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Growth Response of Bambara Groundnut to the Application of *Rhizobium* sp. and *Pseudomonas* sp. in Matriconditioning and Nitrogen-Phosphate Fertilization

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Abstract

Bambara groundnut has the potential to be an alternative food source of protein and carbohydrates. However, the quality of the seeds is not guaranteed, which can affect plant growth. The experiment aimed to determine the growth response of Bambara groundnut to the application of nitrogen fixation bacteria (*Rhizobium* sp.) and phosphate solubilizing bacteria (*Pseudomonas* sp.) integrated into matriconditioning and nitrogen-phosphate (NP) fertilizer. The experiment was conducted in Situraja-District, Sumedang Regency, West Java, Indonesia from November 2020 to April 2021. The experiment was arranged in a split-plot randomized complete block design replicated three times. The main plot was NP fertilizer level (0, 50, and 100% of recommended dose), and the sub-plot was seed invigoration (untreated, matriconditioning + *Rhizobium* KPB2 + *Pseudomonas* BPF9, and matriconditioning + *Rhizobium* KPB5 + *Pseudomonas* BPF9). Corncob biochar was used as the carrier in matriconditioning. The seed invigoration treatments using matriconditioning + *Pseudomonas* BPF9 + *Rhizobium* either KPB2 or KPB5 isolate significantly improved the plant growth (field emergence, plant height, leaf number, canopy diameter, and leaf chlorophyll content). Furthermore, when these invigoration treatments were accompanied by chicken manure 2 $t.ha^{-1}$ significantly increased the leaf number without adding NP fertilizer.

Keywords: biochar, chicken manure, fertilizer, invigoration, nitrogen fixation bacteria, phosphate solubilizing bacteria

Introduction

Bambara groundnut (*Vigna subterranea* (L.) Verdcourt) is an African plant with high carbohydrate, protein, methionine, and low-fat content. In addition, the content of methionine, lysine, and iron in Bambara groundnut is higher than in other legumes (Halimi et al., 2019). As an underutilized legume, Bambara groundnut exhibits superior tolerance to drought, pests, and diseases (Mayes et al., 2019; Mjaika et al., 2024). Therefore, Bambara groundnuts have the potential to play a crucial role in food security by providing essential nutrients and reducing our dependence on staple crops, helping to combat hunger and malnutrition (Feldman et al., 2019; Olatunde et al., 2021; Veldsman et al., 2023). In the health sector, Bambara groundnut contains antioxidants like tannins, flavonoids, and phytic acids, which can help protect against various health issues, including diabetes, stroke, heart disease, cancer, Alzheimer's, and cardiovascular diseases (Ramatsetse et al., 2023). Given its potential, this crop has been identified as a "new millennium crop" (Khan et al., 2021).

In Indonesia, Bambara groundnut is often cultivated as a secondary crop. However, the availability of high-quality seeds remains limited, as farmers frequently rely on seeds from previous seasons. This practice results in suboptimal plant growth and yield. Farmers often produce low-quality seeds due to poor growing conditions, as well as improper seed extraction, processing, and storage. This practice may perpetuate a cycle of inferior seed quality, particularly under water-stressed conditions.

There are several ways to improve seed quality,

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and one of them is invigoration. Invigoration aims to increase the viability and vigor of seeds by seed treatment before planting. One of some invigoration treatments is matriconditioning, the incubation of seeds in moist solid media with high matrix potential and negligible osmotic potential to control the water absorption of seeds (Khan, 1992). It can be integrated with biological agents for advanced benefit (Ilyas, 2012). Matriconditioning is an inexpensive, feasible technique, environmentally friendly, and able to trigger plants' natural defenses, making them more resilient to stress and ultimately boosting crop yield and quality without any negative effect on crop plants (Marthandan et al., 2020).

The growth, yield, and nutritional composition of Bambara groundnut were primarily influenced by the availability of nitrogen (N) and phosphate (P) (Hasan et al., 2019; Hasan et al., 2021). Biological agents that provide NP elements are nitrogen-fixing and phosphate-solubilizing bacteria. Using both biological agents can reduce the use of inorganic fertilizers to maintain soil health (Eickhout et al., 2006), mitigating environmental issues such as biodiversity loss, heavy metal accumulation, eutrophication, and toxicity to beneficial microorganisms. Additionally, it helps reduce greenhouse gas emissions by limiting nitrogen and sulfur gas release (Jote, 2023).

Black seed coat pigmentation in Bambara groundnut treated with *Bradyrhizobium* strain CB756 increased nodulation, N2 fixation, and plant growth (Puozaa et al., 2021). Ilyas et al. (2003) showed that soybean seeds treated with matriconditioning combined with *Bradyrhizobium japonicum*, *Azospirillum lipoferum*, and benomyl fungicide increased plant growth and yield, and reduced N fertilizer usage. Another study also proved that matriconditioning combined with Rhizobium sp. and fungicide reduced the use of N fertilizer by 50% from the recommended fertilization dose (Fitriesa et al., 2016). While the combined application of matriconditioning and biological agents has been shown to enhance seed quality and reduce chemical fertilizer use, their specific impact on Bambara groundnut growth and N-P fertilizer reduction remains unexplored. This study aimed to determine the growth response of Bambara groundnut to nitrogen-fixing bacteria (*Rhizobium* sp.) and phosphate-solubilizing bacteria (*Pseudomonas* sp.) in matriconditioning with various levels of N-P fertilizer.

Material and Methods

This research was conducted from November 2020 to April 2021 in Samoja Village, Situraja-District, Sumedang Regency, West Java, Indonesia (6˚50'53"S and 108˚0'28" E). Soil analysis was tested in Soil Fertility Laboratory, Faculty of Agriculture, IPB University.

Materials

Bambara groundnut seeds with black testa of the Sumedang landrace were produced by farmers in the Situraja District in July 2020. The seeds were stored in containers at room temperature (±27°C) until October 2020 before they were moved to seed storage (±17°C) until November 2020. The seeds had 10 ± 0.5% moisture content and 72% germination.

The microbiological materials used were nitrogen-fixing bacteria (*Rhizobium* sp.) and phosphate solubilizing bacteria (*Pseudomonas* sp.). *Rhizobium* sp. consisted of two isolates (KBP2 and KBP5) taken from plant root nodules of brown testa seed of Bambara groundnut of Sumedang landrace 66 days after planting (DAP) (Lupitasari et al., 2020). Pseudomonas sp. isolate (code BPF9) was obtained from Sukmadewi et al. (2017). BPF9 isolate has the highest ability to dissolve phosphate compared to other isolates from the Soil Biotechnology Laboratory collection, Faculty of Agriculture, IPB University.

Experimental Design

The experiment was arranged in a split-plot randomized complete block design. The main plot was the N and P fertilizer dose, which consisted of three levels i.e 0% (KCl 60 kg.ha^{-1}), 50% (Urea 10 kg.ha^{-1}, SP-36 30 kg.ha$^{-1,}$ KCl 60 kg.ha^{-1}), and 100% (Urea 20 kg.ha^{-1}, SP-36 60 kg.ha$^{-1,}$ KCl 60 kg.ha^{-1}). Sub-plots were seed invigoration treatments consisting of three levels: untreated (without invigoration), matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9, and matriconditioning + *Rhizobium* KBP5 + *Pseudomonas* BPF9. Each treatment was repeated three times.

Matriconditioning

Matriconditioning treatment used corncob biochar as a carrier with a ratio of 5:3:4.5 (seed: corncob biochar: bacterial inoculants). The corncob biochar had a pH of 8.59 and a water content of 4.23%. Corncob biochar was filtered through a 0.5 mm (35 mesh) sieve and sterilized twice within 24 hours using an autoclave at 121°C and 1 atm pressure for 1 hour (Tittabutr et al., 2012). The matriconditioning treatment was performed by mixing seeds with bacterial inoculants in a plastic container (17 cm x 5 cm x 5.5 cm) before adding corncob biochar. The mixture was stored for 3 days at 25°C and 62% relative humidity (Ilyas

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and Sopian, 2013) and was stirred every day. Bambara groundnut seeds gained an average of 52.6% weight after matriconditioning treatments. The matriconditioned seeds were air-dried for 3 hours at room temperature (± 27°C) before planting.

Planting

The planting method was based on the farmers' method of local Sumedang (Alhamdi et al., 2020). Soil preparation and application of chicken manure and rice husks was at a ratio of 2:1 with 2 $t.ha^{-1}$ dosage 2 weeks before planting. The application of fertilizers was done 5 weeks after planting (WAP). Each experimental plot was 4 m x 2 m with a distance between plots of 0.5 m. Plant spacing was 60 cm x 25 cm (Suryati et al., 2019) so each experimental plot contained 42 plant holes. One seed was planted in every planting hole with Furadan 3G (20 $kg.ha^{-1}$). The plant was nurtured by controlling pests, diseases, and weeds, and regulating water from planting to harvest. Earthing up was done twice, during the flowering (35-38 DAP) and the formation of pods (14 days after the appearance of flowers).

Data Collection and Data Analysis

Plants were sampled from 4 m × 2 m subplots to assess morphological growth parameters: field emergence (%), plant height (cm), leaf number, canopy diameter (cm), and chlorophyll content. Field emergence was calculated based on the germination percentage of seeds in each plot at 7, 10, 14, and 21 days after planting (DAP). Plant height, leaf number, canopy diameter, and chlorophyll content were measured on five tagged plants in the middle rows of each plot once a week from 6 to 10 weeks after planting (WAP), except for canopy diameter, which was measured from 11 to 16 WAP. Plant height was measured from the base of the stem to the tip of the uppermost fully expanded leaf. Leaf number was counted based on the number of fully expanded trifoliate leaves. Canopy diameter was measured as the maximum distance between the tips of the outermost leaves. Chlorophyll content was determined using a SPAD-502 chlorophyll meter (Minolta) by measuring the three youngest fully expanded leaves on each plant (Bünger et al., 2021).

The data obtained were analyzed using the SAS 9.0 program with analysis of variance (F test) at a 95% confidence interval. Further tests used DMRT (Duncan Multiple Range Test) at the 5% level to see the differences between treatments. Correlations between measured traits were estimated by computing the Pearson correlation coefficient (r).

Result and Discussion

Field Emergence

Field emergence was influenced solely by invigoration treatment because the NP fertilizer treatment was given at 5 WAP. The invigoration treatments increased field emergence compared to the untreated at 7 and 10 DAP. The untreated control took up to 14 DAP to give the same effect as the invigoration treatment (Table 1). These results support the previous studies by Suryati et al. (2019) and Fitriesa et al. (2016) when Bambara groundnut seeds were treated with matriconditioning combined with *Rhizobium* sp. had higher growth rates than untreated seeds. Khan et al. (1992) stated that matriconditioning could accelerate germination. Matriconditioning softens the hard seed coat of Bambara groundnut, allowing for controlled water absorption and preparing seeds for immediate germination upon planting. Marthandan et al. (2020) stated this process triggers significant cellular changes, including DNA repair, protein synthesis, and energy production, which are essential for successful germination. Additionally, it enhances antioxidant activity, reduces oxidative stress, and increases the abundance of mitochondria and cell division-related proteins like α- and β-tubulin. Primed seeds initiate early cell division, leading to β-tubulin accumulation and DNA replication, facilitating early radical protrusion. According to Hasan et al. (2017), the addition of nitrogen-fixing and phosphate-solubilizing bacteria inoculants has a role in helping the absorption of NP in the soil as a macro-essential element for plant growth. The addition of nitrogen-fixing bacteria helps improve plant root development while phosphate-solubilizing bacteria helps in root formation. Goswami et al. (2013) reported that multi-trait plant growth-promoting bacteria are beneficial organisms that can enhance plant growth by promoting seedling emergence, vigor, and yield.

Plant Height, Leaf number, and Canopy Diameter

Invigoration effectively increased plant height at 6-8 WAP and the fertilizer level had no significant effect. The treatment of matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9 at 6 WAP showed the highest (37.3 cm), with the highest percentage increase (10%) compared to the control in 6 WAP (Table 2). This is in line with the test results of the ability of bacteria to fix nitrogen. Umadi et al. (2023) showed that KBP2 has a higher NH3 concentration (94.46 $mg.L^{-1}$) than KBP5, which has a concentration of NH3 (92.63 $mg.L^{-1}$). In addition, *Rhizobium* KBP2 + *Pseudomonas* BPF9 and *Rhizobium* KBP5 + *Pseudomonas* BPF9 on corncob biochar media could maintain the bacterial population at log 8.2 $cfu.g^{-1}$. The analysis of variance

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showed that both invigoration treatments increased the leaf number compared to the control in each week of observation (6-10 WAP). At the same time, the NP fertilizer dose had no effect (Table 3).

Table 4 shows the interaction of invigoration treatment and NP fertilizer dose on the leaf number at 7 WAP. Matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9 without fertilization increased the leaf number equivalent to 100% fertilizer level (N 20 kg.ha^{-1} and P 60 kg.ha^{-1}). The highest leaf number resulted from matriconditioning + *Rhizobium* KBP5 + *Pseudomonas* BPF9 with a fertilizer level of 50% but not significantly different from the treatment without fertilization. It is suspected that the NP requirement in increasing plant height and the leaf number has been fulfilled by chicken manure at a dose of 2 t.ha^{-1}, which has high organic C, and the invigoration treatment was combined with nitrogen-fixing and phosphate solubilizing bacteria.

The canopy diameter at 11-13 WAP in both invigoration treatments was significantly wider than the untreated (Table 5). Bambara groundnut seeds treated with invigoration increased plant height, leaf number, and canopy diameter (Table 2-4). The addition of *Rhizobium* sp. in matriconditioning plays a role in the availability of N through nitrogen fixation, improving root development to increase vegetative growth. Phosphate solubilizing bacteria (*Pseudomonas* sp.) help produce adenosine triphosphate (ATP) needed in the nitrogen fixation process. This is in line with the previous studies by Suryati et al. (2019), Fitriesa et al. (2016), and Ilyas and Sopian (2013), which stated that matriconditioning combined with *Rhizobium* sp. applied on Bambara groundnut seeds produced higher plant height, leaf number, and canopy diameter than the control.

Table 5 shows the canopy diameter at 11-14 WAP exhibited a positive correlation with increasing fertilizer doses. However, the only significant difference was between the 100% dose and the control. According to Juwita (2012), the canopy diameter of Bambara groundnut has a positive correlation with the yield so the canopy diameter variable can be used as a determining factor for production (number of pods). The research results by Ikenganyia et al. (2017) showed that the fertilization of P 75 kg.ha^{-1} with the addition of *Rhizobium* sp. increased the yield component of Bambara groundnut compared to fertilizer doses of P 0, 25, and 50 kg.ha^{-1} with or without the addition of *Rhizobium*.

Table 1. Effect of invigoration on field emergence per plot

Invigoration treatment	Field emergence (%)			
	7 DAP	10 DAP	14 DAP	21 DAP
Without invigoration	24.9^{b}	50.8^{b}	65.3^{a}	71.4^{a}
Matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9	48.9^{a}	71.4^{a}	73.8^{a}	78.0^{a}
Matriconditioning + *Rhizobium* KBP5 + *Pseudomonas* BPF9	51.1^{a}	75.1^{a}	77.3^{a}	80.4^{a}

Notes: Values followed by the same letters in the same columns show significant differences according to the Duncan Multiple Range Test at α=0.05. DAP = days after planting.

Table 2. Effect of invigoration and NP fertilizer dose on plant height of Bambara groundnut at 6-10 weeks after planting

Treatment	Plant height (cm)				
	6 WAP	7 WAP	8 WAP	9 WAP	10 WAP
Invigoration	**	**	*	ns	ns
Without invigoration	33.9^{c}	35.6^{b}	37.8^{b}	40.6^{a}	41.5^{a}
Matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9	37.3^{a}	38.9^{a}	39.8^{a}	40.5^{a}	41.4^{a}
Matriconditioning + *Rhizobium* KBP5 + *Pseudomonas* BPF9	36.2^{b}	38.1^{a}	39.8^{a}	40.6^{a}	42.3^{a}
NP fertilizer dose	ns	ns	ns	ns	ns
0%	36.6^{a}	37.5^{a}	39.3^{a}	40.8^{a}	41.5^{a}
50%	36.1^{a}	37.3^{a}	39.1^{a}	40.1^{a}	41.4^{a}
100%	35.6^{a}	37.8^{a}	39.0^{a}	40.9^{a}	43.4^{a}

Notes: Notes: Values followed by the same letters in the same columns show significant differences according to the Duncan Multiple Range Test at α=0.05.;** significant in F test level of α<0.01; * significant in F test level of α<0.05; ns: not significant (P>0.05); WAP = weeks after planting.

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Table 3. Effect of invigoration and NP fertilizer dose on the leaf number of Bambara groundnut at 6-10 weeks after planting

Treatment	Leaf number				
	6 WAP	7 WAP	8 WAP	9 WAP	10 WAP
Invigoration	**	**	**	**	**
Without invigoration	24.6^b	38.5^b	52.4^b	63.8^b	71.9^b
Matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9	35.8^a	54.6^a	64.8^a	72.7^a	83.9^a
Matriconditioning + *Rhizobium* KBP5 + *Pseudomonas* BPF9	39.3^a	54.0^a	65.3^a	72.1^a	80.8^a
NP fertilizer dose	ns	ns	ns	ns	ns
0%	32.4^a	50.6^a	60.8^a	67.4^a	81.6^a
50%	33.0^a	49.6^a	59.2^a	70.3^a	78.5^a
100%	34.3^a	47.0^a	62.4^a	71.1^a	76.4^a

Notes: Notes: Values followed by the same letters in the same columns show significant differences according to the Duncan Multiple Range Test at α=0.05.;** significant in F test level of α<0.01; * significant in F test level of α<0.05; ns: not significant (P>0.05); WAP = weeks after planting.

Table 4. Interaction between invigoration and NP fertilizer dose on the leaf number of Bambara groundnut 7 weeks after planting

Treatment	NP fertilizer dose		
	0%	50%	100%
Without invigoration	39.9^Ab	35.1^Ab	40.7^Ab
Matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9	57.5^Aa	54.6^Aa	51.7^Aa
Matriconditioning + Rhizobium KBP5 + *Pseudomonas* BPF9	54.3^ABa	59.0^Aa	48.6^Ba

Values followed by the same letters in the same columns show a significant difference according to the Duncan Multiple Range Test at α=0.05.

Table 5. Effect of invigoration and NP fertilizer dose on canopy diameter of Bambara groundnut at 11-15 weeks after planting

Treatment	Canopy diameter (cm)				
	11 WAP	12 WAP	13 WAP	14 WAP	15 WAP
Invigoration	**	**	**	ns	ns
Without invigoration	62.4^b	65.3^b	66.7^b	69.0^a	70.9^a
Matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9	66.0^a	67.7^a	68.9^a	69.7^a	71.5^a
Matriconditioning + *Rhizobium* KBP5 + *Pseudomonas* BPF9	66.2^a	68.4^a	69.4^a	70.3^a	72.0^a
NP fertilizer dose	*	**	**	*	ns
0%	63.7^b	66.3^b	67.2^b	68.7^b	70.1^a
50%	64.3^ab	66.7^b	68.2^b	69.3^ab	71.4^a
100%	66.3^a	68.6^a	69.6^a	71.0^a	72.9^a

Notes: Notes: Values followed by the same letters in the same columns show significant differences according to the Duncan Multiple Range Test at α=0.05.;** significant in F test level of α<0.01; * significant in F test level of α<0.05; ns: not significant (P>0.05); WAP = weeks after planting.

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Plants and microorganisms have developed a mutually beneficial partnership. Plant microbiome determines plant health and productivity (Ayangbenro et al., 2023). Rhizobial bacteria increase plant productivity and growth by generating a variety of compounds (Ashrafi., et al., 2022). Matriconditioning involves treating seeds with beneficial microbiomes under controlled moisture conditions and priming seeds for germination without premature sprouting. This process stimulates various physiological processes in plants, including increased enzyme activity, enhanced antioxidant defense, and altered hormone levels, ultimately improving plant growth and stress tolerance (Mitra et al., 2021).

Research by Ilyas et al. (2003) showed that combining two types of nitrogen-fixing bacteria (*Bradyrhizobium japonicum* and A*zospirillum lipoferum*) in matriconditioning increased soybean growth and reduced N fertilizer use. According to Hasan et al. (2017), *Rhizobium* sp. can help plants fix nitrogen (N_2) into ammonia (NH_3) which will be converted into nitrogen compounds that plants need. Sufficient nitrogen supply enhances plant growth by stimulating cell division, increasing photosynthetic output, and promoting leaf development (Adeyeye et al., 2019).

A study by Olanrewaju et al. (2022) showed phosphate solubilizing bacteria (*Bacillus*, *Pseudomonas*, and *Streptomyces*) have enormous potential for growth promotion and disease control in Bambara groundnut production. Phosphate solubilizing bacteria play important roles in transforming P and increasing the available P in the soil so that plant roots easily absorb it. The nitrogen fixation process through the symbiosis of the Leguminosae and *Rhizobium* families requires phosphate to produce ATP, the phosphorus supply to sustain the nitrogen fixation process.

Rhizobacteria isolated from the rhizosphere of Bambara groundnut exhibit significant potential as a natural fertilizer, aid in controlling plant diseases, and contribute to enhanced food security (Ajilogba et al., 2020). Bacterial activity in fixing nitrogen and dissolving phosphate can increase the available nitrogen and phosphate nutrients for plants (Li et al., 2020). Provision of nitrogen and phosphorus affects vegetative growth by providing essential proteins and nucleic acids (Hasan et al., 2017).

Leaf Chlorophyll Content (SPAD Value)

Chlorophyll is a pigment that absorbs light in leaves for photosynthetic activity. Changes in chlorophyll content can be seen indirectly through SPAD (soil-plant analysis development) observations. SPAD values indicate the chlorophyll content of leaves. Leaf chlorophyll measurement with SPAD shows the chlorophyll content per unit area of the leaf by measuring the light absorbance in the leaves, which is then calculated into a SPAD value equivalent to the chlorophyll content in the leaves (Bünger et al., 2021).

The results (Table 6) showed that the invigoration treatment and the level of NP fertilizer affected the chlorophyll content of the leaves (SPAD values) except for the invigoration treatment of 10 WAP. Both invigoration treatments increased the chlorophyll content compared to the control. The treatment of NP fertilizer at 6-7 WAP at a dose of 100% showed the highest leaf chlorophyll content, while at 8-10 WAP, a dose of 50% gave the same effect at a dose of 100%. This indicates that the dose of NP fertilizer decreased at 8 WAP, presumably due to the role of nitrogen-fixing and phosphate solubilizer bacteria in providing nitrogen and phosphate to plants.

The invigoration treatment increased nitrogen and magnesium levels, essential for chlorophyll synthesis. This likely enhanced nutrient uptake, leading to increased chlorophyll content and improved growth and photosynthesis (Anwar et al., 2020). Plants with higher N levels in their leaves generally have more chlorophyll, a pigment crucial for photosynthesis. This increase in chlorophyll content contributes to enhanced photosynthetic activity, ultimately leading to improved growth and yield (Fathi, 2022). Purbajanti et al. (2019) demonstrated that a combination of 10 $t.ha^{-1}$ of cow manure and 100 $kg.ha^{-1}$ of NPK fertilizer led to the highest total chlorophyll content in peanuts. Saudy et al. (2020) observed that applying Bacillus megatherium to faba beans increased chlorophyll levels, as measured by SPAD readings. Hasan et al. (2021) further supported the beneficial effects of biofertilizers, finding that they were more effective than P fertilizer in enhancing total chlorophyll content in Bambara groundnuts.

Correlation Analysis

Table 7 shows the correlation matrix showing the relationship between invigoration and fertilizer dose for the determination of growth parameters such as leaf number, plant height, and chlorophyll content. Based on the Pearson correlation coefficient, a significant positive correlation was found in all observed parameters. This finding suggests that plants with a greater leaf number exhibited increased height and chlorophyll content.

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Table 6. Effect of invigoration and NP fertilizer dose on Bambara groundnut leaf chlorophyll content at 6-10 weeks after planting

Treatment	Leaf chlorophyll content (SPAD values)				
	6 WAP	7 WAP	8 WAP	9 WAP	10 WAP
Invigoration	**	**	**	**	ns
Without invigoration	42.0[b]	43.6[b]	45.8[b]	47.1[b]	49.3[a]
Matriconditioning + *Rhizobium* KBP2 + *Pseudomonas* BPF9	44.7[a]	46.2[a]	48.1[a]	49.1[a]	51.2[a]
Matriconditioning + *Rhizobium* KBP5 + *Pseudomonas* BPF9	45.4[a]	46.6[a]	48.2[a]	49.2[a]	50.8[a]
NP fertilizer dose	**	**	**	**	*
0%	43.1[b]	44.1[c]	45.1[b]	46.2[b]	48.7[b]
50%	43.9[b]	45.3[b]	48.3[a]	49.5[a]	51.2[a]
100%	45.2[a]	46.5[a]	48.6[a]	49.7[a]	52.3[a]

Notes: Notes: Values followed by the same letters in the same columns show significant differences according to the Duncan Multiple Range Test at α=0.05.;** significant in F test level of α<0.01; * significant in F test level of α<0.05; ns: not significant (P>0.05); WAP = weeks after planting.

Table 7. Correlation analysis of the invigoration and NP fertilizer dose for growth parameters

	Leaf number	Plant height (cm)	Chlorophyll content
Leaf number			
Plant height (cm)	0.7839***		
Chlorophyll content	0.5317**	0.4312*	

Notes: *= p<0.05; **= p<0.01; ***= p<0.001; ns= not significant (p>0.05).

Conclusion

Seed invigoration treatment using matriconditioning + *Rhizobium* KPB2 + *Pseudomonas* BPF9 or matriconditioning + *Rhizobium* KPB5 + *Pseudomonas* BPF9 effectively increased vegetative growth of Bambara groundnut, including field emergence, plant height, leaf number, canopy diameter, and leaf chlorophyll content. Both invigoration treatments and the application of chicken manure 2 t.ha^{-1} effectively increased the leaf number without adding N and P fertilizer. Our results demonstrate that the synergistic effect of matriconditioning combined with *Rhizobium* and *Pseudomonas* can substantially improve Bambara groundnut growth parameters. This integrated approach, complemented by organic fertilization with chicken manure, offers a promising sustainable alternative to conventional mineral fertilizer-based agriculture. Further research is warranted to explore the potential of a consortium of beneficial microorganisms, including nitrogen-fixing bacteria, phosphate-solubilizing bacteria, and potassium-solubilizing bacteria combined with reduced NPK fertilizer doses, to enhance plant growth and productivity.

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References

Adeyeye, A.S., Dimas, A.E., Olalekan, K.K., Lamidi, W.A., Othman, H.J., and Ishaku, M.A. (2019). The effect of organic and inorganic nutrient sources on the growth and seed yield of Bambara nut (*Vigna Subterranean* (L) Verdc) variety. *World Journal of Agriculture and Soil Science* **2**, 1-9. DOI: https://doi.org/10.33552/WJASS.2019.02.000537.

Ajilogba, C.F., Babalola, O.O., Adebola, P., and Adeleke, R. (2022). Bambara groundnut rhizobacteria antimicrobial and biofertilization potential. *Frontiers in Plant Science* **13**, 854937. DOI: https://doi.org/10.3389/fpls.2022.854937.

Alhamdi, M.F.F., Setiawan, A., Ilyas, S., and Ho, W.K. (2020). Genetic variability of Indonesian landraces of Vigna subterranea: morphological

Received 14/06/2024; Revised 21/11/2024; Accepted 12/12/2024
'https://doi.org/10.29244/jtcs.12.01.37-46

characteristics and molecular analysis using SSR markers. *Biodiversitas* **21**, 3929-3937. DOI: https://doi.org/10.13057/biodiv/d210902.

Anwar, A., Yu, X., Li, Y. (2020). Seed priming as a promising technique to improve growth, chlorophyll, photosynthesis and nutrient contents in cucumber seedlings. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. **48**, 116-127. DOI: https://doi.org/10.15835/nbha48111806.

Ashrafi, S., Kuzmanovic, N., Patz, S., et al. (2022). Two new Rhizobiales species isolated from root nodules of common sainfoin (*Onobrychis viciifolia*) show different plant colonization strategies. *Microbiology Spectrum* **10**, 01099. DOI: https://doi.org/10.1128/spectrum.01099-22.

Ayangbenro, A.S., Adem, M.R., and Babalola, O.O. (2023). Bambara nut root-nodules bacteria from a semi-arid region of South Africa and their plant growth-promoting traits. *International Journal of Microbiology* **2023**, 1-8. DOI: https://doi.org/10.1155/2023/8218721.

Bünger, W., Sarkar, A., Grönemeyer, J.L., Zielinski, J., Revermann, R., Hurek, T., et al. (2021). Root nodule rhizobia from undomesticated shrubs of the dry woodlands of Southern Africa can nodulate Angolan teak *Pterocarpus angolensis*, an important source of timber. *Frontiers in Microbiology* **12**. DOI: https://doi.org/10.3389/fmicb.2021.611704.

Eickhout, B., Bouwman, A.F., and Van Zeijts, H. (2006). The role of nitrogen in world food production and environmental sustainability. *Agriculture Ecosystems & Environment* **116**, 4-14. DOI: https://doi.org/10.1016/j.agee.2006.03.009.

Fathi, A. (2022). Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: A review. *Agrisost* **28**, 1-8.

Feldman, A., Ho, W.K., Massawe, F., and Mayes, S. (2019). Bambara groundnut is a climate-resilient crop: how could a drought-tolerant and nutritious legume improve community resilience in the face of climate change? *In* “Sustainable Solutions for Food Security” (A. Sarkar, S.R. Sensarma and G.W. vanLoon, eds.), pp 151–167. Springer. Cham. DOI: https://doi.org/10.1007/978-3-319-77878-5_8.

Fitriesa, S., Ilyas, S., and Qadir, A. (2016). Invigoration and reduction of N Fertilizer in improving plant growth, yield, and quality of Bambara groundnut seed. *Jurnal Agronomi Indonesia*. **44**,190-196. DOI: https://doi.org/10.24831/jai.v44i2.13489.

Goswami, D., Vaghela, H., Parmar, S., Dhandhukia, P., and Thakker, N.K. (2013). Plant growth promoting potentials of Pseudomonas spp. strain OG isolated from marine water. *Journal of Plant Interactions* **8**, 281–290. DOI: https://doi.org/10.1080/17429145.2013.768360.

Halimi, A.R., Mayes, S., Barkla, B., and King, G. (2019). The potential of the underutilized pulse Bambara groundnut (*Vigna subterranea* (L.) Verdc.) for nutritional food security. *Journal of Food Composition and Analysis* **77**, 47–59. DOI: https://doi.org/10.1016/j.jfca.2018.12.008.

Hasan, M., Uddin, M.K., Mohamed, M.T.M., and Zuan, A.T.K. (2017). Nitrogen and phosphorus management for Bambara groundnut (*Vigna subterranea*) production - A review. *Legume Research* **41**, 483-489. DOI: https://doi.org/10.18805/LR-379.

Hasan, M., Uddin, M.K., Mohamed, M.T.M., Zuan, A.T.K., Motmainna, M., and Haque, A.N.A. (2021). Effect of nitrogen and phosphorus fertilizers on growth, yield, nodulation and nutritional composition of Bambara groundnut [*Vigna subterranea* (L.) Verdc.]. *Legume Research* **44**, 1437-1442. DOI: https://doi.org/10.18805/LR-617.

Hasan, M., Uddin, M.K., Mohammed, M.T.M., Suan, A.T.K., and Motmainna. (2019). Impact of chemical and organic fertilizer on the yield and nutritional composition of Bambara groundnut (*Vigna subterranea* L. Verde.). *Bangladesh Journal of Botany* **48**, 919-924. DOI: https://doi.org/10.3329/bjb.v48i4.48932.

Hasan, M., Uddin, M.K., Mohammed, M.T.M., Zuan, A.T.K., and Motmainna, M. (2021). Growth, yield, nodulation, and amino acid content of Bambara groundnut (*Vigna subterranea*) under Inorganic and organic fertilizer application. *Legume Research* **44**, 322-327. DOI: https://doi.org/10.18805/LR-576.

Ikenganyia, E.E., Anikwe, M.A.N., and Ngwe, O.E. (2017). Influence of rhizobacteria inoculant application methods and phosphate fertilizer rates on dry matter accumulation, the yield

Received 14/06/2024; Revised 21/11/2024; Accepted 12/12/2024
'https://doi.org/10.29244/jtcs.12.01.37-46

of Bambara groundnut [*Vigna subterranea* (L.) Verdc] and soil total nitrogen content in a degraded ultisol in Southeast Nigeria. *Agrotechnology* **6**, 1-7.

Ilyas, S. (2012). “Ilmu dan Teknologi Benih: Teori dan Hasil-Hasil Penelitian”. IPB Press.

Ilyas, S., and Sopian, O. (2013). Effect of seed maturity and invigoration on seed viability and vigor, plant growth, and yield of Bambara groundnut (*Vigna subterranea* (L.) Verdcourt). *Acta Horticulturae* **979**, 695-701. DOI: https://doi.org/10.17660/ActaHortic.2013.979.78.

Ilyas, S., Surahman, M., Saraswati, R., Gunarto, L., and Adisarwanto, T. (2003). "Peningkatan mutu benih dan produktivitas kedelai dengan teknik invigorasi benih menggunakan matriconditioning dan inokulan mikroba". IPB Research Institute and Agricultural Research and Development Agency.

Jote, C.A. (2023). The Impacts of Using Inorganic Chemical Fertilizers on the Environment and Human Health. *Organic and Medicinal Chemistry International Journal* **13**, 555864.

Juwita, L. (2012). “Pembentukan populasi dasar untuk perbaikan produksi kacang Bogor (*Vigna subterranea* L. Verdcourt) asal Darmaga, Sukabumi, dan Parung”. Faculty of Agriculture, IPB University.

Khan, A.A. (1992). Preplant physiological seed conditioning. *Horticultural Reviews* **13**, 131-181. DOI: https://doi.org/10.1002/9780470650509.ch4.

Khan, M.M.H., Rafii, M.Y., Ramlee, S.I., Jusoh, M., and Al-Mamun, M. (2021). Bambara groundnut (*Vigna subterranea* L. Verdc): a crop for the new millennium, Its genetic diversity, and improvements to mitigate future food and nutritional challenges. *Sustainability* **13**, 5530. DOI: https://doi.org/10.3390/su13105530.

Li, Y., Li, Q., Guan, G., and Chen, S. (2020). Phosphate solubilizing bacteria stimulate wheat rhizosphere and endosphere biological nitrogen fixation by improving phosphorus content. *PeerJ Publishing* **8**, 1-15. DOI: https://doi.org/10.7717/peerj.9062.

Lupitasari, E., Ruhimat, R., Umadi, S.S., and Sahana, L.A.A. (2020). Isolation and characterization of Rhizobium bacteria form various roots nodules legumes crop In “Proceeding of the National Seminar on Biotechnology 2020”, pp 78-84, Indonesian Biotechnology and Bioindustry Research Center-PT National Plantation Research.

Marthanda, V., Geetha, R., Kumutha, K., Renganathan, V.G., Karthikeyan, A., Ramalingam, J. (2020). Seed priming: a feasible strategy to enhance drought tolerance in crop plants. *International Journal of Molecular Sciences* **21**, 1-23. DOI: https://doi.org/10.3390/ijms21218258.

Mayes, S., Ho, W.K., Chai, H.H., Gao, X., Kundy, A.C., Mateva, K.I., et al. (2019). Bambara groundnut: an exemplar underutilised legume for resilience under climate change. *Planta* **250**, 803–820. DOI: https://doi.org/10.1007/s00425-019-03191-6.

Mitra, D., Mondal, R., Khoshru, B., Shadangi, S., Mohaptra P.K.D., and Paneerselvam, P. (2021). Rhizobacteria mediated seed bio-priming triggers the resistance and plant growth for sustainable crop production. *Microbial Science* **2**, 100071. DOI: https://doi.org/10.1016/j.crmicr.2021.100071.

Mjaika, N.E., Magaret, C., and Hemba, C.S. (2024). Changing aspects of Bambara groundnut exploitation: 1. limitations and alleyways for future expansion. *Agricultura* **1-2**, 129-130.

Olanrewaju, O.S., and Babalola, O.O. (2022). Plant growth-promoting rhizobacteria for orphan legume production: Focus on yield and disease resistance in Bambara groundnut. *Frontiers in Sustainable Food Systems* **6**, 922156. DOI: https://doi.org/10.3389/fsufs.2022.922156.

Olatunde, S.J., Ogundele, O.M., Oyedokun, J., Chinma, C.E., Shittu, T.A., and Onoja, V. (2021). Traditional food uses of Bambara groundnut. *Springer Nature*, **153-168**. DOI: https://doi.org/10.1007/978-3-030-73920-1_9.

Puozaa, D.K., Jaiswal, S.K., and Dakora, F.D. (2021). Black seedcoat pigmentation is a marker for enhanced nodulation and N2 fixation in Bambara groundnut (*Vigna Subterranea* L. Verdc.) Landraces. *Frontiers in Agronomy* **3**, 692238. DOI: https://doi.org/10.3389/fagro.2021.692238.

Purbajanti, E.D., Slamet, W., Fuskhah, E., and Rosyida. (2019). Effects of organic and inorganic fertilizers on growth, the activity of

Received 14/06/2024; Revised 21/11/2024; Accepted 12/12/2024
'https://doi.org/10.29244/jtcs.12.01.37-46

nitrate reductase, and chlorophyll contents of peanut (*Arachis hypogaea* L.). *IOP Conference Series: Earth and Environmental Science* **250**, 1-7. DOI: https://doi.org/10.1088/1755-1315/250/1/012048.

Ramatsetse, K.E., Ramashia, S.E., and Mashau, M.E. (2023). A review on health benefits, antimicrobial and antioxidant properties of Bambara groundnut (*Vigna subterranean*). *International Journal of Food Properties* **26**, 91-107. DOI: https://doi.org/10.1080/10942912.2022.2153864.

Saudy, H., Noureldin, N., Mubarak, M., Fares, W., and Elsayed, M. (2020). Cultivar selection as a tool for managing soil phosphorus and faba bean yield sustainability. *Archives of Agronomy and Soil Science* **66**, 414-425. DOI: https://doi.org/10.1080/03650340.2019.1619078.

Sukmadewi, D.K.T., Anas, I., Widyastuti, R., and Citraresmini, A. (2017). Test of phytopathogenicity, hemolysis and microbial ability in solubilizing phosphate and potassium. *Jurnal Ilmu Tanah dan Lingkungan (Journal of Soil Science and Environment)* **19**, 68-73. DOI: https://doi.org/10.29244/jitl.19.2.68-73.

Suryati, H., Ilyas, S., Qadir, A., and Budhianto, B. (2019). Effects of invigoration and spacing on growth and seed production of four Bambara groundnut (*Vigna subterranea* (L) Verdc) landraces. *International Journal of Agronomy and Agricultural Research* **19**, 16-25.

Tittabutr, P., Teamthisong, K., Buranabanyat, B., Teaumroong, N., and Boonkerd, N. (2012). Gamma irradiation and autoclave sterilization of peat and compost as the carrier for rhizobial inoculant production. *Journal of Agricultural Science* **4**, 59-67. DOI: https://doi.org/10.5539/jas.v4n12p59.

Umadi, S.S., Ilyas, S., and Widyastuti, R. (2023). Characteristic and viability of nitrogen fixation bacteria and phosphate solubilizing bacteria in biochar carrier media. *Jurnal Ilmu Tanah dan Lingkungan (Journal of Soil Science and Environment)* **25**, 40-45. DOI: https://doi.org/10.29244/jitl.25.2.40-45.

Veldsman, Z., Pretorius, B., and Schönfeldt, H.C. (2023). Examining the contribution of an underutilized food source, Bambara groundnut, in improving protein intake in Sub-Saharan Africa. *Frontiers in Sustainable Food Systems* **7**, 1183890. DOI: https://doi.org/10.3389/fsufs.2023.1183890.