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THE INFLUENCE OF RHIZOBIUM APPLICATION ON THE MORPHOLOGY AND PHYSIOLOGY OF SOYBEAN (*GLYCINE MAX* L. MERRILL) UNDER DROUGHT STRESS CONDITIONS

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ABSTRACT

Soybean (Glycine max L.) is a leguminous plant of significant economic value in providing food and animal feed. However, its productivity is vulnerable to drought stress, which can affect plant growth, metabolism, and osmotic balance. Drought is a major abiotic factor influencing soybean production. This study aims to determine the effects of soybean varieties and Rhizobium japonicum inoculation, as well as the interaction of treatments, on the morphological and physiological responses of soybean plants. The study utilized a Split-Plot Design (SPD) in a Randomized Complete Block Design with three replications. The main plot factor was Soybean Variety (V), consisting of three levels: V_1 (Dega-1), V_2 (Dering-1), V_3 (Devon). The subplot factor was Rhizobium japonicum Inoculation Rate (R), consisting of three levels: R_0 (Without Rhizobium japonicum inoculation), R_1 (3 g/kg soybean seed), R_2 (6 g/kg soybean seed). The observed variables were plant height, leaf number, plant dry weight, shoot dry weight, and root length. The results showed that the variety treatment influenced morphological traits (plant height) and physiology (root length), while Rhizobium japonicum inoculation treatment only affected physiology (root length). The interaction between variety and Rhizobium japonicum inoculation treatments only influenced physiology (root length). Application of 3 g Rhizobium japonicum showed the best effect on enhancing soybean morphological and physiological traits.

KEYWORDS: Plant morphology and physiology, drought stress, soybean varieties.

INTRODUCTION

Soybean, also known as *Glycine max* L., is one of the leguminous plants with significant economic importance. This plant plays a crucial role in providing over a quarter of the food and animal feed needs. However, its productivity often decreases significantly, especially when facing drought pressure. According to (Wang *et. al.*, 2022), domestic soybean production is weakening due to the issue of drought stress, which affects all aspects of plant growth and metabolism, including osmotic balance.

Drought is one of the abiotic factors that significantly impacts soybean production. The importance of drought tolerance has been recognized as a primary area requiring development and enhancement in plant breeding. Although soybean cultivation generally relies on natural rainfall patterns, irregular fluctuations in rainfall can result in significant yield differences in the same agricultural area. Soybean is highly vulnerable to drought, especially during growth phases associated with reproduction (Igiehon *et. al.*, 2019).

Water deficit conditions can reduce the weight and dimensions of soybean seeds during the seed filling process, depending on the timing, duration, and severity of the water deficit. A greenhouse study noted that soybean plants experiencing severe drought at certain stages in their life cycle showed a decrease in individual seed weight, from 0.21 grams to 0.18 grams in one trial, and from 0.20 grams to 0.17 grams in another trial (Sheteiwy *et. al.*, 2021). Another study indicated that soybean plants experiencing drought stress before flowering produced more seeds than those stressed after flowering, as plants experiencing drought at early stages had developed a larger root system before flowering as an adaptation response (Buezo *et. al.*, 2019). Therefore, during flowering and seed filling processes, soybean plants are highly sensitive to water deficit. However, water deficit during pod growth and seed filling stages has a significant negative impact on final yield and yield components (Huang *et. al.*, 2023). Hence, it is crucial to develop practical strategies to mitigate the adverse effects of water deficit on soybean production.

Plant growth-promoting microorganisms such as *rhizobium* can enhance plant resilience to various environmental stresses by increasing the availability of mineral nutrients and water absorption, thereby improving harvest yields (Begum *et. al.*, 2019). Additionally, *rhizobium* also has the ability to influence water balance in adequately irrigated host plants as well as in plants experiencing drought. *Rhizobium* can alter stomatal conductivity, which in turn enhances plant tolerance to drought stress (Tarnabi *et. al.*, 2019), and reduce damage from oxidative stress by stimulating both enzymatic and non-enzymatic antioxidant activities. Furthermore, the utilization of *Rhizobium* can also alter the proline content in leaf tissues (El-Sawah *et. al.*, 2021).

The crucial role of *Rhizobium* in agriculture is primarily attributed to its ability to perform biological nitrogen fixation, which leads to a reduction in the need for chemical nitrogen fertilizers (Mng'ong'o *et. al.*, 2023). However, several factors related to the host plant, bacterial strain, and soil conditions, especially drought, can limit the contribution of nitrogen fixation to plant growth (Tang *et. al.*, 2019). Recent research indicates that soil inoculation with *Rhizobium* strains can enhance nodulation, nitrogen uptake, and yields of leguminous crops (Liu *et. al.*, 2019).

MATERIAL AND METHODS

The research was conducted in a plastic greenhouse located at Jl. Karya Gg. Wonogiri, Medan, with an elevation of 25 meters above sea level (masl), starting from January 2023 until completion. The materials used in this study included soybean seeds of Grobogan, Dering-1, and Devon varieties, polybags sized 30x40 cm², bamboo, UV plastic, raffia rope, urea fertilizer, SP-36, KCL, and other supporting materials. The tools used included a hoe, meter, water sprayer, soil sampler, standard stake, scale, measuring container, measuring cup, manual Vernier caliper, hand sprayer, writing tools, calculator, knife, ruler, and other tools supporting the implementation of this research. The research method used was the Split-Plot Design (SPD) in a Randomized Complete Block Design replicated three times. The first factor as the main plot was Soybean Variety (V) consisting of 3 levels: V_1: Dega-1, V_2: Dering-1, V_3: Devon. The second factor as the subplot was *Rhizobium japonicum* Inoculation Rate (R) consisting of 3 levels: R_0: Without *Rhizobium japonicum* inoculation, R_1: 3 g/kg soybean seed, R_2: 6 g/kg soybean seed.

Preparation of Growing Media

The soil is cleaned and dried for 7-14 days, then placed in polybags sized 30 cm x 40 cm with a quantity of 10 kg after sieving, and given 2.5 g of dolomite per polybag (500 kg/ha), followed by incubation for 3 weeks.

Determination of Field Capacity Water Volume

The determination of field capacity water volume is conducted prior to the research by filling soil into the polybags that will be used as planting media according to the polybag weight, which is 3 kg. Then, watering is performed until saturation condition is reached (water dripping from the holes of the polybags). Next, it is left for 48 hours, and the surface of the polybags is covered with organic mulch derived from surrounding plant residues. After 48 hours, the weight of the wet soil is measured, then the soil inside the polybags is removed and dried in an oven for 48 hours at 105°C. Next, the weight of the dry soil is measured.

The field capacity water volume is the wet soil weight without the polybag minus the oven-dry soil weight without the polybag. For example, if the wet soil weight without the polybag is 3 kg, while the oven-dry soil weight without the polybag is 2.7 kg, then the field capacity water volume is 3 kg - 2.7 kg = 0.3 kg = 0.3 L = 300 mL.

Planting and Fertilization

Before planting, seed selection is carried out, using uniform-sized seeds (without flattening or defects). Planting is done using a dibble to a depth of 3 cm. Two seeds are then placed in each hole, followed by covering with soil. The planting distance is 40 cm between rows and 10-15 cm within rows (Badan Penelitian dan Pengembangan Pertanian, 2016).

Fertilization is done by placing it around the planting hole at a distance of 7-10 cm. The fertilizer dosage includes urea at 75 kg/ha (0.37 g/polybag), SP-36 at 250 kg/ha (1.25 g/polybag), and dolomite at 2 tons/ha (10 g/polybag). The fertilizer application is based on soil analysis results indicating low levels of N, P, K, and pH, thus recommending fertilization at high dosages.

Drought Stress

Drought stress begins at stage V_2 (full open trifoliate leaves on the second node above unifoliolate) until stage R5 (seed size within pod reaches 3 mm on one of the main stem nodes), with watering frequencies according to treatments, namely, daily, every 3 days, and every 6 days.

Weeding and Thinning

Weeding is carried out manually by pulling out weeds. Thinning is done at 2 WAP by cutting the lower stem of poorly growing plants, leaving one plant per polybag.

Pest and Disease Control

Pest infestation is controlled by applying insecticide containing the active ingredient Fipronil at a concentration of 2 mL/L of water, applied in the evening at 4, 6, and 8 WAP.

Harvesting

Harvesting is done by cutting 5 cm above the base of the main stem using scissors. The criteria for harvesting are indicated by most of the leaves turning yellow but not due to pest and disease infestation, the stems are somewhat yellowish-brown, the pods are firm when pressed, and the pod skin has turned yellowish-brown by 95%. The harvested pods are sun-dried for 4 days, and the seeds are removed from the pods (Simanjuntak *et. al.*, 2015).

Observation variables

The observed variables are plant height, leaf count, plant dry weight, shoot dry weight, and root length.

RESULT AND DISCUSSION

Plant Height

Statistical analysis results indicate that the variety treatment significantly influences soybean plant height, while *Rhizobium* inoculation and interactions between treatments have non-significant effects on soybean plant height (Table 1).

Table 1: Plant Height (cm) of Three Soybean Varieties with Rhizobium japonicum Inoculation.

Treatment	Observation Time (WAP)	
	2 WAP	4 WAP
Dega-1 (V1)		
R0 (0 g)	58,51	116,78
R1 (3 g)	63,38	125,83
R2 (6 g)	67,60	125,39
Rataan (V1)	63,16a	122,67a
Dering-1 (V2)		
R0 (0 g)	40,04	83,06
R1 (3 g)	35,72	76,00
R2 (6 g)	36,89	82,39
Rataan (V2)	37,55b	80,48b
Devon (V3)		
R0 (0 g)	41,59	77,23
R1 (3 g)	42,21	74,67
R2 (6 g)	43,38	74,39
Rataan (V3)	42,39b	75,45b

Note: Numbers in the same column followed by different letters indicate significantly different at the 5% level based on the LSD test.

Table 1 presents soybean plant height data at ages 2 and 4 WAP. The variety treatment significantly influences soybean plant height, with the tallest soybean plants obtained in the Dega-1 (V_1) variety treatment, at 63.16 cm at 2 WAP and 122.67 cm at 4 WAP, which are significantly different from the Dering-1 (V_2) and Devon (V_3) variety treatments.

Significant differences in soybean plant height among variety treatments are due to each variety's different morphological characteristics. According to DPKP DIY (2023), the Dega-1 variety exhibits the tallest plant height compared to the Dering-1 and Devon varieties. According to Arsyad *et. al.*, (2007), the ideal soybean plant type (plant-ideotype) with high yield potential and considered suitable in optimal environments typically has a plant height ranging from 60-70 cm. However, in the observations at 4 WAP, soybean plant height has exceeded the ideal plant height range for soybeans, ranging from approximately 74-77 cm for the Devon variety, 76-83 cm for the Dering-1 variety, and 116-125 cm for the Dega-1 variety. This is mainly due to environmental factors during the study.

Leaf Count

Statistical analysis results indicate that variety treatment, *Rhizobium* inoculation, and interactions between treatments have non-significant effects on leaf count (Table 2).

Table 2 presents soybean leaf count data at ages 2 and 4 WAP. The variety treatment with the highest leaf count is obtained in the Devon variety (V_3), while *Rhizobium japonicum* inoculation at 3 g (R_1) at 2 WAP and *Rhizobium japonicum* inoculation at 6 g (R_2) at 4 WAP also exhibit the highest leaf count. The interaction between the Devon variety and *Rhizobium japonicum* inoculation at 3 g (V_3R_1) results in the highest leaf count, at 13.56 leaves at 2 WAP. Meanwhile, the interaction between the Devon variety and *Rhizobium japonicum* inoculation at 6 g (V_3R_2) yields the highest leaf count, at 26.78 leaves at 4 WAP.

Table 2: Leaf Count of Three Soybean Varieties with *Rhizobium japonicum* Inoculation.

Treatment	Observation Time (WAP)	
	2 WAP	4 WAP
Dega-1 (V1)		
R0 (0 g)	12,67	23,00
R1 (3 g)	13,33	23,67
R2 (6 g)	12,67	22,44
Rataan (V1)	12,89	23,04
Dering-1 (V2)		
R0 (0 g)	11,00	22,78
R1 (3 g)	12,33	22,56
R2 (6 g)	11,89	23,22
Rataan (V2)	11,74	22,85
Devon (V3)		
R0 (0 g)	12,89	25,67
R1 (3 g)	13,56	25,44
R2 (6 g)	12,67	26,78
Rataan (V3)	13,04	25,96

Note: Numbers in the same column followed by different letters indicate significantly different at the 5% level based on the LSD test.

Inoculation with *R. japonicum* has a non-significant effect on vegetative growth parameters such as leaf count; however, it has been shown to increase both parameters compared to non-inoculated plants. The highest soybean leaf count is obtained with 6 g of *R. japonicum* inoculation. These findings are consistent with Purwaningsih's research (2015), indicating that *Rhizobium* inoculation can enhance soybean growth compared to non-inoculated soybeans. The increased soybean plant height with *Rhizobium* inoculation is attributed to the effective root nodules in binding N nutrients to support plant growth (Erwin and Mindalisma, 2022). Adisarwanto and Wudianto (2008) revealed that soybeans require a sufficient amount of nitrogen for their growth process. As known, N nutrients can be absorbed by plants directly through the root system and also absorbed through N_2 fixation performed by *Rhizobium* bacteria that symbiotically interact with soybean plants. This is consistent with Adijaya *et. al.*, (2010), stating that nitrogen required by soybean plants originates from the soil and the atmosphere. Nitrogen from the atmosphere is absorbed by soybean plants through symbiosis with *Rhizobium* bacteria. These bacteria form root nodules on soybean roots, and through these nodules, *Rhizobium* bacteria perform N2 fixation from the air, which can then be utilized by the plant. The results of nitrogen fixation are utilized to meet the N requirements needed by soybean plants.

Plant Dry Weight

Statistical analysis results indicate that variety treatment, *Rhizobium* inoculation, and interactions between treatments have non-significant effects on plant dry weight (Table 3).

Table 3 presents soybean plant dry weight data. The variety treatment with the highest plant dry weight is obtained in the Dering-1 variety (V_2) and without *Rhizobium japonicum* inoculation (R_0). The interaction between the Dering-1 variety and no *Rhizobium japonicum* inoculation (V_2R_0) results in the highest plant dry weight, which is 14.00 g.

Table 3: Plant Dry Weight of Three Soybean Varieties with *Rhizobium japonicum* inoculation.

Treatment	Plant Dry Weight
Dega-1 (V1)	
R0 (0 g)	7,00
R1 (3 g)	11,33
R2 (6 g)	8,00
Rataan (V1)	8,78
Dering-1 (V2)	
R0 (0 g)	14,00
R1 (3 g)	7,67
R2 (6 g)	11,00
Rataan (V2)	10,89
Devon (V3)	
R0 (0 g)	6,33
R1 (3 g)	7,67
R2 (6 g)	7,33
Rataan (V3)	7,11

Note: Numbers in the same column followed by different letters indicate significantly different at the 5% level based on the LSD test.

Inoculation with *R. japonicum* has a non-significant effect on several physiological parameters such as plant dry weight, shoot dry weight, root dry weight, and root-to-shoot ratio. Treatment without inoculation exhibits higher plant dry weight, shoot dry weight, root dry weight, and root-to-shoot ratio compared to inoculation treatment. This indicates that *R. japonicum* inoculation has not been effective in enhancing soybean physiological parameters. This inefficacy may be due to several factors affecting the effectiveness of *Rhizobium* in plants, such as competition with native microbial populations and biotic agents such as diseases and insects (Afzal *et. al.*, 2019); the lack of limiting factors in the soil, such as phosphorus and various micronutrients (Kleiber and Tomosa, 2010), and low soil pH (below 5.0). Additionally, the efficiency of *Rhizobium* inoculum also varies for specific legume species (Lindstrom and Mousavi, 2020).

Shoot Dry Weight

Statistical analysis results indicate that variety treatment, *Rhizobium* inoculation, and interactions between treatments have non-significant effects on shoot dry weight (Table 4).

Table 4 presents soybean shoot dry weight data. The variety treatment with the highest shoot dry weight is obtained in the Dering-1 variety (V_2) and without *Rhizobium japonicum* inoculation (R_0). The interaction between the Dering-1 variety and no *Rhizobium japonicum* inoculation (V_2R_0) results in the highest shoot dry weight, which is 9.35 g.

Generally, reduced soil water availability can affect the vegetative growth of soybean plants, leading to inhibited shoot growth. However, the results of this study indicate an increase in several physiological parameters such as plant dry weight, shoot dry weight, and root dry weight with the inoculation of *R. japonicum* under drought stress, resulting in an increase compared to without inoculation. This increase in dry weight is consistent with increased vegetative growth, subsequently increasing the fresh weight of the plant. It suggests that *Rhizobium* bacterial inoculation affects plant resilience mechanisms under drought stress through the production of secondary metabolites. Santi *et. al.*, (2011) explained that the increase in root and shoot wet weight parameters can occur due to bacteria's ability to produce high levels of exopolysaccharides, resulting in the accumulation of exopolysaccharides in the rhizosphere, which can increase water retention around the roots and be easily absorbed by the roots. Absorption of water in large amounts by plant cells can increase photosynthesis rates. Increased photosynthesis rates will enhance the formation of carbohydrates, which will in turn increase the growth of plant organs such as shoots, roots, and leaves, thereby increasing both wet and dry plant weights.

Table 4: Shoot Dry Weight of Three Soybean Varieties with *Rhizobium japonicum* inoculation.

Treatment	Shoot Dry Weight
Dega-1 (V1)	
R0 (0 g)	4,62
R1 (3 g)	7,51
R2 (6 g)	5,40
Rataan (V1)	5,85
Dering-1 (V2)	
R0 (0 g)	9,35
R1 (3 g)	5,19
R2 (6 g)	7,23
Rataan (V2)	7,26
Devon (V3)	
R0 (0 g)	4,22
R1 (3 g)	5,10
R2 (6 g)	4,92
Rataan (V3)	4,74

Note: Numbers in the same column followed by different letters indicate significantly different at the 5% level based on the LSD test.

Root Length

Statistical analysis results indicate that variety treatment, *Rhizobium* inoculation, and interactions between treatments have significant effects on root length (Table 5).

Table 5: Root Length of Three Soybean Varieties With *Rhizobium japonicum* Inoculation

Treatment	Root Length
Dega-1 (V1)	
R0 (0 g)	42,00
R1 (3 g)	68,00
R2 (6 g)	48,00
Rataan (V1)	52,67b
Dering-1 (V2)	
R0 (0 g)	84,00
R1 (3 g)	46,00
R2 (6 g)	66,00
Rataan (V2)	65,3 a
Devon (V3)	
R0 (0 g)	38,00
R1 (3 g)	46,00
R2 (6 g)	44,00
Rataan (V3)	42,67c

Note: Numbers in the same column followed by different letters indicate significantly different at the 5% level based on the LSD test.

Table 5 presents soybean root length data. The variety treatment with the highest root length is obtained in the Dering-1 variety (V_2) and without *Rhizobium japonicum* inoculation (R_0). The interaction between the Dering-1 variety and no *Rhizobium japonicum* inoculation (V_2R_0) results in the highest root length, which is 84.00 cm.

Inoculation with *R. japonicum* does not affect the increase in root length and volume. This is because the primary role of *Rhizobium* is to form root nodules that function to bind nitrogen nutrients. Consistent with the statement by Purwaningsih *et al.* (2021) that *Rhizobium* inoculation plays a crucial role in providing nitrogen nutrients by forming root nodules, which convert atmospheric nitrogen into absorbable nitrogen. Additionally, inoculation will significantly affect growth if there is compatibility between the inoculated *Rhizobium* and its host plant (Santos *et. al.*, 2019). Effective and efficient *Rhizobium* inoculation will be able to compete with native *Rhizobium* and synergize with its host plant (Blanco *et. al.*, 2010).

CONCLUSION

The variety treatment was able to exert influence based on morphological characteristics (plant height) and physiological traits (root length). Meanwhile, the inoculation treatment with *Rhizobium japonicum* only influenced physiology (root length). The interaction between variety treatment and inoculation with *Rhizobium japonicum* only affected physiology (root length). Application of *Rhizobium japonicum* at 3 g showed the best influence on enhancing the morphology and physiology of soybeans.

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