The Effectiveness of Plant Growth Promoting Rhizobacteria (PGPR) on the Growth of Root Nodules of Peanut Genotypes under Water Deficit Condition

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Abstract: The deficiency of water sources and soil fertility are obstacles to producing peanuts in dry land. One of effort to increase production on dry land is the utilization of PGPR biofertilizers. This study aimed to determine the growth of plants and root nodules of several peanut genotypes applied with PGPR, to understand the interaction between several peanut genotypes and PGPR on plant growth and root nodules, and to examine the relationship between water deficit conditions and the formation of root nodules. This experiment used a Complete Randomized Design-split split-plot design with the main plots being D_0 = optimal conditions (no water deficit) and D_1 = water deficit. The subplots were P_0 = without PGPR and P_1 = with PGPR. The sub-subplots consisted of five peanut genotypes: V₁ = Hypoma-I, V₂ = Domba, V₃ = Talam, V₄ = Bison, and V₅ = G300-II. The research result showed that the addition of PGPR resulted in higher plant growth compared to those without PGPR and had more effective root nodules in nitrogen fixation compared to treatments without PGPR. The application of PGPR and genotype did not show significant interaction on plant growth and root nodules under water deficit conditions. Water deficit significantly affected all parameters of plant growth and root nodules. PGPR significantly affected the parameters of leaf number rate, nodule number, and nodule fresh weight. Genotype significantly affected the plant height rate. The interaction of water deficit and genotype significantly affected the leaf number rate, while the interaction of water deficit and PGPR, and the interaction of PGPR and genotype did not significantly affect all observation parameters.

Keywords: PGPR; Root nodules; Water deficit.

Introduction

Drylands agroecosystems are closely related to limited water resources and low soil fertility. The low soil fertility triggers limited availability of nutrients, including nitrogen. Nitrogen is a part of essential macro-nutrients required by plants in large quantities to support plant growth, especially during the vegetative phase. Despite nitrogen being abundant in the atmosphere, constituting approximately 78%, plants cannot directly utilize it because nitrogen is not naturally in a mineral form (Siswanto, 2018). According to Lindsay (1979) cited in Putra et al. (2022), nitrogen is available to plants in two forms: ammonium (NH₄⁺) and nitrate (NO₃⁻). The availability of nitrogen in both forms, ammonium and nitrate, is limited as they are susceptible to loss through leaching (NO_{3}) and evaporation (NH_{4}) .

One approach to enhance the availability of nitrogen nutrients is through the use of biofertilizers. Biofertilizers are defined as materials containing inoculants of microorganisms that facilitate and enhance the availability of specific nutrients for plants (Marom et al., 2017 cited in Jannah et al., 2022). Currently, there is a wide variety of biofertilizers available, with one of the most discussed being Plant Growth Promoting Rhizobacteria (PGPR). PGPR comprises a group of soil microbes that colonize the rhizosphere (root area), including bacteria such as Rhizobium, Pseudomonas, Azobacter, Azospirillum, Acetobacter, and Bacillus, which are believed to contribute to the growth and development of plants (Chandraningtyas & Indrawan, 2023). The selection of

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PGPR biofertilizers aims to increase nitrogen availability in the soil, as PGPR contains nitrogen-fixing bacteria that can symbiotically interact with plant roots.

The symbiotic process between bacteria and plant roots cannot occur in all types of plant commodities, but only in plants from the leguminosae family. This is because only plants that can form nodules or root nodules can engage in symbiosis. The root nodules serve as sites for infection and fixation processes by microorganisms. Nitrogen fixation by microorganisms is carried out by binding free nitrogen in the atmosphere in the form of dinitrogen (N_2) and then fixing it by converting it into NH₄⁺ or NO₃⁻ that can be absorbed by plants (Astija et al., 2022). The success of nitrogen fixation by microorganisms is determined by the effectiveness of the formed root nodules. Imtiyaz and Octavia (2023) state that effective root nodules are pink or red, while green or pale white nodules indicate ineffective root nodules. Ineffectiveness of root nodules can fail the nitrogen nitrification process, as effective nodules require compatibility root between microorganisms and the host plant.

In this study, the tested plant is peanut (Arachis hypogaea L.), which belongs to the leguminosae family. Peanuts are known to have the ability to form root nodules with high capacity, depending on the variety and efficiency of microorganisms (Imtiyaz and Octavia, 2023). The effectiveness of PGPR for plant growth and root nodule formation in various peanut genotypes under water deficit conditions has not been widely studied. The aim of this research is to determine the growth and root nodules of several peanut genotypes applied with PGPR, to understand the interaction between various peanut genotypes and PGPR on plant growth and root nodules, and the relationship of water deficit conditions to root nodule formation.

Method

This experiment was conducted at the Plastic House Teaching Farm owned by Prof. Dr. Ir. A. Farid Hemon, M.Sc., in Sigerongan Village, Lingsar District, West Lombok Regency, West Nusa Tenggara. This study used materials such as peanut seeds, PGPR biofertilizer, NPK fertilizer (15:15:15), and soil. The tools used included polybags, scissors, hoes, bamboo, nails, wire, UV plastic, and stationery.

The design used in this research was a Complete Randomized Design (CRD) with a split-split plot design. The treatment levels were D_0 = optimum conditions (without water deficit) and D_1 = water deficit, subplots were P_0 = without PGPR and P_1 = with PGPR, and the sub-subplots consisted of five peanut ghenotypes, namely V_1 = Hypoma-I, V_2 = Domba, V_3 =

Talam, V_4 = Bison, and V_5 = G300-II. Each treatment was repeated 3 times.

Measurement of Plant Growth and Root Nodules

Measurements of growth and root nodules were made using 40 × 40 cm polybags filled with 10 kg of soil each. The seeds used were peanut seeds collected by Prof. Dr. Ir. Farid Hemon, M.Sc. Planting was done by making holes in the soil in the polybags to a depth of 3 cm, then 1-2 peanut seeds were placed in each hole and covered again with soil. PGPR was applied every 7 days at a dose of 250 ml/polybag. Drought stress treatment began by conditioning the planting media to field capacity from the start of planting until 5 days old. Subsequently, drought stress treatment was applied from seed germination (5 days after sowing) until harvest (60 days after sowing). Plants experiencing drought stress were watered to field capacity every 7-10 days (one day after 70% wilting symptoms appeared on the leaves). Fertilization was done by sprinkling NPK fertilizer (15:15:15) at a dose of 4.16 grams/polybag.

Harvesting was done when the plants were 60 days old. Observations were made on plants in polybags 4 times: at 15, 30, 45, and 60 days after sowing. Parameters observed included plant height (cm), number of leaves (sheets), number of nodules, nodule weight (g), root length (cm), dry root biomass (g), and dry plant biomass (g).

Data Analysis

The obtained data were analyzed using variance analysis (ANOVA) with a split-split plot model at a significance level of 5%. The results of the variance analysis showing significant differences were further tested with Duncan's Multiple Range Test (DMRT) at a significance level of 5%.

Result and Discussion

In this study, the observed characters included plant height growth rate, leaf number growth rate, number of nodules, nodule weight, root length, dry root biomass, and dry plant biomass. In Table 2, it can be seen that the response shown by all observed characters in the three independent factors, namely water deficit (D), PGPR (P), and genotype (V), varied greatly. The main plot deficit (D) showed that all observed characters differed significantly. In contrast, the subplot PGPR (P) showed significant differences in leaf number growth rate, number of nodules, and nodule weight, with the rest not significantly different. On the other hand, the sub-subplot genotypes (V) showed that all observed characters did not differ significantly except for the plant height growth rate.

Table 1. Summary of Anova Test Results for All Observed Parameters

Observation Parameters	Source of Diversity							
Observation rarameters	D	Р	V	DxP	DxV	PxV	DxPxV	
Plant height rate	S	NS	S	NS	NS	NS	NS	
Leaf number rate	S	S	NS	NS	S	NS	NS	
Nodule number	S	S	NS	NS	NS	NS	NS	
Nodule weight	S	S	NS	NS	NS	NS	NS	
Root length	S	NS	NS	NS	NS	NS	NS	
Root dry weight	S	NS	NS	NS	NS	NS	NS	
Plant dry weight	S	NS	NS	NS	NS	NS	NS	

Explanation: S = Significant, NS = Non Significant

Table 2. DMRT further test results at 5% rea	al level of inde	pendent factors
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Treatment Factor	LPTT	LPJD	JB (nodules)	BB (g)	PA (cm)	BBKA (g)	BBKT (g)
Deficit (D)							
D ₀ (Optimum)	1.00b	0.83b	54.30b	0.47b	31.94a	1.35b	21.66b
D_1 (Water deficit)	0.25a	0.54a	32.45a	0.26a	35.63b	1.07a	13.03a
PGPR (P)							
P ₀ (without PGPR)	0.62	0.63a	32.43a	0.27a	31.78	1.18	16.57
P ₁ (with PGPR)	0.63	0.74b	54.32b	0.45b	35.79	1.24	18.12
Varieties (V)							
V ₁ (Hypoma-1)	0.85b	0.71	39.21	0.32	36.59	1.14	17.21
V ₂ (Domba)	0.55a	0.66	41.79	0.35	33.09	1.21	18.31
V ₃ (Talam)	0.59a	0.69	48.58	0.39	33.63	1.06	17.73
V ₄ (Bison)	0.57a	0.67	43.54	0.38	34.09	1.40	16.95
V ₅ (G300-II)	0.57a	0.68	43.75	0.36	31.52	1.23	16.51

Note: LPTT = Plant Height Growth Rate (*b* coefficient), LPJD = Leaf Number Growth Rate (*b* coefficient), JB = Number of Nodules, BB = Fresh Nodule Weight, PA = Root Length, BBKA = Dry Root Biomass, BBKT = Dry Plant Biomass. Numbers followed by the same letter in the same column indicated no significant difference in treatment according to the Duncan 5% test.

Table 2, no observation characteristics were found to be better in the water deficit treatment (D₁) except for root length. Similar results were obtained by Riduan et al. (2005) in Pratiwi (2011), stating that drought stress reduces plant height, and dry weight of shoots and roots, but does not affect root length. This is a strategy used by plants to maximize water use, as the energy required to increase root length is much less compared to shoot elongation (Hemon et al., 2021). Nasrudin and Firmansyah (2020) added that an increase in root length under water deficit conditions is often associated with drought tolerance, where the root elongation mechanism is suspected to be an effort to search for water and nutrients to support plant growth and development.

The application of Plant Growth growthpromoting rhizobacteria (PGPR) significantly affects the characteristics of the rate of leaf number, nodule number, and nodule weight. The presence of rhizobium bacteria contained in PGPR is believed to increase the growth of leaf number and root nodules compared to treatments without PGPR. Rhizobium bacteria in PGPR are known to form a mutualistic symbiosis with legume plants (peanuts) by infecting the plant roots, and to compensate for the energy provided by the host, rhizobium bacteria make nitrogen available to the plants. Furthermore, Sayekti et al. (2016) in Purba and Sadiarso (2020) stated that leaf growth is influenced by nitrogen, as nitrogen plays a role in protein synthesis, which is closely related to plant growth processes, especially the leaf parts.

The number of root nodules is known to be linear with the amount of nitrogen obtained by the plants, meaning that the more root nodules, the more nitrogen available to the plants. According to Nursayuti (2021), root nodules formed on plants are responsible for fixing nitrogen from the air, making it usable by the plants to support growth and increase plant fertility. Research results in Table 2 show that the number of nodules formed is directly proportional to the weight of the root nodules. These results are consistent with the statement by Hodiyah and Milati (2022) that the weight of root nodules is a result of the number of root nodules.

Observations showed that all genotypes did not significantly affect the rate of leaf number, nodule number, nodule weight, root length, root dry weight, and plant dry weight, except for the rate of plant height. The rate of plant height for each genotype showed varied responses. The tallest plant was found in the Hypoma-1 (V₁) reaching 0.85 cm, while the shortest plant was 0.55 cm in the Domba (V₂), and these results were not significantly different from the Talam (V₃), Bison (V₄), or G300-II (V₅) genotypes. These results are most likely due to genetic differences between genotypes, as the environmental conditions and treatments applied were relatively the same (Hidayat et al., 2023).

Table 3. DMRT follow-up test results at a 5% level of significance for the interaction between Water Deficit and
PGPR (DP)

Deficit x PGPR (DP)	LPTT	LPJD	JB (nodules)	BB (g)	PA (cm)	BBKA (g)	BBKT (g)
D_0P_0	15.34	11.61	617.50	5.60	441.50	20.05	315.05
D_0P_1	14.66	13.23	1011.50	8.50	516.70	20.30	334.70
D_1P_0	3.21	7.23	355.50	2.55	511.80	15.20	181.95
D_1P_1	4.24	8.86	618.00	5.10	556.95	17.00	208.80

The research results show that the interaction factor of deficit with PGPR (Table 3) does not show a significant difference for all observation characteristics, as well as the interaction factor of deficit and variety (Table 4). Although deficit and PGPR (DP) did not interact significantly, the D_1P_1 (Deficit and PGPR) treatment tended to show a better increase in the number of nodules compared to the D_0P_0 (Optimum and Without PGPR) treatment. This is possible because the application of PGPR further increases the number of soil microbial populations, which also play a role in

nutrient supply through the decomposition of soil organic matter.

For the root length character in the D_1P_1 treatment, it was also found to be longer compared to other treatments. The formation of a deep root system in plants is a form of morphological adaptation to avoid drought, as long roots are used by plants to help absorb water and nutrients. Hund et al. (2009) in Hemon et al. (2021) stated that changes in plant structure, such as physiological, metabolic, and morphological adaptations, can be induced when water sources are limited.

Table 4. Results of further DMRT test at 5% real level of PGPR and Variety (PV) interaction

PGPR x Varieties (PV)	LPTT	LPJD	JB (nodules)	BB (g)	PA (cm)	BBKA (g)	BBKT (g)
P_0V_1	0.92	0.66	24.33	0.23	31.86	1.15	16.39
P_0V_2	0.61	0.63	30.67	0.26	33.93	1.08	16.09
P_0V_3	0.60	0.60	40.33	0.31	28.66	1.03	16.53
P_0V_4	0.51	0.64	34.42	0.29	32.74	1.33	17.68
P_0V_5	0.45	0.62	32.42	0.27	31.70	1.28	16.13
P_1V_1	0.77	0.77	54.08	0.41	41.32	1.13	18.03
P_1V_2	0.49	0.69	52.92	0.45	32.25	1.34	20.53
P_1V_3	0.57	0.79	56.83	0.48	38.61	1.09	18.93
P_1V_4	0.63	0.70	52.67	0.48	35.43	1.48	16.21
P_1V_5	0.69	0.74	55.08	0.46	31.33	1.17	16.88

The observation characteristics available in Table 4 show varied results for PGPR and genotype treatments. On average, treatments with PGPR tend to produce better characteristics for all observation characteristics except for plant height, which was highest in the P_0V_1 treatment. This result is suspected to be due to the

genetic factors of the genotype used, as in all treatments, whether independent factors, two-factor interactions, or even three-factor interactions involving genotype treatments, the Hypoma-1 (V_1) always produced the tallest plants among the other treatments.

Deficit x Varieties (DV)	LPTT	LPJD	JB (nodules)	BB (g)	PA (cm)	BBKA (g)	BBKT (g)
$\overline{D_0V_1}$	1.30	0.76cd	48.17	0.43	30.67	1.18	20.47
D_0V_2	0.89	0.79cd	52.58	0.45	30.36	1.39	23.77
D_0V_3	1.02	0.93d	59.25	0.48	33.70	1.08	22.45
D_0V_4	0.84	0.86d	58.67	0.55	34.83	1.65	21.90
D_0V_5	0.95	0.81cd	52.83	0.44	30.14	1.43	19.71
D_1V_1	0.39	0.67bc	30.25	0.22	42.51	1.10	13.95
D_1V_2	0.21	0.52ab	31.00	0.26	35.82	1.03	12.86
D_1V_3	0.15	0.46a	37.92	0.30	33.57	1.05	13.02
D_1V_4	0.29	0.48a	28.42	0.22	33.34	1.16	11.99
D_1V_5	0.19	0.55ab	34.67	0.28	32.89	1.03	13.31

The interaction between deficit and genotype (DxV) significantly affects the rate of leaf number. The highest leaf number rate of 0.93 leaves was obtained in the D_0V_3 treatment, while the lowest number was found in the D_1V_3 treatment with 0.46 leaves. The best leaf number rate obtained in D_0V_3 did not show a significant difference with the optimal water treatment (D_0) for each variety. The same applies to the water deficit treatment (D_1), where the treatments D_1V_2 , D_1V_4 , and D_1V_5 did not result in a higher leaf number rate

compared to D_1V_3 . Based on these results, it can be seen that the high or low number of leaves formed is greatly influenced by the presence of water, as water plays an important role in facilitating plant metabolism processes (Hasanah and Erdiansyah, 2020). This can be proven in other characteristics such as plant height rate, nodule number, nodule weight, root dry weight, and plant dry weight, which tend to show better results in each genotype when water conditions are optimal.

Table 6. DMRT further test result	lts with 5% real level interaction of	Deficit, PGPR and Genotype (DPV)
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Deficit x PGPR x Varieties (DPV)	LPTT	LPJD	JB (nodules)	BB (g)	PA (cm)	BBKA (g)	BBKT (g)
$D_0P_0V_1$	1.43	0.71	31.33	0.35	26.13	1.25	20.00
$D_0P_0V_2$	1.06	0.79	41.00	0.37	28.88	1.13	21.20
$D_0P_0V_3$	1.08	0.79	44.50	0.33	27.28	1.08	22.15
$D_0P_0V_4$	0.82	0.87	47.00	0.43	34.38	1.60	22.95
$D_0P_0V_5$	0.72	0.71	42.00	0.38	30.48	1.62	18.72
$D_0P_1V_1$	1.17	0.81	65.00	0.50	35.20	1.12	20.93
$D_0P_1V_2$	0.73	0.79	64.17	0.53	31.83	1.65	26.33
$D_0P_1V_3$	0.95	1.06	74.00	0.63	40.12	1.07	22.75
$D_0P_1V_4$	0.87	0.85	70.33	0.67	35.28	1.70	20.85
$D_0P_1V_5$	1.18	0.91	63.67	0.50	29.80	1.23	20.70
$D_1P_0V_1$	0.41	0.60	17.33	0.12	37.58	1.05	12.78
$D_1P_0V_2$	0.16	0.46	20.33	0.15	38.97	1.03	10.98
$D_1P_0V_3$	0.11	0.41	36.17	0.28	30.03	0.98	10.92
$D_1P_0V_4$	0.20	0.41	21.83	0.15	31.10	1.05	12.42
$D_1P_0V_5$	0.19	0.52	22.83	0.15	32.92	0.95	13.55
$D_1P_1V_1$	0.37	0.74	43.17	0.32	47.43	1.15	15.12
$D_1P_1V_2$	0.26	0.58	41.67	0.37	32.67	1.03	14.73
$D_1P_1V_3$	0.20	0.51	39.67	0.32	37.10	1.12	15.12
$D_1P_1V_4$	0.39	0.55	35.00	0.28	35.58	1.27	11.57
$D_1P_1V_5$	0.20	0.58	46.50	0.42	32.87	1.10	13.07

The interaction between water deficit, PGPR, and genotype (DPV) showed no significant differences in all observation factors. Table 6 shows that the $D_1P_1V_5$ treatment was higher than the $D_0P_0V_5$ treatment in two observation parameters, namely nodule number and nodule weight. This is because, under water deficit conditions, PGPR can be associated with plant roots to form more root nodules. However, from the data, it can generally be seen that optimal water conditions and PGPR application produce better characteristics. The presence of water in the soil helps the absorption of nutrients from the soil. Brutu et al. (2019) explained that water is very important for plant growth and development because it dissolves nutrients in the soil and transports nutrients into plant tissues. Additionally, water indirectly affects soil microorganisms that play an important role in the decomposition of nutrients in the soil. Water in the soil accelerates the decomposition of soil organic matter, thus increasing the availability of food for soil microorganisms.

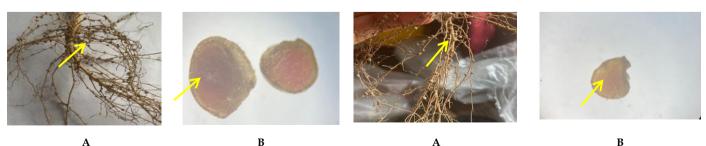


Figure 1. A. Distribution of root nodules with PGPR **Figure 1.** B. Color of root nodules with PGPR under microscope observation (40 x 40)

Figure 2.A. Distribution of root nodules without PGPR **Figure 2.B.** Color of root nodules without PGPR under microscope observation (40 x 40)

The effectiveness of nitrogen fixation due to Plant Growth Promoting Rhizobacteria (PGPR) treatment can be determined by the color of the root nodules. Hartanti and Octavia (2023) informed that nodules that are pink, red, or brown (containing leghemoglobin pigment) indicate that the nodules formed are effective. Conversely, if the nodules are green, pale white, or gravish (lacking leghemoglobin pigment), it means the nodules are ineffective. The effectiveness of root nodules greatly determines the success of the nitrogen fixation process by rhizobia bacteria. Essentially, legume plants can form root nodules naturally without the help of PGPR or other biofertilizers. However, the number and diameter of root nodules, as stated by Survati (2013) in Astija et al. (2022), influence nitrogen fixation. Generally, effective root nodules will form on plants provided that the bacteria and host are compatible, as mutual compatibility is a determinant of nitrogen fixation. If there is no compatibility, the nitrogenase enzyme protected by leghemoglobin protein cannot form, resulting in nitrogen fixation failure (Imtiyaz & Octavia, 2023).

Astija et al. (2022) stated that the color shown by the nodules is due to the presence of leghemoglobin, which functions to bind oxygen needed by rhizobia bacteria. Figure 1 and Figure 2 show the appearance of root nodule color in treatments with PGPR (P1) and without PGPR (P₀). It is seen that the P0 treatment (Figure 2) shows a somewhat pale color, while the P_1 treatment (Figure 1) tends to produce a reddish color. This indicates that the root nodules formed are effective, meaning the rhizobia bacteria successfully symbiosis with the peanut plant roots. Based on these results, the application of PGPR (P₁) successfully helps the plants provide nitrogen for the peanut plants through the assistance of rhizobia bacteria that symbiosis mutually with the host plant, in this case, peanuts.

Conclusion

The research result showed that the addition of PGPR resulted in higher plant growth compared to those without PGPR and had more effective root nodules in nitrogen fixation compared to treatments without PGPR. The application of PGPR and genotype did not show significant interaction on plant growth and root nodules under water deficit conditions. Water deficit significantly affected all parameters of plant growth and root nodule fresh weight. Genotype significantly affected the plant height rate. The interaction of water deficit and genotype significantly affected the leaf number rate, while the interaction of water deficit and PGPR,

and the interaction of PGPR and genotype did not significantly affect all observation parameters.

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None

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