BIOFERTILIZER AND CHEMICAL FERTILIZER APPLICATIONS TO INCREASE THE GROWTH AND YIELD OF FOXTAIL MILLET (*Setaria italica* (L.) P. Beauv.) IN A POT EXPERIMENT

APLIKASI PUPUK HAYATI DAN PUPUK KIMIA UNTUK MENINGKATKAN PERTUMBUHAN DAN HASIL JEWAWUT (*Setaria italica* (L.) P. Beauv.) PADA PERCOBAAN POT

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Accepted: 10 August 2023 / Approved: 27 December 2023

ABSTRACT

Biofertilizers are recommended to improve crops yield but researches regarding biofertilizer inoculation on foxtail millet in Indonesia is still Tlimited. The objective of the experiment was to analyze the growth and yield responses of five local-millet accessions to Bacillus biofertilizer with reduced NPK fertilizer dose; and the germination rate of millet seeds. The pot experiment was arranged in randomized block design with 10 treatments and 10 replications. The treatments were combination of five millet accessions with one dose of NPK fertilizer, and half dose of NPK fertilizer with biofertilizer. Generally, half dose of NPK with Bacillus inoculation did not change the height of six-weeks old plant, and panicle weight and length of millet compared to one dose of NPK. However, this combined fertilizer increased plant height and panicle length of Mani-Mani 79. Reduced dose NPK with Bacillus inoculation increased panicles number of J3 but reduced that of Polman 3; moreover, this combined fertilizer increased the grain weight of J3 and J4, but decreased the yield of Polman 3. The Enrekang and Polman 3 accession had better germination rate; 92% and 89.33%, respectively. This research considered that Bacillus biofertilizer reduced NPK fertilizer dose by 50% and is suggested for growing millet.

Key words: Bacillus, Biofertilizer, Foxtail millet, NPK Fertilizer

ABSTRAK

Pupuk hayati banyak disarankan untuk meningkatkan produksi tanaman tetapi riset mengenai inokulasi pupuk hayati pada jewawut aksesi lokal Indonesia belum banyak dilakukan. Percobaan ini bertujuan untuk menganalisis respons pertumbuhan dan hasil lima aksesi jewawut lokal terhadap inokulasi pupuk hayati Bacillus dan pengurangan dosis pupuk

Cite this as: Hindersah, R., Chotimah A., Matsuura, A., Dewi, Y. W., & Karuniawan, A. (2023). Biofertilizers and chemical fertilizer application for increasing the growth and yield of foxtail millet (*Setaria italica* (L.) P. Beauv.) in a pot experiment. *Jurnal Agro*, *10*(2), 349-360. https://doi.org/10.15575/28983

NPK; serta daya berkecambah biji jewawut setelah panen. Percobaan pot disusun dalam rancangan acak kelompok dengan 10 perlakuan dan 10 ulangan. Perlakuan percobaan adalah kombinasi lima aksesi jewawut dengan satu dosis pupuk NPK, dan setengah dosis pupuk NPK disertai pupuk hayati. Umumnya, tinggi tanaman umur enam minggu, serta bobot dan panjang malai jewawut dengan setengah dosis NPK disertai inokulasi Bacillus sama dengan tanaman yang diberi satu dosis NPK. Namun, tinggi tanaman dan panjang malai Mani-Mani 79 meningkat setelah aplikasi setengah dosis NPK dengan inokulasi Bacillus. Jumlah malai aksesi J3 meningkat sedangkan Polman 3 menurun dengan aplikasi setengah dosis NPK disertai inokulasi Bacillus. Kombinasi pupuk ini meningkatkan bobot biji J3 dan J4 tetapi menurunkan hasil Polman 3. Aksesi Enrekang dan Polman 3 memiliki daya berkecambah yang lebih baik; masing-masing sebesar 92% dan 89,33%. Penelitian ini menunjukkan bahwa pupuk hayati Bacillus dapat mengurangi dosis pupuk NPK sampai 50% dan dapat disarakan untuk tanaman jewawut.

Kata kunci: Bacillus, Jewawut, Pupuk Hayati, Pupuk NPK

INTRODUCTION

Currently, global food availability and security are facing the challenge of losing the diversity of local food crops. Despite its high nutrient value, millet (*Setaria italica* L.) is underutilized in most countries. The largest global millet producer is India with 41.05% market share (APEDA, 2022). From 1961 to 2018 about 25.71% of millets cultivation area has been reduced even though global millet productivity has increased up to 900 kg ha^{-1} on 2018 compared to 575 kg ha^{-1} on 1961 (Meena et al., 2020). Foxtail millets are used to be a staple food in certain dry region of Indonesia; the millet consumption is ceased due to the presence of rice as staple food but millets have still cultivated in some region in limited area (Juhaeti et al., 2020).

Foxtail millet is a Gramineae with intensive roots, and is resistant to drought (Liu et al., 2019), high temperatures (Aidoo et al., 2016), and grows well in soils with low nitrogen and phosphorus (Nadeem et al., 2020). These characteristics are compatible with general condition of dryland ecosystem in Indonesia. The constraint of crops cultivation in tropics is low levels of organic carbon (C), total Nitrogen (N) and available phosphor (P). In common, farmers apply inorganic fertilizer to correct the soil nutrient status. For maintaining the soil quality, the used of inorganic fertilizer combined with organic matter and biofertilizer is better. The biofertilizers usually contain plant growth promoting rhizobacteria (PGPR) as active ingredients to benefice plant growth and yield by increasing nutrient availability and provide bio-stimulant substances.

The prominent PGPR widely used in biofertilizer formulation is the Bacillus that have a mixed function as biofertilizer as well as bio-stimulant. The Bacillus form endospore to withstand the abiotic stress such as drought environment. The mechanisms by which Bacillus may promote millet growth are nitrogen fixation, phosphate solubilization and phytohormone production (Poveda & González-Andrés, 2021; Saeid et al., 2018). The Bacillus has reported to produce exopolysaccharide (Petrova et al., 2021) to increase aggregation by binding soil particle (Vardharajula & Sk, 2014), and to avoid metal toxicity in bacterial cell (Raj et al., 2018). Phytohormones synthesis by *B. methylotrophicus*. *B. subtilis* and *B. megaterium* were reported (Hindersah et al., 2020; Radhakrishnan et al., 2017).

Despite a lot of millet local accessions was grown in certain province, the researchs of biofertilizers application for growing millet in Indonesia are limited. The response of local millet to biofertilizer inoculation along with NPK fertilizer application has not yet been studied. Researchers in other countries concluded that the response of millet to fertilization is determined by the agroecosystem zone (Dicko et al., 2018; Guan et al., 2022). Mixed of chemical fertilizer, organic matter and rhizobacteria are reported to contribute to grain yield of foxtail millet (Monisha et al., 2019; Selectstar Marwein et al., 2019; Vishnu et al., 2022). The inoculation of Bacillus increases biomass, nutrient uptake and yield of foxtail millet (Khatri et al*.,* 2016). However, the information of foxtail millet response to PGPR Bacillus in tropical agroecosystem is still limited. The objective of this pot experiment was to observe the effect of Bacillus biofertilizer consortium and reduced dose of NPK fertilizer on the growth, yield and seed germination of five local millet accession in a pot experiment.

MATERIALS AND METHODS

Pot experiment was conducted in the greenhouse of Faculty of Agriculture, Universitas Padjadjaran located in tropics at 752 m above sea level from July to November 2022. The five millet accessions used in experiment were J3 and J4 from Nusa Tenggara Timur; while Enrekang, Mani-Mani 79 and Polman collected from Papua. The J3 is black millet while the seed color of others was white. Bacillus liquid inoculant was prepared in the Soil Biology Laboratory of the Faculty of Agriculture Universitas Padjadjaran in collaboration with Pupuk Kujang Hold. Co, a national fertilizer company. The population of total Bacillus in liquid inoculant was approximately 10^9 CFU mL^{-1}.

Bacterial inoculant preparation

The *Bacillus Safensis* MDL5 and *B. altitudinis* RPW2 were isolated from Broccoli rhizosphere, Bacillus sp. SZ057 for strawberry rhizosphere, and *B. subtilis* YPS4 was obtained from sweetcorn rhizosphere. Pure culture of each species was grown in Tryptic Soy slant for 72 h at 30°C. One loop of bacterial colony was inoculated to 100 mL of Tryptic soy broth for 48 h prior to inoculate 5 % of liquid inoculant on organic liquid media. The culture then put on the 115-rpm gyratory shaker at room temperature for 72 h. Liquid culture of individual species was mixed and put in the gyratory shaker at room temperature. The inoculant of Bacillus consortium was stored at room temperature without direct sun light prior to millet seedlings inoculation.

Experimental Design

The experimental design was Randomized Block Design to test 10 combination treatments of five millet accession and two fertilization method. All accessions were treated with 1) a dose of recommended NPK compound fertilizer (16:16:16) without Bacillus inoculation; and

2) a half dose of NPK fertilizer with Bacillus. Each treatment was replicated 10 times. The recommended dose of NPK fertilizer for millet is 200 kg ha^{-1}; the recommended and half dose NPK treatments received 0.75 and 0.375 g per pot. The NPK fertilizer was split for two applications at one and four weeks after planting.

Experimental Establishment

Prior to transplanting, the seeds were germinated in 5-cm deep tray filled with mineral soil for 7 days. The millet seedlings were planted in perforated 10 kg plastic pots filled with Inceptisols collected from Ciparanje of Jatinangor Campus. The soil reaction was acid (pH of 4.92) and contained organic-C of 1.5% (medium), total-N of 0.28% (medium), C/N of 5.36 (low), available P_2O_5 of 6.09 mg kg^{-1} (low), potential P_20_5 of 20.43 mg 100 g^{-1} (low), potential K_2O 7.53 mg 100 g^{-1} (low). The Cation Exchange Capacity and base saturation of soil were low. In general, the soil is unfertile.

Three days before planting, the soil in the pots was enriched with cow dung equivalent to 20 t ha^{-1}. A total of 10 millet seedlings were grown in each pot. A week after, the NPK and inoculation of Bacillus biofertilizer consortium were applied by soil treatment. The NPK fertilizer was put in the 2-cm deep hole around the stem plants and then covered by soil. Liquid biofertilizer of Bacillus was applied by soil dressing as much as 10 mL per pot; the inoculant was diluted in 90 mL of ground water before application. The pots were kept in the greenhouse and received 200 mL of water every day; at 5^{th} and 6^{th} week, watering is conducted twice a day; as much as 200 mL each pot for each application.

***In Vitro* Germination Test**

In Indonesia, the harvest time of foxtail millet is 3-4 month after planting. After harvest, the panicles were air-dried at the greenhouse for 4 days, and the seeds were manually detached from the panicles. Seeds were sterilized by dilute (0.01%) $HgCl_2$ and 70% ethanol, and then grown in 9 cm Petri dish containing two layers of sterilized gauze (perforated cotton fabrics) and watering with 10 mL sterilized water. Each Petri dish which contained 25 seeds of one accession stored at 30°C. The assay was replicated three times; the number of germinated seed was observed every day and at day three the germination rates were calculated for three replications.

Parameters and Statistical Analysis

The plant height was measured each week from 3 to 6 weeks after planting. The population of Bacillus in millet rhizosphere was counted by serial dilution plate method on Tryptic soy agar at 6^{th} week. Meanwhile, the number, length and fresh weight of panicle as well as grain weight in a pot were measured at harvest time which depend on the accession. All data of pot experiment were subjected to analysis of variance at $p<0.05$. If the treatment was significantly affected the parameter, then the Duncan multiple Range (DMR) Test at $p<0.05$ was conducted. All statistical analysis was performed using Statistical Product and Service Solutions (SPSS) Ver 20. The average and standard deviation of germinated seed and germination rate were calculated from three replication. The data generated from seed germination test was presented in histogram with standard deviation.

RESULTS AND DISCUSSION

Based on analysis of variance, the plant height of millet was affected by the combination of accession and fertilizer. Table 1 showed that the highest plant at 3rd weeks were Enrekang with recommended NPK dose, while at 4th and 5th were Enrekang with both one dose of NPK as well as half dose of NPK + biofertilizer. The highest plant at 6th week was J3 and Enrekang received both fertilizer treatments. The Bacillus biofertilizer combined with reduced dose of NPK fertilizer did not show significant change of plant height at 3rd week compared to recommended dose (Table 1). However, at 4th week, reduced NPK fertilizer combined with biofertilizer decreased the plant height of Polman 3 but did not affect height of other accessions. At 5 and 6 weeks after transplanting, the height of all millet accessions received reduced dose of NPK + Bacillus was similar with plant treated with recommended dose of NPK. This experiment showed that J3 and Enrekang have higher shoot at 6 weeks after transplanting (Table 1); while the plant height of Polman 3 was the lowest.

Tabel 1. Plant height of various accession of millet grown with NPK, and combination of NPK fertilizer and Bacillus inoculant

Millet Accession and Fertilizer	Plant height (cm) at week			
	3	4	5	6
J3, NPK	63.5 ± 8.2 abc	86.1 ± 12.8 b	119.2 ± 13.7 bcd	139.1 ± 13.0 d
J3, 1/2 dose NPK, BB*	65.3 ± 6.9 abc	88.9 ± 8.8 bc	123.2 ± 9.1 cd	145.2 ± 9.8 d
J4, NPK	56.5 ± 2.8 a	72.8 ± 4.5 a	104.4 ± 4.6 a	121.2 ± 5.8 ab
J4, 1/2 dose NPK, BB	60.3 ± 2.6 abc	73.8 ± 3.4 a	105.6 ± 6.4 a	122.1 ± 6.7 ab
Enrekang, NPK	71.6 ± 9.6 c	94.2 ± 11.5 c	125.1 ± 17.4 d	139.8 ± 14.6 d
Enrekang, 1/2 dose NPK, BB	70.5 ± 6.3 bc	94.5 ± 4.3 c	125.9 ± 8.6 d	141.5 ± 11.6 d
Mani-Mani 79, NPK	59.9 ± 4.7 abc	89.1 ± 7.7 bc	110.5 ± 8.9 ab	122.2 ± 13.6 ab
Mani-Mani 79, 1/2 dose NPK, BB	58.9 ± 4.3 ab	89.2 ± 9.0 bc	115.5 ± 10.6 bc	129.1 ± 13.3 bc
Polman 3, NPK	54.2 ± 3.4 a	82.0 ± 5.1 b	110.9 ± 8.6 ab	116.2 ± 10.6 a
Polman 3, 1/2 dose NPK, BB	53.8 ± 9.3 a	73.9 ± 11.91 a	104.8 ± 1.5 a	113.4 ± 14.47 a

Numbers in a column followed by the same letters are not significantly difference based on DMR test at p≤0.05. *BI: Bacillus Biofertilizer.

This experiment clearly showed that reduced doses of NPK combined with Bacillus enabled to maintain plant height at 6th week. Bacillus species are well known P-solubilizer and N-fixer (Saeid et al., 2018; Singh *et al.,* 2020). The N and P content of soil dictate the nitrogen fixation and P solubilization. Nitrogen fixation inhibition in soil by nitrogen fertilizers are reported in common bean (Reinprecht et al., 2020). The activity of phosphate solubilizing bacteria was reduced in soil with high available P content but stimulated in soil with high insoluble P (De Bolle et al., 2013; Long & Wasaki, 2023). In this experiment, reduced dose of NPK lower the N and P content in soil and enhance the capacity of Bacillus to fix N and solubilize P and hence provide N and P for plant height increment (Table 1).

At the end of vegetative stadia, the Bacillus population in the millet rhizosphere was approximately 10^7 CFU g^{-1} which is 7 in log_{10} (Figure 1). The Bacillus population of J3, J4 and Polman 3 rhizosphere was lower after inoculation because none of Bacillus used in this experiment was isolated from millet rhizosphere. It is likely that reduced dose of NPK increase the C/N and C/P soil which then reduce the Bacillus proliferation. All Bacillus in biofertilizer were isolated from vegetables rhizosphere. Since plants have different exudates composition (Dhungana *et al.,* 2023), Bacillus proliferation in the rhizosphere might be differ. In this experiment, lower Bacillus population in rhizosphere of millet J3, J4 and Polman received half dose of NPK fertilizer mixed with biofertilizer (Figure 1) is possibly related to the different compound and amounts of root exudates. However, the different of Bacillus count of all accession and treatments was only less than 1 of log_{10} (Figure 1).

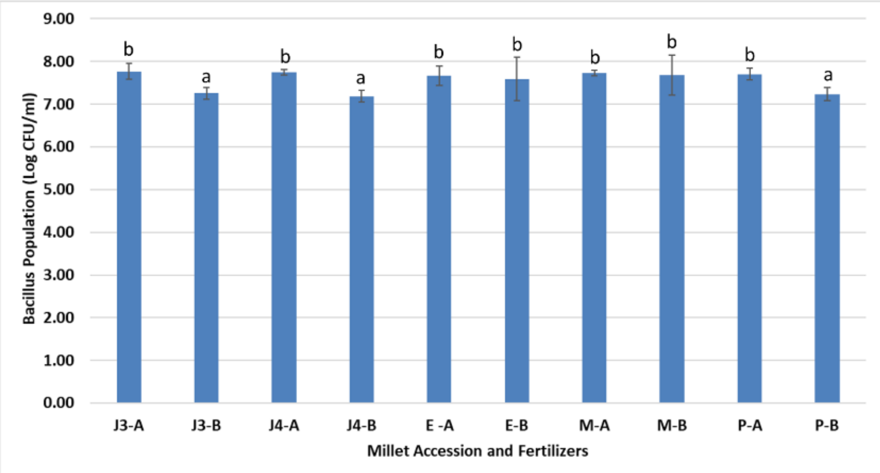

Figure 1. The population of Bacillus in the rhizosphere of different accession of millet grown with A: recommended dose of NPK fertilizer, B: half dose of NPK fertilizer with biofertilizer. J3, J4, E (Enrekang), M (Mani-mani 79), P (Polman 3) were the accession.

Tabel 2. Flowering and harvest time of five millet accession grown in potted-soil

Accession	Flowering	Harvest
J3	47	106
J4	51	106
Enrekang	43	86
Mani-Mani 79	53	106
Polman	41	82

The flowering time of millet depended on the accession so that the mature panicle was harvested in different time (Table 2). The millets were transplanted on 27th July and the last harvest was on 10 November for J3, J4 and Mani-Mani 79 accessions. At the flowering stadia, the plants still green and performed panicle without physiological disorder and pest attack (Figure 2).

Based on analysis of variance, the panicle number, weight and length were determined by the combination of accession and fertilizer composition. The higher panicle number was demonstrated by J4 accession but their weight and length were significantly lower than Mani-Mani 79 (Table 3). Certain accession demonstrated positive response on bacterial inoculation combined with reduced dose of NPK. The panicle number of J3 was slightly increased due to Bacillus inoculation; but Polman 3 has lower panicle number when bacterial inoculation and reduced fertilizer doses is applied. The panicle length of Mani-mani 79 was clearly increased by the application of half dose NPK combined with bacterial inoculation. The phosphor is prominent for the plant generative stadia (Hidayat *et al.,* 2018). In this experiment, the Bacillus population in Mani-mani rhizosphere was not affected by reduced NPK. The difference response of each accession to fertilizer treatment was likely due to genetic properties. The morphological and yield characteristics of each millet accession usually determined by their genetic properties but the morphological data of tested accession is not available.

Figure 2. The flowering of millet plant Polman 3 accession (a) and grain filling of Enrekang accession (b)

The grain yield of each treatment depended on the combination of accession and fertilization (Figure 3). In general, Bacillus inoculant combined with 50% NPK fertilizer resulted in the similar plant height (Table 1) indicated that bacterial inoculant enables to replace half dose of NPK. In contrast, combination of biofertilizer and 50% of NPK fertilizer significantly influenced the grain weight of certain accession (Figure 3). Application of half dose of NPK combined with biofertilizer significantly increased the grain weight of J3, slightly increased that of J4, but clearly decreased that of Polman 3.

Millet researches are usually performed in the field. In various agroecosystem, chemical fertilization consistently increased the yield. In various Mali-African agroecological zones, an application of 22.5 kg P combined with 40 or 60 kg N, and 30 kg P without N and K significantly increased millet grain yields (Dicko et al., 2018). In warm-temperate sub-humid continental monsoon climate of China, the optimal crop yield (3927.0 kg ha^{-1}) was showed in plants with 130.4–173.5 kg ha^{-1} N, 83.5–103.8 kg ha^{-1} P, and 133.4–153.2 kg ha^{-1} K (Guan et al., 2022). In the Andhra Pradesh tropical wet and dry climate agro-climatic zone, of the four varieties, millet SiA 3085 enhanced growth and yield after application of 50 kg ha^{-1} of N (Jyothi et al., 2015).

Table 3. Panicle number per plant of various millet accession grown with NPK, and combination of NPK fertilizer and Bacillus biofertilizer.

Accession and Fertilizer treatments	Panicle number	Panicle weight (g)	Panicle length (cm)
J3, NPK	12.8 ± 2.5 ab	21.17 ± 4.1 ab	9.5 ± 2.7 ab
J3, 1/2 dose NPK, BB*	16.1 ± 3.5 a	22.72 ± 4.2 ab	9.0 ± 2.4 a
J4, NPK	19.4 ± 2.5 d	19.56 ± 6.0 ab	11.2 ± 1.6 abc
J4, 1/2 dose NPK, BB	18.9 ± 3.9 d	19.56 ± 5.4 ab	10.5 ± 1.5 abc
Enrekang, NPK	13.3 ± 2.7 abc	22.23 ± 4.9 ab	14.2 ± 2.6 de
Enrekang, 1/2 dose NPK, BB	14.8 ± 2.7 abc	24.49 ± 5.4 b	12.5 ± 2.4 cd
Mani-Mani 79, NPK	15.4 ± 2.8 bc	24.69 ± 3.9 b	12.1 ± 2.2 cd
Mani-Mani 79, 1/2 dose NPK, BB	14.2 ± 1.9 abc	24.37 ± 3.6 b	14.8 ± 3.9 e
Polman 3, NPK	14.4 ± 2.6 abc	19.81 ± 3.3 ab	11.7 ± 2.1 bc
Polman 3, 1/2 dose NPK, BB	12.1 ± 1.7 a	18.71 ± 5.3 a	11.0 ± 1.8 abc

Numbers in a column followed by the same letters are not significantly difference based on DMR test at p≤0.05. *BI: Bacillus Biofertilizer

The grain weight of other accession was not changed. Lower P availability in soil induce the P solubilization by Bacillus resulted in the increase of fertilizer efficiency used. Moreover, Bacillus enable to provide Indole acetic acid and Cytokinin (Poveda & González-Andrés, 2021) for better root growth and hence nutrient uptake and plant growth. Irrespective of the fertilizer treatments, the grain yield of Polman 3 was higher while that of Enrekang was the lowest. The variation of grain weight might be caused by its genetic characteristics.

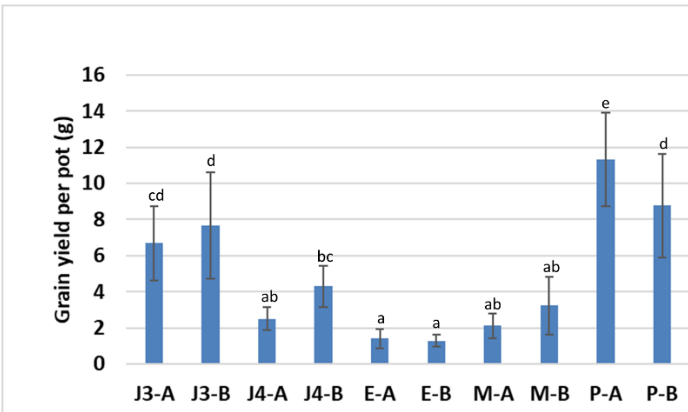

Figure 3. Grain weight of five millet accession grown in pot with A: recommended dose of NPK fertilizer, B: half dose of NPK fertilizer with Bacillus biofertilizer. J3, J4, E (Enrekang), M (Mani-mani 79), P (Polman 3) were the accession.

The application of beneficial microbes to agroecosystems is supposed to improve millet plant growth and yield. Mixed application of 75% recycled-derived fertilizer with biofertilizer of 5 kg ha^{-1} incubated in vermicompost recorded higher grain yield of millet (Divya et al., 2017). The *B. megaterium* UFMG50 and other P-solubilizations increased foliar area, plant height, root, shoot, plant biomass, and P content in the shoot of pearl millet (*Pennisetum glaucum*) in Brazil (Silva et al., 2021). Current study agrees with the increase of millet grain yield in pot culture after seeds inoculation with *Bacillus* sp. before sowing with or without 50 g of tri-calcium phosphate by 51.39% and 36.55% respectively (Khatri et al., 2016). The result showed that foxtail millet grains are about 1-1.5 mm in length and the color of glumes (husk) was brown (Figure 4). The color of all seeds was brown but J4 was black.

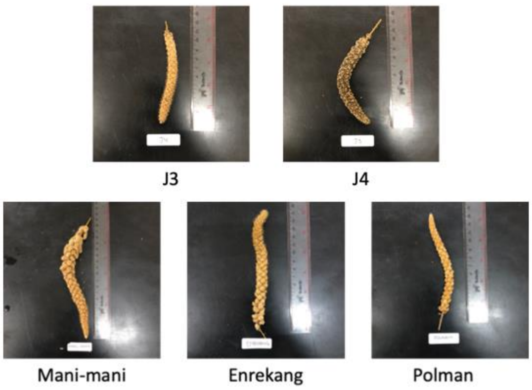

Figure 4. The tassel shape and color of five millet accession grown in potted soil

Seed Germination Percentage

Based on in vitro germination test by using 25 seeds in a Petri dish, the number and percentage of germinated seeds determined by the accession (Table 4). The germination percentage is an estimate of the viability of a population of seeds when they grow in the field for corps production.

The rate of germination is an important indicator of seed vigor. High germination percentage increases the chance of the millet seed to establish in the field which in turn assure the growth and production. In general, recommended germination percentage for good crops production is more than 90%. The J3, J4 and Mani-mani 79 accession have germination rate less than 90%. Both J3 and J4 accession showed lowest germination rate.

Table 4. Millet seed germination based on *in vitro* test

Millet Accession	Number of germinated seeds	Germination rate (%)
J3	6.33 ± 1.15	25.33 ± 4.62
J4	11.33 ± 0.58	45.33 ± 2.31
Enrekang	23.00 ± 1.00	92.00 ± 4.00
Mani-Mani 79	17.67 ± 0.58	70.67 ± 2.31
Polman 3	22.33 ± 0.58	89.33 ± 2.31

CONCLUSION

The pot experiments verified that some accessions were responsive on the application of half dose of NPK with Bacillus inoculation. This study concluded that

- Half dose of NPK + biofertilizer did not change the plant height of 6-weeks old accession compared to recommended dose of NPK; but Mani-mani 79 plant height was slightly increased by that treatment.
- The panicle number of J4 has not affected by fertilization method but

was higher compared to other accession. The panicle number of J3 was increased due to reduced NPK fertilizer combined with Bacillus but Polman 3 has lower panicle number when combined fertilizer was applied.

- The panicle weight and length of each accession have not influence by reduced dose of NPK + biofertilizer. Nonetheless, panicle length of Mani-mani 79 was clearly increased by the application of half dose NPK combined with Bacillus.
- Half dose of NPK combined with Bacillus biofertilizer increased the grain weight of J3 and J4 but clearly decreased the Polman 3 yield.
- The germination rate of Enrekang (92%) and Polman 3 (89.33%) was higher compared to other accession.

ACKNOWLEDGEMENT

The research was funded by Academic Leadership Grant of Universitas Padjadjaran; contract number 2203/UN6.3/PT.00/2022. We appreciate Balai Besar Pengembangan Pertanian (Agricultural Development Office) of Papua Province for providing some millet accessions.

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