of the flowering period has been observed to result in a reduction in the number of pods produced. Stress experienced subsequent to the onset of flowering and throughout the period of full bloom has been demonstrated to result in a reduction in both pod number and grain size. Limited irrigation has been demonstrated to result in a notable decline in grain yield and its constituent components (Korte et al., 2023). Additionally, statistical differences were observed in the number of pods produced under different *Rhizobia* inoculation applications. The application of the USDA-110 inoculant resulted in a higher mean number of pods plant^{-1} (62.96) than the control application (54.41). Gumus and Beyyavas (2020) reported that the number of pods in soybean varieties ranged from 81.50 to 133.90. Kumagai et al. (2022) reported a pod count of 57-101. Cevheri and Yilmaz (2018) reported a pod count of 103 in soybean under semi-arid climate conditions. The number of pods per plant was reported by Ilker et al. (2010) to be 50.92-111.11, by Boydak et al. (2018) to be 32.17-72.10, and by Surgun Acar (2022) to be 62.03-118.40. A comparison of the values in the literature with those obtained in our study reveals a high degree of consistency.

Number of seeds pod^{-1}

The impact of water stress on soybean yield is dependent on the developmental stage at which it occurs. While water stress can reduce yield at all developmental stages, the severity of this effect varies depending on the specific stage of development that is affected. Flowering is susceptible to drought stress, which can result in flower shedding. However, since flowering occurs over an extended period (approximately 3-4 weeks) along the length of the stem, it is possible to offset losses resulting from flower shedding. This evidence suggests that drought stress during the pod-setting period may be more severe than during the flowering period (Mandic et al., 2015).

The analysis revealed that the inoculant*variety*irrigation interaction was a statistically significant factor ($p \leq 0.01$) influencing the number of seeds per pod. In particular, the variety and irrigation factors were found to exert a significant influence on the number of seeds per pod. Table 1 illustrates that there is no statistically significant difference in the mean number of seeds per pod across the trial years. The application of the *Rhizobia* inoculant resulted in a significantly higher number of seeds pod^{-1}, with an average of 2.65 seeds pod^{-1}, compared to the control application, which yielded an average of 2.51 seeds pod^{-1}. It was observed that 50% limited irrigation (2.68 per plant) may be sufficient to achieve the highest value for the number of seeds pod^{-1}. The Altinay variety exhibited a higher seed number (2.67) than the Cinsoy variety (2.60). The primary discrepancies were noted during the final stages of pod formation and the initial phase of seed filling in both experimental years (BBCH 75-85). In 2021, the temperatures recorded at the conclusion of the pod setting period (August 10) and the onset of seed filling (August 22) resulted in notable discrepancies. During the specified period, daily maximum temperatures in 2021 exceeded 40°C, whereas in 2020, these values ranged between 35 and 37°C. The duration of phenological stages that coincided with these months was shortened due to the elevated temperatures, resulting in a reduction in maturity time in the initial year. Drought stress can be particularly impactful during the flowering, seed formation, and seed filling stages. The reduction in seed size is attributed to the shorter period of seed filling and the earlier onset of ripening (Ashley and Ethridge, 1978). Boydak et al. (2018) observed that the number of seeds pod^{-1} in soybeans was 2.78 in the first year and 2.83 in the second year of his two-year study. In contrast, Altinyuzuk (2017) reported values between 2.23 and 2.83, Ilker et al. (2010) between 2.83 and 3.00, Kobraee et al. (2011) between 2.3 and 3.5, and Shamima and Farid (2006) obtained values between 2.2 and 3.0. The number of seeds pod^{-1} obtained in our study is comparable to the values reported in other studies. Furthermore, Kumagai et al. (2022) reported that the number of seeds in soybean pods ranged from 1.62 to 2.07 and Surgun Acar (2022) from 1.99 to 2.54. These values were generally below those obtained in our study.

1000 seed weight (g)

Extreme environmental conditions, such as drought, accelerate the transition from the vegetative to the generative phase in plant development. This phenomenon is evidenced by a reduction in the duration of the generative and grain filling periods, which can be attributed to elevated temperatures and restricted irrigation. Consequently, there is a decline in photosynthesis and nutrient production, as well as a reduction in grain weight and, consequently, yield (Yigit and Chmielewski, 2024). The abiotic stress conditions experienced during the grain filling period has been demonstrated to result in a reduction in grain size, which in turn leads to a decline in grain weight and yield (Desclaux et al., 2000). The study revealed that during the experimental years, limited irrigation, variety, and inoculant treatments resulted in notable variations in the 1000-seed weight of soybean (Table 1). The highest 1000 seed weight was observed in 2020 (147.35 g), while the lowest 1000 seed weight was recorded in 2021 (134.27 g). It can be posited that the reason for this is that the elevated temperatures experienced during the pod-setting period in the second year of the experiment had an adverse impact on the filling period, resulting in a reduction in seed weight. A comparison of the varieties yielded mean values of 135.86 g for the Cinsoy variety and 145.76 g for the Altinay variety. The experiment revealed significant effects of average limited irrigation treatments (50 and 25%) on 1000-seed weight. Irrigation applications resulted in a range of 1000 seed weight values, with the highest observed in the 75% and 100%irrigation amount (144.42 and 142.68 g) and the lowest in the 25% and 50% limited irrigation amount (139.40 and 136.65 g), respectively.

In the context of the USDA-110 inoculant application, the highest value was observed among the inoculant applications, reaching a value of 145.16 g. Upon examination of the studies conducted, it was found that the 1000-seed weight values of soybean ranged from 135.4 to 167.4 g (Yetkin and Arioglu, 2010), and from 140.5 to 180.4 g (Ilker et al., 2010). Boydak et al. (2018) obtained 1000 seed weight values of 117.10 to 156.96 g in the first year and 100.71 to 128.18 g in the second year in the soybean experiment. The data obtained in the present study are comparable to those of previous studies conducted in this area.

Seed yield (kg ha^{-1})

Restricted irrigation conditions result in a linear reduction in the average soybean yield. This is attributed to the shortening of the vegetative and generative periods caused by drought stress (Borowska and Prusiński, 2021). The period during which soybeans require the greatest quantity of water is that of flowering and pod maturation. Furthermore, this period is regarded as one of the most crucial due to the fact that insufficient water during the seed filling phase results in a reduction in the number of seeds and seed weight within the pod, ultimately leading to a decline in yield (Staniak et al., 2023).

The study revealed that the effects of year, inoculant, irrigation, and the interaction between inoculant, variety and irrigation were of particular significance ($p \leq 0.01$). The irrigation amounts resulted in notable alterations in seed yield. As illustrated in Table 2, the mean seed yield exhibited a notable increase from 441.5 kg ha^{-1} in limited irrigation (25%) to 3086.7 kg ha^{-1} in 100% full irrigation. Significant differences were observed in the mean yield values among the trial years. In 2020, the mean seed yield was 1700.7 kg ha^{-1}, while in 2021, it increased significantly to 2165.6 kg ha^{-1}.

It is hypothesized that increased irrigation amounts result in notable increases in seed yield, attributed to the elevated temperatures observed during the trial years, which frequently exceed the norm. Additionally, the impact of intermittent periods of exceptionally high daily temperatures is a contributing factor. In applications of *Rhizobia* inoculant, the highest grain yield was obtained with the USDA-110 inoculant (2083.1 kg ha^{-1}). In studies conducted with soybean, seed yields have been reported to vary considerably. For instance, Kresovic et al. (2017) reported yields between 1630 and 3210 kg ha^{-1}, while Kabraee et al. (2011) reported yields between 2266.9 and 3700.1 kg ha^{-1}. Ozer (2021) reported yields between 1434 and 3801 kg ha^{-1}, while Feng et al. (2022) reported yields between 1600 and 4100 kg ha^{-1}. Istemil (2015) reported that the grain yield of soybean exhibited considerable variation, with values ranging between 2709 and 3553 kg ha^{-1}. Kumagai et al. (2022) observed a similar trend, with soybean grain yield values ranging between 2570 and 3640 kg ha^{-1}. Similarly, Gojic et al. (2018) reported that the highest grain yield was achieved when 100% water was applied to soybean in four distinct irrigation treatments (control, 40%, 60%, and 100%). The highest yield was observed in the 100% water treatment, with a value of 3690 kg ha^{-1}.

Table 2. Average values of yield, leaf area and quality traits for the combined trial years

Factor	Seed Yield (kg ha^{-1})	Leaf Area (FP) (cm^2 plant^{-1}) (BBCH 65)	Leaf Area (PD) (cm^2 plant^{-1}) (BBCH 75)	Crude Protein (%)	Crude Oil (%)
Mean Year (A)					
2020	1700.7b	1061.64	1699.19	35.47a	20.85b
2021	2165.6a	1764.22	1691.01	32.59b	22.67a
Mean Bacteria (B)					
Control	1719.6b	1611.21a	1906.99a	32.65	22.36a
Rhizobium	1996.7a	1601.91a	1497.74b	34.48	21.55b
USDA 110	2083.1a	1025.67b	1680.57ab	34.59	21.38b
Mean Variety (C)					
Cinsoy	1942.1	1324.56	1661.63	34.21	21.10b
Altinay	1924.1	1501.30	1728.54	33.81	22.42a
Mean Irrigation (D)					
%25	441.5d	927.67c	1042.96c	35.00a	21.54
%50	1795.4c	1551.15ab	1467.31b	33.85ab	21.84
%75	2409.0b	1338.71b	2219.84a	34.47a	21.76
%100	3086.7a	1843.19a	2050.29a	32.72b	22.30
LSD values					
A	75.8**	ns	ns	0.97**	0.97*
B	158.7**	308.85**	287.94*	ns	0.41**
C	ns	ns	ns	ns	0.59**
D	118.6**	347.56**	365.69**	1.59*	ns
A*B*C	ns	ns	633.40**	ns	ns
A*B*D	290.6**	602.00**	895.77*	ns	ns
A*C*D	237.3*	ns	ns	ns	11.88*
B*C*D	290.6**	ns	ns	ns	ns
A*B*C*D	ns	ns	ns	ns	ns

ns: non-significant; *: significant at 0.05 level; **: significant at 0.01 level; Leaf Area (FP): Leaf Area amount in flowering period; Leaf Area (PD): Leaf Area amount in pod formation period

Leaf area (cm² plant⁻¹)

Leaf area is an important factor affecting light retention and biomass production in plants (Yao et al., 2017). Reducing the leaf area of the plant under drought and water stress conditions is considered as a strategy to reduce water loss in stress conditions. This situation leads to a decrease in biomass accumulation in soybeans in the following stages (Dong et al., 2019). It is known that drought conditions during the vegetative period reduce morphological characteristics such as leaf area, plant height, and first pod height (Staniak et al., 2023).

Leaf area amounts were quantified on two occasions during the course of the experiment, once during the flowering period (Leaf Area $_{FP}$) and once during the pod formation period (Leaf Area $_{PD}$). Table 2 illustrates the statistically significant impact of restricted irrigation and *Rhizobia* inoculant applications on leaf area amounts during the flowering period (BBCH 65). The observed effects were significant at the $p \leq 0.01$ level. As irrigation amounts increased, a linear increase in leaf area was observed in soybean plants. The greatest leaf area was observed in the treatment group that received 100% irrigation, with a value of 1843.19 cm² plant^{-1}. In the case of *Rhizobia* inoculant applications, the highest leaf area was observed in the control application, while the AZOTEK-2 bacteria application was found to be statistically similar to the control group (1611.21 cm² plant^{-1}). The effects of irrigation and *Rhizobia* inoculant applications on leaf area measurements taken during the pod formation period (BBCH 75) were found to be statistically significant (Table 2). The greatest quantity of leaf area (2219.84 cm² plant^{-1}) was observed in the irrigated condition (75% irrigation). Drought conditions restrict stomatal conductance, which in turn impairs photosynthetic activity. Furthermore, the leaf areas of plants subjected to water stress are markedly inferior to those of unstressed plants (Mangena, 2018).

In a study investigating the effects of inoculants and different doses of nitrogen fertilizer applications on yield and growth in soybeans, the combination of *Rhizobia* application and 50% nitrogen fertilizer application yielded the most optimal results. In the study, the leaf area per plant was determined to be 138.75 cm² (Herliana et al., 2019).

Protein Content (%)

Restricted irrigation conditions result in a deficiency of water in the tissues, which in turn impedes a number of physiological processes, including photosynthesis, transpiration, and stomatal conductance. This situation has an impact on plant growth and development, as well as seed yield and grain composition (Staniak et al., 2023). In arid conditions, there is a decrease in the ratio of crude oil and an increase in the protein content of soy seeds. These ratios change linearly with an increase in stress (Sobko et al., 2020). Ravelombola (2022) posited that drought can impact protein structure and protein synthesis. Additionally, the decomposed proteins are hindered from transferring amino acids to the leaf due to the effects of hydration.

As demonstrated in Table 2, the factors of year ($p \leq 0.01$) and irrigation ($p \leq 0.05$) were found to have a statistically significant impact on protein values. The mean protein values were found to be 35.47% in 2020 and 32.59% in 2021 among the trial years. The highest protein value among the irrigation amounts was observed in the 25% limited irrigation treatment (35.00%). As reported by Poeta et al. (2016), an increase in protein content was observed to be positively and linearly related to water stress and negatively related to oil content. While Unal and Onder (2008) reported a grain protein ratio of 34.40-38.61%, and Devi et al. (2013) reported a ratio of 34.40-36.71%, Cevheri and Yilmaz (2018) reported a protein ratio of 39.31-41.74%. Alsajri et al. (2020) reported that the oil and protein content of the seed is closely related to environmental factors, particularly with increasing temperature during the growth period, which has been observed to result in increased oil content and decreased protein content. The results of our study indicate that the highest protein content was achieved with a 25% limited irrigation amount, while the highest crude oil content was observed with a 100% full irrigated condition. These findings are largely consistent with those of other significant studies in the field.

Crude oil content (%)

High daily temperatures reduce oil content and increase protein content in soybean seeds (Gibson and Mullen,1996; Dornbos, 2020). It is established that water limitation influences the nutrient content of soybean seeds and accumulation to seeds during the flowering and seed development stages. Borowski and Michalek (2014) reported a decrease in crude oil content (13.8%) and an increase in grain protein content (6.2%) in soybean varieties with limited irrigation under drought conditions. The statistical analysis revealed that the applications of year ($p \leq 0.05$), variety and inoculant ($p \leq 0.01$) had a statistically significant impact on crude oil ratios (Table 2). Additionally, significant discrepancies were observed between the trial years. The mean crude oil content was 20.85% in 2020 and 22.67% in 2021. The differences in crude oil content between the two experimental years was approximately 2%. The effects of the *Rhizobia* inoculants on the crude oil ratio in the seed exhibited statistical differences, with the highest crude oil ratio observed in the control plot (22.36%). The applications had a notable impact on the variety averages. The mean crude oil ratio for the varieties was determined to be 21.10% for the Cinsoy variety and 22.42% for the Altinay variety.

The impact of restricted irrigation on the crude oil value of soybean was found to be relatively similar. The average crude oil contents of the irrigation treatments varied very little and exhibited similar results. Upon examination of previous studies on crude oil ratios, it was found that the crude oil ratio value was reported to be between 19.15 and 19.89% by Temory (2014), between 16.66 and 19.30% by Ay (2012), 18.65% by Kılınc and Arioglu (2018), and between 7.20 and 18.60% by Gaweda et al. (2017). In comparison to previous studies, our study yielded higher oil content values (20.85-22.36%), and our findings indicate that oil content is not affected by irrigation treatments.

CONCLUSION

The objective of this study was to investigate the nodule formation performance of *Rhizobia* strains in soybean, a crop of paramount importance to sustainable agriculture, under conditions of limited and irrigated, as well as to evaluate the performance of soybean varieties in terms of yield and quality under conditions of Mediterranean climate. The study revealed significant insights into the interactions between the yield and quality potentials of different applications and related high temperatures in soybean varieties grown in the Aydin province. The experimental results demonstrated that the nodule formation performance of the *Rhizobia* inoculant was markedly influenced by irrigation practices and high temperatures. In the first year of the experiment, only USDA 110 *Bradyrhizobium* inoculant was applied, resulting in enhanced nodule formation in both the Cinsoy and Altinay varieties when 75% to 100% irrigation amount applied. However, almost no nodulation history was observed in any of the plants evaluated. This phenomenon was observed in a limited number of sampled areas and only in a subset of plant roots. It was observed that daily high temperatures were experienced in both trial years. However, the 2021 trial year exhibited longer and higher daily maximum temperatures in comparison to the 2020 trial year. It is hypothesized that during certain periods of plant development when elevated temperatures are present, *Rhizobia* inoculants can infect soybean roots, thereby affecting their capacity to form nodules. It has been observed that the average values obtained are significantly affected by limited water supply and varieties. The statistical significance ($p \leq 0.01$) of variety and irrigation in many parameters examined depending on the applications revealed that yield and quality traits were not influenced by a single factor. The highest values obtained in the examined parameters indicated that 100% irrigation amount was optimal, with the Altinay variety demonstrating superior performance. Furthermore, the global average yield for soybeans was achieved with a 75% irrigation amount in our experiment (2409.0 kg ha^{-1}).

The results demonstrated that a sufficient average yield can be achieved with 75% irrigation application (612.73-644.05 mm) under the ecological conditions of the limited water supply regions. Upon examination of both experimental years, it was observed that the period of highest water consumption (combined with high temperature) in soybeans was the developmental phase between the pod formation period and the seed filling period. The study revealed important results, especially regarding the significant effects of high temperatures and limited water applications on *Rhizobia* inoculation and nodulation performance during the vegetative period and the end of pod formation and grain filling periods.

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