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Viability, productivity, and anatomical response of groundnuts (*Arachis hypogaea* L.) to biofertilizer-sludge biogas applications

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ABSTRACT. Groundnuts (*Arachis hypogaea* L.) is one of the essential food commodities in Indonesia. The use of biofertilizer has been applied to various types of crops. Meanwhile, the effect of using biofertilizer-sludge biogas on groundnuts is yet unknown. This study aims to analyze the seed viability and vigour, yield productivity, the anatomical response of groundnuts, and optimum concentration that could increase the values of each parameter. Treatments given include applying biofertilizer-sludge with 15 levels of treatment concentration compared to groundnuts without biofertilizer-sludge application as a control. The land was divided into 16 beds for each treatment consisting of control, biofertilizer from 10, 15, 30 L/ha, sludge from 12, 24, to 36 ml, and variations dosage of biofertilizer and sludge combined. The parameters observed for viability and vigour include the percentage of seed germination (GP), seed vigor index (SVI) for yield, the value of harvest index (HI), dry weight of the harvest, and root-shoot ratio (R/S). Anatomical responses were observed with stem diameter, stem's metaxylem diameter, root diameter, root's metaxylem diameter, and seed diameter. The biofertilizer-sludge results significantly affected HI, R/S values, stem diameter, root's metaxylem diameter, and seed diameter. This research concluded that the application of biofertilizer-sludge did not significantly affect the seed viability and vigour and the dry weight of the harvest. The application of biofertilizer-sludge in various doses of concentration resulted in a decrease in the stem metaxylem diameter and root diameter compared to the control. A total of 10 L/ha biofertilizer + 24 ml sludge was an optimum concentration to increasing HI and R/S values. For the increasing stem, root metaxylem, and seed diameter, biofertilizer 30 L/ha + sludge 12 ml, sludge 24 ml, and biofertilizer 15 L/ha + sludge 12 ml were the optimum concentrations, respectively.

Keywords: biofertilizer-sludge; germination; harvest index; root-shoot ratio; seed viability and vigour

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INTRODUCTION

Groundnuts (*Arachis hypogaea* L.) is a family of Fabaceae originating from Brazil, South America, as one of the essential commodities in the food crop sub-sector (Kementerian Pertanian, 2015). According to Sumarno (2015), groundnuts production in Indonesia is still low, and it cannot meet domestic needs, especially for the food industry. It was reported that in 2010, the total production of groundnuts only reached 770228 tons/year, while the total consumption and industrial needs were around 800000 tons/year (Kementerian Pertanian, 2011). In fact, Indonesia is the second-largest country in groundnuts imports (Kementerian Pertanian, 2015). Based on the data, efforts are needed to increase domestic groundnuts' productivity to meet production and consumption needs and reduce the number of imports from abroad.

Climatic conditions and the planting environment strongly influence plant productivity. The use of chemical fertilizers continuously and erratically can cause decreased soil fertility and the efficiency of absorption for nutrients needed of plants, which results in stagnation or decreased yields (Dhar *et al*., 2015; Kumbar *et al*., 2017; Lin *et al.*, 2019). Replacing chemical fertilizer with biofertilizers can prevent the negative impact of chemical fertilizers, which can provide nutrients for plants and increase the level of sustainability of the agronomic system in the long term (Moradi *et al*., 2011; Suhag, 2016; Mahanty *et al*. 2017; Kizito *et al*., 2019).

The advantage of biofertilizer application is no or minimum adverse effect on the ecosystem as it has longer shelf life than a chemical one (Kawalekar, 2013; Sahoo *et al*., 2014; Saha & Bauddh, 2020). Biofertilizers applied as seed and soil inoculants can multiply and participate in nutrient cycling, providing a safe environment, and thus benefiting crop productivity (Singh *et al*., 2011; Herrmann & Lesueur, 2013). Biofertilizer can be applied with the biogas sludge that contains many materials and nutrients (Nguyen *et al*., 2013; Kirchmann *et al.*, 2017). The high nutrient content in biogas sludge can increase soil fertility by improving soil physical, chemical, and biological properties (Du *et al*., 2018; Xu *et al.*, 2019). Biogas sludge has undergone anaerobic fermentation to be directly used to fertilize plants, so the use of biofertilizer together with biogas sludge can optimize the increase in plant productivity (Asam *et al.*, 2011; Thorin *et al.*, 2012; Liu *et al.*, 2014; Zhang *et al*., 2017).

As the previous study reported by El-Sayed *et al.* (2017), El-Sayed *et al.* (2018), and Bertham *et al.* (2019), biofertilizers could increase the quality of fennel plant productivity, essential fat content and cause an anatomical response, which the mericarpium cells in fennel to become more compressed. Each cremocarpium produces one sterile seed and one fertile seed. The treatment of sludge 2000 ml/100m^2 can provide the most excellent productivity gains in rice (*Oryza sativa* L. cv. Segreg) in our previous studies (Siswanti *et al*., 2018). The effect of biofertilizer and biogas sludge on productivity and the anatomical response of groundnuts is still unknown, so this research needs to be conducted well.

MATERIALS AND METHODS

The research methods include cultivation of groundnuts (*A. hypogaea* L.), maintenance, harvesting, testing the viability and vigour, drying of harvest product, making slide preparations of groundnuts organs, and observing the anatomical parameters. Chemical analysis of soil, water, and fertilizer was conducted by Laboratory of Soil, BPTP, Yogyakarta. The cultivation to harvesting process is carried out in Wukirsari Village, Cangkringan, Sleman, Yogyakarta. The seed viability and vigour testing, and weight measurement were carried out at the Greenhouse of Faculty of Biology, Universitas Gadjah Mada. The making and observing anatomical slide preparations were carried out at the Laboratory of Plant Development Structure, Faculty of Biology, Universitas Gadjah Mada.

Table 1. The concentration of biological fertilizers, sludge, and a combination of both were used in this study.

Treatments	
P0	Control (without biofertilizer or sludge)
P1	Biofertilizer 10 L/Ha
P2	Biofertilizer 15 L/Ha
P3	Biofertilizer 30 L/Ha
P4	Sludge 12 ml
P5	Sludge 24 ml
P6	Sludge 36 ml
P7	Biofertilizer 10 L/Ha + Sludge 12 ml
P8	Biofertilizer 10 L/Ha + Sludge 24 ml
P9	Biofertilizer 10 L/Ha + Sludge 36 ml
P10	Biofertilizer 15 L/Ha + Sludge 12 ml
P11	Biofertilizer 15 L/Ha + Sludge 24 ml
P12	Biofertilizer 15 L/Ha + Sludge 36 ml
P13	Biofertilizer 30 L/Ha + Sludge 12 ml
P14	Biofertilizer 30 L/Ha + Sludge 24 ml
P15	Biofertilizer 30 L/Ha + Sludge 36 ml

Notes: The treatments were applied to each demonstration plot with 10 replications each.

Cultivation and seed testing. The materials needed for cultivation are 110 L of biogas sludges, 1.5 L of biofertilizer (cow urine processed with bio-organic fertilizer POMI brand) stored since 2017, and 2 kg of groundnut seeds. Seed viability testing is carried out directly on top of some thin paper media (Top of Paper) (Copeland & McDonald, 1995; ISTA, 2016). A total of 50 seeds harvested from each treatment were placed on trays coated with three scrap papers on top of it, which were remotely in wet condition and observed for ten days at room temperature. Germination percentage (%) is determined by counting seeds that normally germinate after seven days of incubation (ISTA, 2016), then calculated by the following formula:

$$\textit{Germination percentage}\ (\%) = \frac{\sum a + \sum b}{\sum number\ of\ seeds\ set\ for\ germination}\ x\ 100\%$$

Notes:
∑ a= ∑ number of normal seedling I is number of seeds germinated on the 5th day
∑ b= ∑ number of normal seedling II is number of seeds germinated on the 10th day

The seedling vigor index (%) was determined by counting germination in the first five days (ISTA, 2016). Each part of the plant is dried, regularly weighing every two days until a constant weight is obtained (Junjittakarn *et al.*, 2014; Yusuf *et al.*, 2014). The measured constant weight is recorded as dry weight. Furthermore, it is used to determine the value of the crop Harvest Index with the following formula (Bewly & Black, 1982; Sharma & Smith, 1986).

$$\text{SVI (\%)} = \frac{\sum number\ of normal\ seedling\ I}{\sum nmber\ of\ seeds\ set\ for\ germination}\ x\ 100\%$$

Notes:
∑ normal germinated seeds I is number of seeds germinated on the 5th day

$$\text{Harvest index (HI)} = \frac{Pods\ dry\ weight}{Pods\ dry\ weight + plant\ dry\ matter}\ x\ 100\%$$

$$R/S = \frac{Root\ dry\ weight}{Shoot\ dry\ weight}$$

Anatomical slide preparations. The fresh semi-permanent slide preparations is conducted by non-embedding technique. Each treatment preparation of seeds, roots, and stems made three replications. Then samples are put in a 70% alcohol solution for fixation purposes. The making of cross slices was carried out using a sliding microtome with a thickness of 6-12 µm, then glued to a glass object with a mixture of glycerin. The preparations were stained using 1% safranin which has been dissolved in 70% alcohol. The tag label was given to the slide preparations. The semi-permanent slides were then immediately measured (Image Raster ver. 3.0) and photographed (Optilab ver. 2.2).

Data Analysis. Data on germination percentage (%), seedling vigor index (%), harvest index (%), dry weight of yield (g), root-shoot ratio (R/S), and the measurement of anatomical parameters were analyzed by ANOVA (Analysis of Variance) with a confidence level of 0.05, followed by the DMRT test with a confidence level of 95% (α= 0.05) (Steel & Torrie, 1984). The data analysis of quantitative performed using SPSS software ver. 16.0.

RESULTS AND DISCUSSION

Environmental condition. The research was conducted on a field located in Wukirsari Village, Cangkringan District, Sleman Regency at an altitude of 400 m above sea level (masl), with the highest recorded temperature being 32°C and the lowest temperature being 18°C (Kapanewon Cangkringan, 2020). The average temperature during the research was about 26.75°C, the average sunlight intensity of 441.75 Lux, with pH 7, and soil moisture is relatively dry conditions. This condition can be stated as a good condition to support the growth of groundnuts (*A. hypogaea* L.), that grow well on with an altitude < 500 masl, optimal growth temperatures ranging from 25-32°C, and pH is almost neutral 6.5-7.0 (Ramadani *et al*., 2015).

The biofertilizer-sludge biogas treatment to the soil was tested for the available nitrogen content (N-NH_4) using the Kjeldahl method. The results showed that the available N content of the soil in this study was classified as very low (<0.1%) (Table 2). This category is based on research criteria resulting from soil analysis listed in the technical guidelines for chemical analysis of soil, plants, water, and fertilizers by BPPT (2020).

Table 2. N-available contained in soil.

Treatments	N-available
P0	0.0191*
P3	0.0188*
P6	0.0270*
P9	0.0227*
P12	0.0209*
P15	0.0227*

Notes: *= Very low (< 0.1%). Source: Laboratory of Soil, BPTP, Yogyakarta.

The application of biofertilizer-sludge did not have an optimal effect on increasing nitrogen levels by the N-fixing microbes. The N-available in the soil only depends on the results of N_2 fixation from the environment by the microbial association of *Rhizobium* sp., which is symbiotic with the groundnut plant's root system. In addition, the effectiveness of the biofertilizer plays a role due to the long storage time. Biofertilizer used in this research was made in 2017 and stored since that time. Thus a high possibility of microbes contained in biofertilizer partly has analyzed as *Rhizobium* sp. The results showed *Rhizobium* sp. was not found inside the sample, the opposite with the starter. It is expected that these microbes have died during the storage period.

Seeds viability and vigour. The percentage of seed germination (GP) and seed vigor index (SVI) obtained after testing seed viability was shown in Table 3.

Table 3. N-available contained in soil.

Treatments	Parameters	
	GP(%)	SVI(%)
P0	42 ± 20.6^{ab}	14 ± 9.3^{a}
P1	16 ± 11.2^{a}	6 ± 6^{a}
P2	20 ± 10.5^{ab}	6 ± 4^{a}
P3	36 ± 17.2^{ab}	18 ± 8.6^{a}
P4	60 ± 14.8^{ab}	12 ± 5.8^{a}
P5	**72 ± 8.6^{ab}**	20 ± 4.5^{a}
P6	52 ± 22.7^{ab}	20 ± 8.4^{a}
P7	58 ± 18^{ab}	20 ± 7.1^{a}
P8	32 ± 11.6^{ab}	6 ± 2.4^{a}
P9	42 ± 27.3^{ab}	16 ± 13.6^{a}
P10	28 ± 9.7^{ab}	8 ± 5.8^{a}
P11	28 ± 3.7^{ab}	6 ± 2.4^{a}
P12	24 ± 14.7^{ab}	8 ± 3.7^{a}
P13	44 ± 10.8^{ab}	10 ± 3.2^{a}
P14	26 ± 10.8^{ab}	12 ± 5.8^{a}
P15	36 ± 18.3^{ab}	6 ± 4^{a}

Notes: The number followed by the same letter in one column shows that it is not significantly different from the DMRT test with the 95% confidence level; Figures in bold indicate the most optimal treatment results.

The average seed germination percentage showed no significant difference between the control with various dosages of biofertilizer-sludge. P5 with sludge 24 ml provides the highest GP among all treatments. The average of SVI was not significantly different between control and various concentrations of biofertilizer-sludge. P5 with 24 ml of sludge, P6 with 36 ml of sludge, and P7 with biofertilizer 10 L/ha + sludge 12, respectively, gave the highest SVI value compared to all treatments. The GP in the biofertilizer treatment increased with the gain of biofertilizer doses (P1, P2, P3), but this was not in line with the sludge and the biofertilizer-sludge combination. According to Silitonga *et al.* (2018), combining the two treatments can often trigger inhibition or cause plants do not to respond to the treatment at all. This condition can occur because the response of plants is influenced by plant genetic and environmental conditions as interrelated factors.

The percentage of seed germination has the same correlation with the size of the seed diameter. The internal factors, including the ABA accumulation during the seed maturity, the lifespan, size, longevity, and dormancy of the seeds, and the presence of a germination inhibitor, play a role in seed germination (Chiang *et al.*, 2011; Rajjou *et al.*, 2012; Long *et al.*, 2015; Zhang *et al.*, 2015).

Table 4. Average value of dry weight of *A. hypogaea* L. application of biofertilizer-sludge in various dosage concentrations.

Treatments	Parameters			
	Total dry weight (g)	Shoot dry weight (g)	Root dry weight (g)	Pods dry weight (g)
P0	46 ± 5.7^{a}	17 ± 3.3^{abc}	0.78 ± 0.08^{a}	28.2 ± 3.4^{ab}
P1	41.3 ± 4.5^{a}	12 ± 1.1^{ab}	0.7 ± 0.05^{a}	27.8 ± 3.6^{ab}
P2	42.36 ± 4.7^{a}	15.2 ± 2.2^{ab}	0.76 ± 0.07^{a}	26.4 ± 3.0^{ab}
P3	42.84 ± 3.3^{a}	12.4 ± 1.7^{ab}	0.64 ± 0.04^{a}	29.8 ± 3.0^{ab}
P4	36.74 ± 6.0^{a}	17.2 ± 1.8^{abc}	0.74 ± 0.06^{a}	18.8 ± 5.0^{a}
P5	37.6 ± 4.0^{a}	11.4 ± 2.0^{ab}	0.6 ± 0.10^{a}	25.6 ± 2.7^{ab}
P6	55.26 ± 6.1^{a}	**25.4 ± 4.3^{c}**	0.86 ± 0.09^{a}	29 ± 4.5^{ab}
P7	36.82 ± 5.8^{a}	15.8 ± 4.0^{ab}	0.62 ± 0.04^{a}	20.4 ± 3.6^{ab}
P8	36.92 ± 1.5^{a}	10.2 ± 1.5^{a}	0.72 ± 0.09^{a}	26 ± 0.8^{ab}
P9	50.02 ± 6.0^{a}	20.2 ± 2.3^{bc}	0.82 ± 0.12^{a}	29 ± 4.2^{ab}
P10	38.1 ± 4.0^{a}	12.4 ± 2.6^{ab}	0.7 ± 0.07^{a}	25 ± 2^{ab}
P11	53.1 ± 9.1^{a}	19 ± 3.5^{abc}	0.9 ± 0.10^{a}	**33.2 ± 6.2^{b}**
P12	47.46 ± 6.2^{a}	16.6 ± 2.9^{abc}	0.86 ± 0.10^{a}	30 ± 3.5^{ab}
P13	46.26 ± 6.0^{a}	16.6 ± 3.5^{abc}	0.86 ± 0.10^{a}	28.8 ± 2.9^{ab}
P14	41.82 ± 6.0^{a}	15 ± 2.6^{ab}	0.82 ± 0.12^{a}	26 ± 3.4^{ab}
P15	43.64 ± 8.4^{a}	17.6 ± 1.9^{abc}	0.84 ± 0.11^{a}	25.2 ± 6.5^{ab}

Notes: The number followed by the same letter in one column shows that it is not significantly different from the DMRT test with the 95% confidence level; Figures in bold indicate the most optimal treatment results.

Yield productivity. Based on the results obtained in Table 4 that the highest dry weight was obtained in sludge 36 ml (P6) with pods dry weight of 29 g, root dry weight of 0.9 g, and shoot dry weight of 25 g. The lowest dry weight was in the sludge12 ml (P4) with a pod + seed dry weight of 19 g, a root dry weight of 0.8 g, and a shoot dry weight of 17 g. The application of biofertilizer-sludge fertilizer, which was not significantly different and had no significant effect on dry weight or yield biomass, was also reported in previous studies by Priambodo *et al.* (2019) on spinach (*Amaranthus tricolor*) with the treatment of biofertilizer and inorganic fertilizers. Cahyadi & Widodo (2017) stated that the dry weight of caisin biomass (*Brassica chinensis*) treated by biofertilizer is not significantly different from NPK fertilizer (control). The concentration of biofertilizer might not provide the nutrients needed by plants, thus the concentration of biofertilizer needed to be increased.

The distribution pattern of photosynthate is different during the vegetative and the generative phase (Sarawa & Baco, 2014). In the vegetative phase, the allocation of photosynthate prioritizes the canopy part, while in the generative phase, the allocation of photosynthate focuses on supplying nutrients to the reproductive parts of plants such as fruit and seeds. In line with the results of this study, the dry weight of pods + seeds tended to be greater than the canopy dry weight (Table 4). The application of biological fertilizers could not increase the growth of groundnuts. The lack of nitrogen in the plant is shown in soil analysis with a very low N-available (Table 2). Nitrogen deficiency interferes with the growth process, causing stunted plants, reduced yields of dry weight. Also causing the older leaves to turn yellow, and eventually, the plant's growth stops (Awadalla & Abbas, 2017).

Based on Table 5, P8 with biofertilizer 10 L/ha + sludge 24 ml was the most effective in increasing R/S and HI in *A. hypogaea* L. The average soil moisture during this study was recorded dry. However, the R/S result obtained was an average of 0.055. This R/S is low when compared to previous studies with the same dry condition or water stress. Srivalli *et al.* (2016) reported that the R/S value of peanuts under water stress conditions is increased with the value from 0.2 to 0.44. The low R/S value can be caused by low soil moisture and deficient levels of N-available (Table 2). Jagana *et al.* (2012) also reported in their research that a better R/S increase in grounds was positively correlated with an increase in pod production and HI under drought conditions.

Table 5. Average value of root-shoot ratio (R/S) and harvest index (HI) of *A. hypogaea* L. application of biofertilizer-sludge in various dosage concentrations.

Treatments	Parameters	
	R/S	HI (%)
P0	0.053 ± 0.008^{ab}	61.4 ± 4.46^{abcd}
P1	0.060 ± 0.008^{abc}	66.2 ± 1.96^{bcd}
P2	0.054 ± 0.004^{ab}	61.6 ± 3.17^{bcd}
P3	0.058 ± 0.007^{abc}	68.8 ± 4.04^{cd}
P4	0.045 ± 0.004^{ab}	47.8 ± 6.65^{a}
P5	0.058 ± 0.005^{abc}	67.6 ± 3.50^{cd}
P6	0.037 ± 0.004^{a}	52.2 ± 5.01^{ab}
P7	0.050 ± 0.010^{ab}	55 ± 6.98^{abc}
P8	$\mathbf{0.078 \pm 0.010^{c}}$	$\mathbf{70.4 \pm 3.34^{d}}$
P9	0.043 ± 0.006^{ab}	57 ± 2.64^{abcd}
P10	0.066 ± 0.009^{bc}	66.2 ± 3.76^{bcd}
P11	0.053 ± 0.005^{ab}	61.4 ± 4.31^{abcd}
P12	0.058 ± 0.006^{abc}	63.6 ± 3.07^{bcd}
P13	0.062 ± 0.010^{bc}	63.4 ± 4.82^{bcd}
P14	0.061 ± 0.006^{bc}	62.2 ± 2.48^{bcd}
P15	0.049 ± 0.005^{ab}	54.8 ± 3.75^{abc}
Σ	0.055 ± 0.002	61.7 ± 1.16

Notes: The number followed by the same letter in one column shows that it is not significantly different from the DMRT test with the 95% confidence level; Figures in bold indicate the most optimal treatment results.

In this study, there was also a positive correlation between the average R/S and HI. It can be observed in P13, P14, and P15. The decrease in R/S also causes a decrease in HI and the dry weight of pods (Table 4). The effective treatment in increasing R/S and HI is the same (P8) with 10 L/ha biofertilizer + 24 ml sludge (Table 5). The HI of *A. hypogaea* L. ranged from 61.7 ± 1.16% or about 61%, is higher than reported by Bell & Wright (1998) that the average HI of *A. hypogaea* L. in Indonesia is only 0.31 or 31%. A high HI value manifests the capability of plants to increase the partition of more assimilates into the pods to maintain HI in drought conditions. The high HI value of this study is probably the response of plants to dry environmental conditions during the research conducted.

Anatomical response. According to Table 6, P13 with a biofertilizer 30 L/ha + sludge 12 ml provided the highest average diameter. The highest mean of root metaxylem diameter was obtained from P5 24 ml sludge. P10 biofertilizer 15 L/ha + sludge 12 ml is considered the most optimal in increasing the seed's diameter. The root metaxylem sludge provided a better response than biofertilizer or both combinations.

Table 6. Anatomical response of stem, root, and seed of *A. hypogaea* L. to biofertilizer-sludge applications.

Treatments	Parameters (diameter of-)(µm)				
	Stem	Stem metaxilem	Root	Root metaxilem	Seed
P0	342 ± 7.1^{cd}	**3.70 ± 0.16^{g}**	395.77 ± 0.30^{i}	3.41 ± 0.09^{bcde}	742.97 ± 5.6^{bcd}
P1	327.3 ± 24.2^{bc}	3.21 ± 0.25^{ef}	355.07 ± 2.63^{de}	2.59 ± 0.12^{a}	629.71 ± 20.9^{a}
P2	344 ± 6.3^{cd}	3.53 ± 0.14^{fg}	373.87 ± 1.22^{fg}	3.09 ± 0.11^{bc}	830.35 ± 88.7^{de}
P3	243.7 ± 6.4^{a}	2.98 ± 0.16^{de}	350.97 ± 0.98^{de}	2.53 ± 0.13^{a}	832.07 ± 3.4^{de}
P4	335 ± 14^{cd}	2.3 ± 0.30^{abc}	367.07 ± 9.7^{f}	**4.12 ± 0.19^{gh}**	624.75 ± 19.8^{a}
P5	299 ± 4.9^{b}	3.19 ± 0.19^{af}	347.87 ± 0.78^{cd}	4.42 ± 0.21^{h}	619.58 ± 36.7^{a}
P6	370 ± 7.5^{de}	2.39 ± 0.12^{abc}	380.23 ± 2.22^{gh}	4.08 ± 0.23^{gh}	582.35 ± 39.3^{a}
P7	346.3 ± 18.5^{cd}	2.44 ± 0.09^{abc}	391.1 ± 0.78^{hi}	3.41 ± 0.10^{bcde}	656.61 ± 18.1^{ab}
P8	349.3 ± 3.7^{cd}	2.12 ± 0.20^{ab}	337.73 ± 1.12^{bc}	3.93 ± 0.15^{efg}	594.83 ± 11.2^{a}
P9	300 ± 4.0^{b}	1.98 ± 0.14^{a}	262.6 ± 10.16^{a}	3.49 ± 0.17^{cde}	749.41 ± 8.8^{bcd}
P10	341.3 ± 0.9^{cd}	2.71 ± 0.02^{cde}	379.93 ± 3.68^{gh}	3.58 ± 0.03^{cdef}	883.95 ± 27.1^{e}
P11	340 ± 2.1^{de}	2.34 ± 0.06^{abc}	354.7 ± 0.90^{de}	3.74 ± 0.12^{defg}	783.63 ± 7.9^{cd}
P12	370.3 ± 7.8^{de}	2.62 ± 0.23^{bcd}	372.8 ± 3.47^{fg}	2.96 ± 0.14^{ab}	721.63 ± 3.8^{bc}
P13	**384 ± 9.2^{e}**	2.70 ± 0.05^{cde}	352.6 ± 1.70^{de}	3.39 ± 0.28^{bcd}	726.8 ± 6.6^{bc}
P14	362.3 ± 16.2^{cd}	2.52 ± 0.11^{bcd}	362.37 ± 1.13^{ef}	3.47 ± 0.15^{bcde}	792.45 ± 3.2^{cde}
P15	347 ± 2.6^{cd}	2.41 ± 0.16^{abc}	328.67 ± 1.66^{b}	3.68 ± 0.13^{defg}	752.19 ± 37.7^{bcd}

Notes: The number followed by the same letter in one column shows that it is not significantly different from the DMRT test with the 95% confidence level; Figures in bold indicate the most optimal treatment results.

There was a negative response by several treatments on stem diameter, stem metaxylem, and root diameter due to the need for transport of nutrients and water by plants. The plants responded with changes, especially in the xylem anatomy, both in the stem and roots. The functions of the stem and root organs in plants are equal to supporting organs for nutrient and water transport. Hence, the availability of nutrients and water will have the same effect on these two organs. In line with Rosawanti *et al.* (2015), the changes in the anatomical roots of plants, especially xylem, can be utilized as an important variable to predict plant tolerance to drought stress. Based on the results obtained, it was also observed that metaxylem diameter was negatively correlated with HI, which is also an important variable for the defense response of groundnut plants to drought. The seed diameter in the biofertilizer increased along with increasing the dose of biofertilizer (P1, P2, P3) (Table 6). It is correlated with the percentage of GP (Table 3). The seed diameter decreased with the increasing dose of sludge (P4, P5, P6). The sludge in various doses also gave a negative response to the size of the seed diameter and the results of the combination of biofertilizer-sludge. The biofertilizer treatment is better than the sludge or both combination in increasing the seed diameter.

CONCLUSION

The biofertilizer-sludge application did not significantly affect GP, SVI, and dry weight of yields. However, the significant effect showed on harvest index (HI) and root-shoot ratio (R/S). HI reaches 61%, higher than the average HI in Indonesia. The anatomical response of A. hypogaea L. to biofertilizer-sludge application showed a significant effect on stem diameter, root metaxylem diameter, and seed diameter. The biofertilizer-sludge in various doses of concentration decreased in the stem metaxylem diameter and root diameter compared with the control. The biofertilizer 10 L/ha + 24 ml sludge application is the most optimum concentration for increasing HI and R/S values. For the increasing stem, root metaxylem, and seed diameter, biofertilizer 30 L/ha + sludge 12 ml, sludge 24 ml, and biofertilizer 15 L/ha + sludge 12 ml are the optimum concentrations, respectively.

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