GROWTH AND YIELD OF KOMAK BEANS (*Lablab purpureus* (L.) Sweet) WITH APPLICATION OF PHOTOSYNTHETIC BACTERIA PNSB UNDER SHADE

A B S T R A C T
The research was conducted to determine the effect of the application of PNSB on the growth and yield of komak beans under shade. The experiment was arranged in a Split Plot Design, consisting of two factors, namely shade as the Main Plot and concentration of PNSB as Sub-Plots. The shade consists of two levels (no shade and 50% shade). The PNSB concentration consists of three levels (0; 5; and 10 mL L^{-1}). Each treatment was carried out with four replications. Data were analyzed using analysis of variance and continued with Duncan's Multiple Range Test at a 5% significance level. The results showed that the effect of shade was significantly different on stem diameter at 70 DAP, chlorophyll content (a, b, and total), and the number of dry seeds, very significantly different to stem diameter at 105 DAP, dry pod weight, number of dry pods, dry seed weight, and light intensity. 50% shade reduces the yield component even if PNSB is provided. The effect of PNSB was not significantly different on all variables, except for the number of leaves at 35 DAP and total N content. Application of PNSB 10 mL L^{-1} increased the number of leaves aged 35 DAP and the total N content, both without shade and with shade, increased total chlorophyll content with 50% shade, but decreased without shade.

KEYWORDS:
Komak Beans, Photosynthetic Bacteria, Purple Non-Sulfur Bacteria, Shade.

I. INTRODUCTION

Indonesia has legume plant resources that have the potential to be developed as an alternative food ingredient for soybeans through a food diversification program because they have nutritional quality, taste, and image that are not inferior to soybeans, including komak *(Lablab purpureus* (L.) Sweet*)*, alternative raw material for making tempe (Antara News Megapolitan 2022; Ekafitri and Rhestu 2014; Haliza *et al.* 2010; Sari and Yunita 2020). Komak is rich in nutrients: carbohydrates, protein, fat, vitamins (A, B_1, B_2, C), β-carotene, P, K, Ca, Zn, and Cu, can be used as a substitute for processed food ingredients such as yogurt, sweet soy sauce, and protein isolate raw materials (Arifin 2014; Purwitasari *et al.* 2014; Suwarno and Maryani 2003; Widiastuti 2017), are beneficial for health (circulatory, reproductive, immune, digestive and skeletal systems), so they are called functional foods (Andra Farm 2022; Hartoyo *et al.* 2010; Makayasa 2020; Republika Online 2022; Singh and Abhilash 2019; Wardani *et al.* 2015).

Intensive agricultural practices that use agrochemicals to increase crop yields can cause environmental and public health disturbances that affect food security and agricultural sustainability. The use of biofertilizers is the solution to overcome this problem, such as the use of photosynthetic bacteria PNSB. Biofertilizers have great potential and play an important role in increasing plant biomass and productivity. The relationship between plants and microbes is important for sustainable agricultural development because sustainable and ecologically innovative technologies are the best way to maintain soil fertility and increase crop yields (Kumar *et al.* 2021).

Photosynthetic bacteria (PSB) are bacteria that can photosynthesize, can convert organic materials into amino acids or bioactive substances with the help of sunlight. These bacteria are very beneficial for agriculture because they can fix N_2, add H_2S to the soil, are a source of minerals for amino acids, nucleic acids, physiologically active compounds, and polysaccharides, increase root and plant growth and plant quality, reduce the cost of using chemical fertilizers, and increase resistance. against pests and diseases (Bali Province Agriculture and Food Security Service, 2022).

One of the PSB groups is Purple Non-Sulfur Bacteria (PNSB), prokaryotes that have versatile anaerobic metabolism as photoautotrophic, photoheterotrophic, chemoautotrophic, and chemoheterotrophic, depending on the presence of nutrients, O_2 concentration, and light intensity. This metabolic flexibility recommends it for biotechnological applications such as environmental bioremediation, bioenergy production, biopolymers such as polyhydroxybutyrate (PHB) as an alternative to petroleum-based plastics, and in agriculture as a biofertilizer (Carlozzi and Sacchi 2001; du Toit and Pott 2020; Lee *et al.* 2021). Androga *et al*. (2012) added, PNSB is a gram-negative prokaryote, facultative anoxygenic

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phototroph, belonging to the class Alphaproteobacteria and includes several genera in the orders *Rhodobacterales*, *Rhodospirales*, and *Rhizobiales*.

In the agricultural sector, PNSB is widely used in biofertilization, biostimulation, and biocontrol to encourage plant growth which contributes to increased nutrition, production of IAA hormones, induction of the immune system against pathogens, production of carotenoid pigments, vitamins, able to interact with other beneficial microorganisms in the root area (rhizosphere), and produces endogenous 5-aminolevulinic acid (5-ALA) which functions to suppress abiotic stress and improve plant quality (Madigan and Jung 2009). These bacteria can grow in both aerobic and anaerobic conditions and can use organic or inorganic materials as electron donors for biological CO_2 and N_2 fixation (Larimer *et al.* 2004; Pechter *et al.* 2015), can live on the leaf surface (phyllosphere) and increase the activity of other phyllosphere microbial species. Around the roots increases the metabolic activity of other beneficial bacteria to promote growth and increase nutrient absorption by the roots (Androga *et al.* 2012).

Photosynthesis in PNSB occurs in the intracytoplasmic membrane which consists of photosystems, transport proteins, cytochrome complexes, and ATP synthase proteins. The photosystem transfers energy to the reaction center and initiates cyclic electron transfer (Androga *et al*. 2012). Photosynthesis biosynthesis is mainly controlled by O_2 (bacteriochlorophyll synthesis is suppressed during aerobic conditions) and light, at low light intensity, photosystem biosynthesis increases to collect more light energy, and vice versa (Firsow and Drews 1977; Pemberton *et al*. 1998; Zhu and Hearst 1986). Changes in sunlight intensity and temperature greatly influence PNSB growth and hydrogen production (Androga *et al*. 2012).

The research aimed to determine the effect of the application of photosynthetic bacteria PNSB on the growth and yield of komak bean plants under shade.

2. MATERIALS AND METHODS

2.1. Time and place

The experiment was carried out at the Para-para Garden, Agroecotechnology Laboratory, Faculty of Agriculture, Mulawarman University from March to October 2022.

2.2. Materials and tools

The materials used in the experiment consisted of black komak bean seeds, topsoil, chicken manure, photosynthetic bacteria PNSB solution, vegetable pesticide Neem Oil, turmeric, garlic, lemongrass, and papaya leaves, pesticides Clinten and Metin (used when uncontrolled aphid pest attacks), and compound NPK (16:16:16) as basic fertilizer.

The tools used in the research consisted of polybags, Jewelry Scale 3 kg 0.1G 0.1 gram scales, hand sprayer, XENON electric disinfectant pest sprayer and mist blower booster fogging, self-locking wire zip cable ties, Lux Meter Photometer Light Meter LX1010B, stationery and NETAC U352 64GB flash disk, double tape foam, plastic container box, plant labels, Digital Moisture Meter, plant scissors, bucket, stake, shading net 50%, hoe, shovel, LCD digital caliper Vernier Sigmat caliper, soil pH and moist KS06, WS-A7 Mini Hygrometer Thermometer.

2.3. Experimental Design

The experiment was arranged in a Split Plot Design consisting of two factors and four replications, namely: shade (N) as the Main Plot, consisting of two levels, namely: n_0 = no shade and n_1 = 50% shade; and PNSB concentration (B) as the Sub Plot, consists of four levels, namely: b_0 = 0 mL L^{-1}; b_1 = 5 mL L^{-1}, and b_2 = 10 mL L^{-1}. Each treatment consisted of five plants, so there were 120 experimental units.

2.4. Experimental Procedures

The stages of experimental activities consist of preparing shade and planting media, planting seeds, and applying basic fertilizer. Shading uses a shading net with a density of 50% which is installed by the research environment design. The planting medium is a mixture of soil and chicken manure in a 1:1 ratio which is mixed evenly, then put into polybags, each weighing 12 kg. Three (3) komak bean seeds are planted per planting hole, after one week of age, one healthy plant is left as experimental material. Compound NPK fertilizer (16:16:16) as a basic fertilizer is given when the plants are one week after planting by immersing the fertilizer around the root area at a dose of 25 kg per hectare.

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2.5. Administration of PNSB Photosynthetic Bacteria

The photosynthetic bacteria used were a mixed culture of PNSB bacteria, given 5 (five) times, namely when the plants were 15, 30, 45, 60, and 75 days after planting according to the treatment concentration by spraying all parts of the plant. Spraying was carried out at 11.00 because PNSB is active during the day in light conditions.

2.6. Maintenance

Maintenance includes watering and controlling plant pests (aphids). Pest control is carried out by applying the vegetable pesticide Neem Oil and a mixture of turmeric, garlic, lemongrass, and papaya leaves. Chemical pesticides were used once due to an aphid attack due to high rainfall and humidity during the study.

2.7. Observation Variables

Observation variables included the number of leaves at 35 days after planting; stem diameter at 35, 70, and 105 days after planting; chlorophyll a, chlorophyll b, and total chlorophyll content at the age of 70 days after planting; N total; dry pod weight; the number of dry pods; the number of dry seeds; dry seed weight; the weight of 100 dry seeds; light intensity, temperature, and air humidity.

Chlorophyll content was measured using a spectrophotometer at wavelengths of 649 and 665 nm. Calculation of chlorophyll content uses the following formula:

Chlorophyll a = 13.7 D-665 – 5.76-D-649 (mg L^{-1});

Chlorophyll b = 25.8 D-649 – 7.60 D-665 (mg L^{-1});

Total chlorophyll = 20.0 D-649 + 6.10 D-665 (mg L^{-1}) (Wintermans and de Mots, 1965).

2.8. Data Analysis

Data were analyzed using analysis of variance, followed by Duncan's Multiple Range Test at the 5% level to compare two treatment averages.

3. RESULTS AND DISCUSSION

3.1. Results

3.1.1 Application of photosynthetic bacteria PNSB with shading on number of leaves, stem diameter, chlorophyll content, and total N of komak bean plants

The results of the analysis of variance and Duncan's Multiple Range Test on the number of leaves, stem diameter, chlorophyll content, and total N of komak bean plants are shown in Table 1.

Table 1. Recapitulation of data from research on the application of photosynthetic bacteria PNSB with shade on the number of leaves, stem diameter, amount of chlorophyll, and total N of komak beans (*Lablab purpureus* (L.) Sweet)

Treatments	Number of leaves on a plant (sheet)	Stem diameter per plant (mm)			Chlorophyll content per plant (mg L^{-1})			N total (%)
	35 DAP	35 DAP	70 DAP	105 DAP	a	b	total	
Shade	ns	ns	*	**	*	*	*	ns
n_0 (0%)	21.88 a	3.98 a	6.29 b	7.14 b	10.95 a	3.40 a	14.34 a	5.25 a
n_1 (50%)	21.32 a	3.60 a	5.54 a	6.23 a	15.27 b	5.49 b	20.34 b	5.66 a
PNSB (mL L^{-1})	**	ns	ns	ns	ns	ns	ns	*
b_0 (0)	21.88 b	3.63 a	5.83 a	6.34 a	12.52 a	4.45 a	16.36 a	4.87 a
b_1 (5)	18.25 a	3.89 a	5.79 a	6.99 a	12.46 a	3.93 a	16.37 a	5.24 a
b_2 (10)	24.66 b	3.85 a	6.12 a	6.73 a	14.36 a	4.96 a	19.29 b	6.26 b
Interaction	ns	*	ns	ns	**	ns	**	ns
n_0b_0	20.75 ab	3.98 ab	6.34 c	6.91 ab	9.16 a	3.16 a	12.30 a	4.51 a
n_0b_1	18.63 ab	4.38 b	6.32 c	7.20 b	12.92 b	3.74 a	16.63 ab	5.21 ab

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n_0b_2	26.25 c	3.59 ab	6.20 c	7.31 b	10.78 ab	3.31 a	14.08 a	6.05 b
n_1b_0	23.00 bc	3.29 a	5.33 ab	5.77 a	15.88 c	5.75 ab	20.42 b	5.24 ab
n_1b_1	17.88 a	3.40 a	5.27 a	6.78 ab	12.00 ab	4.12 ab	16.10 a	5.27 ab
n_1b_2	23.08 bc	4.10 ab	6.04 bc	6.14 ab	17.93 c	6.60 b	24.50 c	6.48 b

Note: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at the 5% significance level. ns = not significantly different; * = significantly different; ** = very significantly different

Chlorophyll a is the main photosynthetic pigment in photosynthetic organisms, especially higher plants, has the molecular formula $C_{55}H_{72}O_5N_4Mg$, absorbs purple, blue, orange and red light, reflects greenish blue, maximum absorption at λ 673 nm, found mostly in Photosystem II, plays a role directly in the reaction converting radiation energy into chemical energy as well as absorbing and transporting energy to the molecular reaction center. Chlorophyll b together with chlorophyll a are the main types of chlorophyll found in higher plants and green algae, have the molecular formula $C_{55}H_{70}O_6N_4Mg$, absorb red and orange light, reflect greenish yellow light, maximum absorption at λ 455-640 nm, mostly found in Photosystem I, functions as an absorber of radiation energy which is then passed on to chlorophyll a.

a. Number of leaves per plant

The effect of different shades was not significant on the number of leaves at 35 days after planting. The effect of different PNSB applications was very significant on the number of leaves. The number of leaves increased significantly in the 10 mL PNSB L^{-1} treatment compared to the 5 mL PNSB L^{-1} treatment, but not significantly with 0 mL PNSB L^{-1}, while the interaction between shade and PNSB was not significantly different. The interaction without shade and 10 mL of PNSB L^{-1} showed the highest number of leaves, not significantly different from the interaction of shade with 0 and 10 mL of PNSB L^{-1}, but significantly different from the interaction of shade with 5 mL of PNSB L^{-1} and the application of PNSB 0 and 5 mL L^{-1} without shading. The lowest number of leaves was obtained from the interaction between shade and 5 mL PNSB L^{-1}.

b. Stem diameter per plant

Shade had a non-significantly different effect on stem diameter at 35 DAP, but was significantly different at 70 days after planting and very significantly different at 105 days after planting. 50% shade provides a smaller stem diameter. The effect of PNSB application was not significantly different on stem diameter at all observation times, but tended to increase the size of the stem diameter, while the interaction between shade and PNSB was significantly different at 35 days after planting, but not significantly different at 70 and 105 days after planting.

The interaction between 5 mL PNSB L^{-1} without shade resulted in a larger stem diameter at 35 DAP, not significantly different from 0 and 10 mL PNSB L^{-1} without shade and the application of 10 mL PNSB L^{-1} with shade, but significantly different from 0 and 5 mL PNSB L^{-1} with shade. At 70 days after planting, the interaction between treatment without shade at all levels of PNSB application and shade at 10 mL PNSB L^{-1} was not significantly different but significantly different from shade at 0 and 5 mL PNSB L^{-1}. At 105 days after planting, the interaction between the treatment without shade at 5 and 10 mL PNSB L^{-1} was not significantly different from the treatment without shade at 0 mL PNSB L^{-1} and the shade treatment at 5 and 10 mL PNSB L^{-1}, but significantly different from the treatment shade at 0 mL PNSB L^{-1}. The increase in stem diameter during shade interaction at 10 mL PNSB L^{-1} compared to shade interaction at 0 mL PNSB L^{-1} at ages 35, 70, and 105 days after planting was 19.76 respectively; 11.75; and 6.03% although the difference was not significant at the ages of 70 and 105 days after planting.

c. Chlorophyll content per plant

The effect of shade was significantly different on the content of chlorophyll a, chlorophyll b, and total chlorophyll. 50% shade increases the content of chlorophyll a, chlorophyll b, and total chlorophyll. The application of PNSB had no significantly different effect, but the application of 10 mL L^{-1} PNSB tended to increase the content of chlorophyll a, chlorophyll b, and significantly the total chlorophyll. The interaction between shade and PNSB was not significantly different for chlorophyll a and total chlorophyll content but was very significantly different for chlorophyll b.

The interaction between shade and 10 mL PNSB L^{-1} significantly increased chlorophyll compared to the interaction without shade at all levels of PNSB application and shade at 5 mL PNSB L^{-1}, but not significantly different from the shade treatment at 0 mL PNSB L^{-1}. The interaction of the treatment without shade at all levels of PNSB application showed a lower chlorophyll b content and not significantly different

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compared to the other interactions, but significantly different from the interaction of the shade treatment at 10 mL of PNSB L^{-1}. The interaction of shade with 10 mL of PNSB L^{-1} significantly increased the total chlorophyll content compared to other treatment interactions.

d. N Total

The effect of different shades was not significant on the percentage of total plant N, but shade increased the total N content of plants. The percentage of total plant N was significantly influenced by the application of PNSB. Application of 10 mL PNSB L^{-1} significantly increased the percentage of total plant N compared to 0 and 5 mL PNSB L^{-1}. The interaction between shade and different PNSB was not significant, but the application of 10 mL PNSB L^{-1} with shade showed the highest percentage of total plant N and was significantly different from the interaction without shade and 0 mL PNSB L^{-1}, but not significant with the other interactions.

3.1.2 Application of PNSB photosynthetic bacteria with shading on dry pod weight, number of dry pods, number of dry seeds, dry seed weight and weight of 100 dry seeds of komak beans

The results of the analysis of variance and Duncan's Multiple Range Test on dry pod weight, number of dry pods, number of dry beans, dry bean weight, and weight of 100 dry beans of komak beans are presented in Table 2.

Table 2. Recapitulation of data from research on the application of photosynthetic bacteria PNSB with shade on dry pod weight, number of dry pods, number of dry seeds, dry seed weight, and weight of 100 dry seeds of komak beans (*Lablab purpureus* (L.) Sweet)

Treatments	Dry pod weight per plant (g)	Number of dry pods per plant (fruit)	Number of seeds dry per plant (grains)	Weight seed dry per plant (g)	Weight 100 dry seeds per plant (g)
Shade	**	**	*	**	ns
n_0 (0%)	133.13 b	119.42 b	429.26 b	98.70 b	25.51 a
n_1 (50%)	60.00 a	58.33 a	201.58 a	50.33 a	24.64 a
PNSB (mL L^{-1})	ns	ns	ns	ns	ns
b_0 (0)	96.20 a	89.88 a	318.17 a	72.60 a	24.30 a
b_1 (5)	99.98 a	88.13 a	334.47 a	74.11 a	25.62 a
b_2 (10)	93.51 a	88.63 a	293.63 a	76.84 a	25.31 a
Interaction	ns	ns	ns	ns	ns
n_0b_0	130.63 bc	118.00 cd	425.33 b	95.45 cd	24.70 a
n_0b_1	154.73 c	135.00 d	430.68 b	88.28 bcd	26.54 a
n_0b_2	114.03 bc	105.25 cd	431.75 b	112.38 d	25.28 a
n_1b_0	61.78 a	61.75 ab	211.00 a	49.75 ab	23.89 a
n_1b_1	45.23 a	41.25 a	238.25 a	59.95 abc	24.71 a
n_1b_2	73.00 ab	72.00 bc	155.50 a	41.30 a	25.34 a

Note: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at the 5% significance level. ns = not significantly different; * = significantly different; ** = very significantly different

a. Dry pod weight per plant

The effect of different shades was very significant and reduced dry pod weight by 54.93%. The effect of the PNSB application and the interaction between shade and PNSB were not significantly different. The interaction without shade with all levels of the PNSB application showed heavier pod weight compared to the interaction with shade at all levels of the PNSB application. The interaction without shade with 5 mL PNSB L^{-1} application showed the highest pod weight, not significantly different from 0 and 10 mL PNSB L^{-1} without shade and the interaction between shade at 10 mL PNSB L^{-1}, but significantly different from the interaction between shade at the application of PNSB 0 and 5 mL L^{-1}.

b. Number of dry pods per plant

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The effect of different shades was very significant and reduced the number of dry pods by 51.16%. PNSB application and the interaction between shade and PNSB application were not significantly different from the number of dry pods. The interaction between PNSB application without shade provided a greater number of dry pods compared to the interaction with shade. The highest number of dry pods was obtained in the interaction without shade with the application of 5 mL PNSB L^{-1}, not significantly different from 0 and 10 mL PNSB L^{-1}, but significantly different from the interaction between shade at all levels of PNSB application.

c. Number of dry seeds per plant

Shading had a significantly different effect and reduced the number of dry seeds by 53.40%. The effect of the PNSB application and the interaction between the two was not significantly different in the number of dry seeds. The interaction without shade at all levels of PNSB gave a greater number of dry seeds and was significantly different from the interaction between shade at all levels of PNSB concentration.

d. Dry seed weight per plant

The effect of different shades is very significant and reduces dry bean weight by 49.00%. PNSB application showed no significant effect on dry seed weight but tended to increase dry seed weight when applying 10 mL PNSB L^{-1}. The interaction between no shade at all levels of PNSB gave higher dry seed weight than the interaction between shade at all levels of PNSB. The interaction without shade and the application of 10 mL PNSB L^{-1} showed the highest dry seed weight and increased the dry seed weight by 15.06% although it was not significantly different from 0 and 5 mL PNSB L^{-1}, but significantly different from the interaction with shade at all application levels PNSB.

e. Weight of 100 dry seeds per plant

The effects of shade, PNSB, and the interaction between the two were not significantly different on the weight of 100 dry seeds.

3.1.3 Effect of shade on light intensity, air temperature, and air humidity

The results of the analysis of variance showed that the effect of shade is very significantly different on light intensity, but not significantly different on air temperature and humidity as presented in Table 3.

Table 3. Effect of shade on light intensity, temperature, and air humidity

Treatments	Light Intensity (mmol m^{-2} s^{-1})	Air Temperature (^{0}C)	Air Humidity (%)
Shade	**	ns	ns
n_0 (0%)	1,358.05b	29.08a	55.51a
n_1 (50%)	812.43a	28.60a	55.50a

Note: The average value followed by the same letter is not significantly different based on Duncan's Multiple Range Test at the 5% significance level. ns = not significantly different; ** = very significantly different

3.1.4. Application of PNSB photosynthetic bacteria with shading on pH, temperature, and humidity in planting media for komak beans

The results of the analysis of variance for the pH, temperature, and humidity of the growing medium for komak bean plants were not significantly different between the shade treatments, PNSB, or the interaction between the two as shown in Table 4.

Table 4. Application of PNSB photosynthetic bacteria with shade on pH, temperature, and humidity of planting media

Treatments	pH	Temperature (^{o}C)	Humidity (%)
Shade	ns	ns	ns
n_0 (0%)	6.36	31.04	65.83
n_1 (50%)	6.46	30.88	63.33
PNSB (mL^{-1})	ns	ns	ns

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b_0 (0)	6.48	31.06	61.56
b_1 (5)	6.41	30.94	64.06
b_2 (10)	6.34	30.88	68.13
Interaction	ns	ns	ns
n_0b_0	6.53	31.00	61.25
n_0b_1	6.40	31.25	64.38
n_0b_2	6.15	30.88	71.88
n_1b_0	6.43	31.13	61.88
n_1b_1	6.43	30.63	63.75
n_1b_2	6.53	30.88	64.38

3.2. Discussion

3.2.1 Interaction between shade and PNSB photosynthetic bacteria on the growth and yield of komak beans

The results of the analysis of variance showed that the interaction between shade and PNSB application was not significantly different on the number of leaves at 35 days after planting, stem diameter at 70 and 105 days after planting, chlorophyll b, and total N content of plants (Table 1), dry pod weight, number of dry pods, number of dry seeds, weight of dry seeds, and weight of 100 dry seeds (Table 2). Shade significantly influences light intensity, but its influence on other environmental conditions (air temperature and humidity) is not significantly different. The light intensity received by plants in conditions without shade is greater (1,358.05 mmol m^{-2} s^{-1}) compared to shade conditions (812.43 mmol m^{-2} s^{-1}) as presented in Table 3, but the effect is not linear on The rate of plant photosynthesis is thought to be because the komak bean plant is shade-tolerant. Utomo (2007) stated that the high intensity of sunlight is not directly proportional to the rate of photosynthesis, several plant species reach different compensation points, depending on the type of plant and its level of tolerance. In addition, PNSB photosynthetic bacteria can collect more sunlight energy at low light intensity by increasing the biosynthesis of their photosystem, so that the rate of plant photosynthesis does not decrease. According to Darma *et al.* (2012); Pemberton *et al*. (1998); and Zhu and Hearst (1986), light intensity and quality control the photosynthesis of PNSB bacteria, at low light intensity, the photosystem biosynthesis will increase to collect more light energy, whereas at high light intensity the photosystem biosynthesis will decrease. Therefore, even with low light intensity, the photosynthesis process continues to run actively and produces sufficient photosynthesis for plant growth, development, and yield.

The interaction between shade and PNSB application had a significantly different effect on stem diameter at 35 days after planting, very significantly different on the chlorophyll a and total chlorophyll content of komak plant. The interaction of shade treatment and 10 mL PNSB L^{-1} significantly increased the content of chlorophyll a (48.91%), chlorophyll b (52.00%), and total chlorophyll (49.80%) compared to the interaction without shade and 0 mL PNSB L^{-1}. Although the difference was not significant, the interaction between shade and 10 mL PNSB L^{-1} increased the total N content of plants by 6.64% compared to the interaction without shade and 10 mL PNSB L^{-1}, while the interaction between shade and 10 mL PNSB L^{-1} was significantly different and increased the total N content by 30.40% compared to the interaction without shade and 0 mL PNSB L^{-1}. This is thought to be because the komak bean plant is shade tolerant, according to the research results of Darma *et al*. (2012) which showed an increase in the chlorophyll a (5.99%) and chlorophyll b (13.7%) content of soybeans of the shade-tolerant Petek variety at 50% shade. Apart from that, the photosynthetic bacteria PNSB can convert N_2 from the air into a form that can be absorbed by plants (NH_4^+) by the nitrogenase enzyme. Although the nitrogenase enzyme can be inhibited in the presence of oxygen, Larimer *et al*. (2004) reported that the genus *Rhodopseudomonas* and *Rhodobacter* are very tolerant to oxygen and can live in conditions of minimal oxygen. Elbadry *et al.* (1999) added that the nitrogen content in rice increased by 7.10% with PNSB (*R. capsulatus* DSM 155) inoculation on plant roots. The total N content is thought to also be influenced by the association of plants with the N_2-fixing bacteria *Rhizobium*.

3.2.2 The effect of shading on the growth and yield of komak beans

The effect of shade was significantly different on stem diameter at 70 days after planting, chlorophyll a, chlorophyll b, and total chlorophyll content, as well as the number of dry seeds dry, but not significantly different in the number of leaves and stem diameter at 35 days after planting, total N content, and weight of 100 dry seeds. Komak bean plants with shade produce a lower number of leaves, stem diameter, dry

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pod weight, dry pod number, dry seed number, dry seed weight, and dry weight of 100 seeds, but the chlorophyll a, chlorophyll b, and total N content of the plant is higher (Tables 1 and 2).

Table 1 shows that the different shading treatments were not significant and reduced the number of leaves and stem diameter of komak bean plants 35 days after planting compared to without shading. The results of this research are in line with the research results of Komariah *et al.* (2017) on red bean plants and Suparwata (2018) on green bean plants which reported that the number of leaves on plants that were shaded was less than on plants without shade. The number of leaves and stem diameter at 35 days after planting were not significantly different from those in shade, presumably because plant growth and development at that age were not yet optimal, branches and leaves did not cover each other, so the plants could use light optimally. Apart from that, legume plants belong to the C3 group, namely plants that have a lower light saturation level compared to C4 group plants, so these plants are tolerant of low light intensity. This is the opinion of Sundari *et al*. (2005) who reported the results of their research on the effect of shade on green beans, that C3 plants had a lower light saturation level than C4 plants, so they were tolerant of low light intensity.

Stem diameter was significantly different and decreased significantly with shading by 11.92% at 70 days after planting and 12.75% at 105 days after planting. This is because at the age of 70 and 105 days after planting the branches and leaves have developed further and cover each other, there is competition between plant parts for light which is one of the factors that greatly influences the rate of photosynthesis. Apart from that, at this age the plant has entered the phase of formation and filling of pods and seeds, photosynthesis resulting from photosynthesis is more distributed to the pods and seeds. According to Harjadi (1979), at the beginning of growth, there is maximum use of light, but in the end, the appearance of the plant decreases due to competition between light and other growth factors.

The effect of different shades was very significant and reduced the weight of dry pods, number of dry pods, number of dry beans, and weight of dry beans, but was not significantly different on the weight of 100 dry beans (Table 2). Shade causes reduced light received by plants, thus affecting plant physiological processes (opening and closing of stomata, transpiration, and photosynthesis). Kurosaki and Yumoto (2003) reported the results of their research on soybeans, there was a decrease in yield components and soybean yield with shade due to a decrease in the rate of photosynthesis and the level of light saturation. The lack of light received causes fewer pods to be produced and yields decrease by up to 75%, depending on the plant variety. Arsyad (1995) *in* Rendy (2012) added that the critical phases of komak beans are almost the same as soybeans, namely the vegetative growth phase before entering the exponential phase, the flower formation phase, the initial pod formation phase, and the initial pod filling phase (65-70 days after planting).

Shading significantly increased chlorophyll a, chlorophyll b, and total chlorophyll content, as well as total N content not significantly. The contents of chlorophyll a, chlorophyll b, and total chlorophyll increased significantly with 50% shade, respectively 28.29; 38.0; and 29.50% compared to plants without shade. In conditions of lack of light, plants try to keep photosynthesis running. Shade-tolerant plants have thinner and wider leaves, and contain higher chlorophyll b and a lower chlorophyll a/b ratio compared to shade-sensitive plants (Soepandi *et al.* 2003 *in* Komariah *et al.* 2017).

3.2.3 Effect of application of photosynthetic bacteria PNSB on growth and yield of komak beans

The effect of different PNSB applications was not significant on all variables observed, except for the number of leaves at 35 days after planting and total N content (Tables 1 and 2). Application of PNSB with a concentration of 10 mL L^{-1} significantly increased the number of leaves at 35 days after planting by 25.99% compared to a concentration of 5 mL PNSB L^{-1}, but the increase in leaf number was only 11.27% when compared to the control (0 mL PNSB L^{-1}). Application of 10 mL PNSB L^{-1} significantly increased the total chlorophyll content by 15.19% and total N content by 22.20% compared to the control (0 mL PNSB L^{-1}).

The number of leaves at 35 days after planting and the total N content were significantly different from the application of PNSB, presumably because PNSB was able to collect N_2 from the air and dissolve P to make it available to plants as stated by Lee *et al*. (2021) and Sakarika *et al.* (2020), providing live PNSB cells will indirectly increase N availability because they can fix N_2 in the air and function as a P solvent, making it available to plants. According to Mitchell (1970), plants need N in the vegetative and generative phases, because N is a component of nucleic acids and proteins needed for cell formation. Gardner *et al.* (1991) added that a lack of N will inhibit cell division and enlargement, while a lack of P will reduce the number and length of roots. Hardjowigeno (2007) states that P plays a role in the formation of

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albumin, carbohydrate metabolism, root development, resistance to disease, strengthening stems, formation of flowers, fruit, and seeds, and accelerating ripening. Wong *et al.* (2014) reported the results of their research that the application of PNSB increased the growth response of pak choi plants.

PNSB application did not significantly different on stem diameter (35, 70, and 105 days after planting), chlorophyll content (a, b, and total), dry pod weight, number of dry pods, number of dry seeds, dry seed weight, and weight of 100 dry seeds, perhaps influenced by the compatibility/suitability between the genotype or plant variety and the photosynthetic bacteria PNSB, environmental factors, and inoculation which ensures the formation of an association between plants and PNSB such as soybeans and *Rhizobium*. According to Sumarno *et al.* (1989), the ability of soybean plants to fix N_2 depends on the compatibility of the soybean genotype/variety with Rhizobia, environmental factors that encourage N fixation, and inoculation that ensures the formation of *Rhizobium* colonies. In this study, the environmental conditions (pH of the planting medium and temperature and humidity of the air and planting medium, except light intensity, were not significantly different) (Tables 3 and 4), so it is suspected that PNSB growth is influenced by light intensity as stated by Androga *et al.* (2012), PNSB growth and hydrogen production are influenced by changes in sunlight intensity and temperature.

3.2.4 Effect of shade on light intensity, air temperature, and air humidity

Light intensity was very significantly different from the shade treatment, while air temperature and humidity were not significantly different (Table 3). Shade affects the intensity of light received by plants, apart from directly affecting plants, it indirectly affects the microclimate around the plants (Reskynawati 2014). According to Bunyamin (2010), microclimate is the climate in the air layer near the earth's surface (± 2 m). Microclimate directly influences the physical conditions of an environment. Lakitan (2002) added that microclimate (temperature, humidity, air pressure, shade, and the dynamics of sunlight energy) is important for human, plant, and animal life because it influences the behavior and metabolism of living creatures.

4. CONCLUSION

1. The effect of shade was significantly different on stem diameter at 70 DAP, chlorophyll a, chlorophyll b, and total chlorophyll content, as well as the number of dry seeds, very significantly different to stem diameter at 105 DAP, dry pod weight, number of dry pods, dry seed weight, and light intensity. 50% shade reduces the yield component, even if PNSB is provided.
2. The effect of PNSB was not significantly different on all variables observed, except for the number of leaves at 35 DAP and total N content.
3. Application of PNSB 10 mL L^{-1} increased the number of leaves aged 35 DAP and the total N content of komak bean plants, both without shade and with shade, increased total chlorophyll content with 50% shade, but decreased under without shade.

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