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

Nur Hidayah Pangestuti¹, Dwi Umi Siswanti¹*

¹Laboratory of Plant Physiology, Faculty of Biology, Universitas Gadjah Mada
Jl. Teknika Selatan, Sekip Utara, Sleman, D.I.Yogyakarta, Indonesia. 55281
*Email: dwiumi@ugm.ac.id

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 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

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/100m² 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:

\[
\text{Germination percentage} (\%) = \left( \frac{\sum a + \sum b}{\text{number of seeds set for germination}} \right) \times 100\% 
\]

**Notes:**

\[\sum a = \sum \text{number of normal seedling I is number of seeds germinated on the 5th day}\]

\[\sum b = \sum \text{number of normal seedling II is number of seeds germinated on the 10th day}\]
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).

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

Notes: 
\(\sum\) normal germinated seeds \(I\) is number of seeds germinated on the 5th day

Harvest index (HI) = \(\text{Pods dry weight} \times 100\%\) / \(\text{Pods dry weight} + \text{plant dry matter}\)

\(R/S = \frac{\text{Root dry weight}}{\text{Shoot dry weight}}\)

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 \(\mu\)m, 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% (\(\alpha = 0.05\)) (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, BPPT, 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.

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

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).

**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 sludge 12 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 & Bacó, 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<sup>ab</sup> | 61.4 ± 4.46<sup>abcd</sup> |
| P1         |            | 0.060 ± 0.008<sup>abc</sup> | 66.2 ± 1.96<sup>cd</sup> |
| P2         |            | 0.054 ± 0.004<sup>ab</sup> | 61.6 ± 3.17<sup>bcd</sup> |
| P3         |            | 0.058 ± 0.007<sup>abc</sup> | 68.8 ± 4.04<sup>d</sup> |
| P4         |            | 0.045 ± 0.004<sup>ab</sup> | 47.8 ± 6.65<sup>a</sup> |
| P5         |            | 0.058 ± 0.005<sup>abc</sup> | 67.6 ± 3.50<sup>d</sup> |
| P6         |            | 0.037 ± 0.004<sup>ab</sup> | 52.2 ± 5.01<sup>b</sup> |
| P7         |            | 0.050 ± 0.010<sup>ab</sup> | 55 ± 6.98<sup>abc</sup> |
| P8         |            | **0.078 ± 0.010<sup>d</sup>** | **70.4 ± 3.34<sup>d</sup>** |
| P9         |            | 0.043 ± 0.006<sup>abc</sup> | 57 ± 2.64<sup>abcd</sup> |
| P10        |            | 0.066 ± 0.009<sup>abc</sup> | 66.2 ± 3.76<sup>abcd</sup> |
| P11        |            | 0.053 ± 0.005<sup>abc</sup> | 61.4 ± 4.31<sup>abcd</sup> |
| P12        |            | 0.058 ± 0.006<sup>abc</sup> | 63.6 ± 3.07<sup>abcd</sup> |
| P13        |            | 0.062 ± 0.010<sup>abc</sup> | 63.4 ± 4.82<sup>abcd</sup> |
| P14        |            | 0.061 ± 0.006<sup>abc</sup> | 62.2 ± 2.48<sup>abcd</sup> |
| P15        |            | 0.049 ± 0.005<sup>ab</sup> | 54.8 ± 3.75<sup>bc</sup> |
| Σ          |            | 0.055 ± 0.002<sup>ab</sup> | 61.7 ± 1.16<sup>a</sup> |

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 ± 7.13<sup>cd</sup>       | 3.70 ± 0.16<sup>d</sup> | 395.77 ± 0.30<sup>d</sup> | 3.41 ± 0.09<sup>bcde</sup> | 742.97 ± 5.6<sup>cd</sup> |
| P1         | 327.3 ± 24.2<sup>bc</sup>    | 3.21 ± 0.25<sup>ef</sup> | 355.07 ± 2.63<sup>de</sup> | 2.59 ± 0.12<sup>a</sup>  | 629.71 ± 20.9<sup>a</sup>  |
| P2         | 344 ± 6.3<sup>cd</sup>       | 3.53 ± 0.14<sup>fg</sup> | 373.87 ± 1.22<sup>fg</sup> | 3.09 ± 0.11<sup>bc</sup>  | 830.35 ± 88.7<sup>de</sup> |
| P3         | 243.7 ± 6.4<sup>e</sup>      | 2.98 ± 0.16<sup>de</sup> | 350.97 ± 0.98<sup>de</sup> | 2.53 ± 0.13<sup>a</sup>  | 832.07 ± 3.4<sup>de</sup>  |
| P4         | 335 ± 14<sup>cd</sup>        | 2.3 ± 0.30<sup>abc</sup> | 367.07 ± 9.7<sup>f</sup>  | 4.12 ± 0.19<sup>gh</sup> | 624.75 ± 19.8<sup>a</sup>  |
| P5         | 299 ± 4.9<sup>b</sup>        | 3.19 ± 0.19<sup>ef</sup> | 347.87 ± 0.78<sup>cd</sup> | 4.42 ± 0.21<sup>b</sup>  | 619.58 ± 36.7<sup>a</sup>  |
| P6         | 370 ± 7.5<sup>de</sup>       | 2.39 ± 0.12<sup>abc</sup> | 380.23 ± 2.22<sup>gh</sup> | 4.08 ± 0.23<sup>gh</sup> | 582.35 ± 39.3<sup>a</sup>  |
| P7         | 346.3 ± 18.5<sup>cd</sup>    | 2.44 ± 0.09<sup>abc</sup> | 391.1 ± 0.78<sup>bc</sup> | 3.41 ± 0.10<sup>bcde</sup> | 656.61 ± 18.1<sup>ab</sup> |
| P8         | 349.3 ± 3.7<sup>d</sup>      | 2.12 ± 0.20<sup>ab</sup> | 337.73 ± 1.12<sup>bc</sup> | 3.93 ± 0.15<sup>fg</sup> | 594.83 ± 11.2<sup>a</sup>  |
| P9         | 300 ± 4.0<sup>b</sup>        | 1.98 ± 0.14<sup>a</sup> | 262.6 ± 10.16<sup>a</sup> | 3.49 ± 0.17<sup>de</sup> | 749.41 ± 8.8<sup>bcd</sup> |
| P10        | 341.3 ± 0.9<sup>d</sup>      | 2.71 ± 0.02<sup>de</sup> | 379.93 ± 3.68<sup>gh</sup> | 3.58 ± 0.03<sup>cdef</sup> | 883.95 ± 27.1<sup>c</sup>  |
| P11        | 340 ± 2.1<sup>de</sup>       | 2.34 ± 0.06<sup>abc</sup> | 354.7 ± 0.90<sup>de</sup> | 3.74 ± 0.12<sup>defg</sup> | 783.63 ± 7.9<sup>c</sup>  |
| P12        | 370.3 ± 7.85<sup>bc</sup>    | 2.62 ± 0.23<sup>bcde</sup> | 372.8 ± 3.47<sup>fg</sup> | 2.96 ± 0.14<sup>ab</sup> | 721.63 ± 3.8<sup>bc</sup>  |
| P13        | 384 ± 9.2<sup>e</sup>       | 2.70 ± 0.05<sup>cd</sup> | 352.6 ± 1.70<sup>bcde</sup> | 3.39 ± 0.28<sup>def</sup> | 726.8 ± 6.6<sup>bc</sup>  |
| P14        | 362.3 ± 16.2<sup>cd</sup>    | 2.52 ± 0.11<sup>bcd</sup> | 362.37 ± 1.13<sup>ef</sup> | 3.47 ± 0.13<sup>bde</sup> | 792.45 ± 3.2<sup>de</sup>  |
| P15        | 347 ± 2.6<sup>cd</sup>       | 2.41 ± 0.16<sup>abc</sup> | 328.67 ± 1.66<sup>b</sup> | 3.68 ± 0.13<sup>defg</sup> | 752.19 ± 37.7<sup>bcd</sup> |

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.

ACKNOWLEDGEMENTS

The authors would like to thank Vice Chancellor for Research and Community Service of UGM. This study was supported by RTA Grant UGM No. 2019/UN1/DITLIT/DITLIT/LT/2019. The author thank to Niken Wulansari for the cooperation during research.

REFERENCES

Asam ZUZ, Poulsen TG, Nizami AS, Rafique R, Kiely G, Murphy JD. 2011. How can we improve biomethane production per unit of feedstock in biogas plants?. Applied Energy. vol 88(6): 2013–2018. doi: https://doi.org/10.1016/j.apenergy.2010.12.036.

Awadalla AO, Abbas MT. 2017. Peanut (Arachis hypogaea L.) yield and its components as affected by N-fertilization and diazotroph inoculation in Toshka desert soil-South Valley-Egypt. Environmental Risk Assessment and Remediation. vol (1): 39–55. doi: https://doi.org/10.4066/2529-8046.100023.

Bell MJ, Wright GC. 1998. Groundnut growth and development in contrasting environments. 1. Growth and plant density responses. Experimental Agriculture. vol 34(1): 99–112. doi: https://doi.org/10.1017/S001447979800101X.

Bertham RR, Arifin Z, Nusantara AD. 2019. The improvement of yield and quality of soybeans in a coastal area using low input technology based on biofertilizers. International Journal on Advance Science Engineering Information Technology. vol 9(3): 787–791.

Bewly JD, Black BM. 1982. Germination of seeds. In: Physiology and biochemistry of seed germination. Ed. Khan AA. New York: Springer Verlag. pp 40-80.

Cahyadi D, Widodo WD. 2017. Efektivitas pupuk hayati terhadap pertumbuhan dan hasil tanaman cain (Brassica Chinensis L.). Buletin Agrohorti. vol 5(3): 292–300. doi: https://doi.org/10.29244/agrob.v5i3.16466.

Chiang GC, Bartsch M, Barua D, Nakabayashi K, Debieu M, Kronholm I, Koornneef M, Soppe WJ, Donohue K, De Meaux J. 2011. DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in Arabidopsis thaliana. Molecular Ecology. vol 20(16): 3336–3349. doi: https://doi.org/10.1111/j.1365-294X.2011.05181.x.

Copeland LO, McDonald MB. 1995. Principles of seed science and technology. 3rd ed. New York: Chapman and Hall. pp 409.

Dhar DW, Prasanna R, Pabbi S, Vishwakarma R. 2015. Significance of cyanobacteria as inoculants in agriculture. In Algal biorefinery: An integrated approach. New York: Springer, Cham: pp 339–374. doi: https://doi.org/10.1007/978-3-319-22813-6_16.

Du Z, Xiao Y, Qi X, Liu Y, Fan X, Li Z. 2018. Peanut-shell biochar and biogas slurry improve soil properties in the North China Plain: a four-year field study. Scientific Reports. vol 8(1): 1–9. doi: https://doi.org/10.1038/s41598-018-31942-0.

El-Sayed AEGA, Darwish MA, Aoz SN, Abd-Alla AM, Elsayed SIM. 2017. Effect of mineral, bio and organic fertilizers on productivity, essential oil composition and fruit anatomy of two dill cultivars (Anethum graveolens L.). Middle East Journal of Applied Sciences. vol 7(3): 532–550.

El-Sayed AA, El-Leithy AS, Swaeef YM, Senossi ZFM. 2018. Effect of NPK, bio and organic fertilizers on growth, herb yield, oil production and anatomical structure of (Cymbopogon citratus, Stapf) plant. Annual Research & Review in Biology. vol 26(2): 1–15. doi: https://doi.org/10.9734/ARRB/2018/41038.

Herrmann L, Lesueur D. 2013. Challenges of formulation and quality of biofertilizers for successful inoculation. Applied Microbiology and Biotechnology. vol 97(20): 8859–8873. doi: https://doi.org/10.1007/s00253-013-5228-8.

ISTA. 2016. International rules for seed testing 2016. Wallisellen: International Seed Testing Association. https://www.seedtest.org/.

Jagara SR, Vadez V, Bhatnagar-Mathur P, Narasu ML, Sharma KK. 2012. Better root:shoot ratio conferred enhanced harvest index in transgenic groundnut overexpressing the Rd29a:DREB1A gene under intermittent drought stress in an outdoor lysimetric dry-down trial. Journal of SAT Agricultural. vol 10: 1–7.

Junjittakarn J, Girdthai T, Jogl Dhar DW, Prasanna R, Pabbi S, Vishwakarma R. 2015. Significance of cyanobacteria as inoculants in agriculture. In Algal biorefinery: An integrated approach. New York: Springer, Cham: pp 339–374. doi: https://doi.org/10.1007/978-3-319-22813-6_16.

Kapanewon Cangkrikan. 2020. Profil Kecamatan Cangkrikan. Yogyakarta: Pemerintah Kabupaten Sleman. https://cangkrikankec.sleman.go.id/.

Kawalekar JS. 2013. Role of biofertilizers and biopesticides for sustainable agriculture. Journal of Bio Innovation. vol 2(3): 73–78.

Kementerian Pertanian. 2015. Outlook komoditas pertanian tanaman pangan, kacang tanah. Jakarta: Pusat Data dan Sistem Informasi Pertanian, Kementerian Pertanian Republik Indonesia. http://epublikasi.setjen.pertanian.go.id/.

Kirchmann H, Börjesson G, Kätterer T, Cohen Y. 2017. From agricultural use of sewage sludge to nutrient extraction: A soil science outlook. Ambio. vol 37(3): 265–275.
Ramadani S, Linda R, Setyawati TR. 2015. Pertumbuhan tanaman kacang tanah (Arachis hypogaea L.) pada tanah gubum yang diaplikasikan dengan bokashi jerami dan pupuk petrikhaphos. Protobiont. vol 4(1): 1–9. doi: https://dx.doi.org/10.26418/protobiont.v4i1.8740.

Rosawanti P, Ghulamahdi M, Khumaida N. 2015. Respon anatom ti dan fisologi akar kedelai terhadap cekaman kekeringan. Jurnal Agromoni Indonesia. vol 43(3): 186–192. doi: https://doi.org/10.24831/jai.v43i3.11243.

Saha L, Baudh K. 2020. Sustainable agricultural approaches for enhanced crop productivity, better soil health, and improved ecosystem services. In Ecological and Practical Applications for Sustainable Agriculture. Singapore: Springer. pp. 1-23. doi: https://doi.org/10.1007/978-981-15-3372-3_1.

Sahoo RK, Ansari MW, Dangar TK, Mohanty S, Tuteja N. 2014. Phenotypic and molecular characterisation of efficient nitrogen-fixing Azotobacter strains from rice fields for crop improvement. Protoplasma. vol 251: 511–523. doi: https://doi.org/10.1007/s00709-013-0547-2.

Sarawas, S. Baco AR. 2014. Partisi fotosintat beberapa kultivar kedelai (Glicine max. (L.) Merr.) pada ultisol. Jurnal Agroteknos. vol 4(3): 152–159.

Sharma RC, Smith EL. 1986. Selection for high and low harvest index in three winter wheat populations 1. Crop Science. vol 26(6): 1147–1150. doi: https://doi.org/10.2135/cropsci1986.0011183X0002600060013x.

Silitonga L, Turmudi E, Wido W. 2018. Growth and yield response of peanut (Arachis hypogaea L.) to cow manure dosage and phosphorus fertilizer on ultisol. Akta Agrosia. vol 21(1): 11–18. doi: https://doi.org/10.31186/aa.21.1.11-18.

Singh JS, Pandey VC, Singh DP. 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agriculture, Ecosystems & Environment. vol 140(3–4): 339–353. doi: http://doi.org/10.1016/j.ages.2011.01.017.

Siswanti DU, Syahidah A, Sudjino S. 2018. Produktivitas Tanaman padi (Oryza sativa L.) Segregen Terhadap Aplikasi Sludge Biogas di Lahan Sawah Desa Wukirsari, Cangkringan, Sleman. Biogenesis: Jurnal Ilmiah Biologi. vol 6(1): 64–70. doi: https://doi.org/10.24252/bio.v6i1.4241.

Srivalli P, Nadaf HL, Rajeev R. 2016. Association between root traits and drought tolerance under intermittent drought stress conditions in groundnut (Arachis hypogaea L.). International Journal of Agricultural Science And Research (IJASR). vol 6(6): 151–162.

Steel RGD, Torrie JH. 1984. Principles and procedures of statistics. Singapore: Mc Graw Hill Book C., Inc. pp 172–177.

Suhag M. 2016. Potential of biofertilizers to replace...
chemical fertilizers. *International Advanced Research Journal in Science, Engineering and Technology*, vol 3(5): 163–167. doi: https://doi.org/10.17148/IARJSET.2016.3534.

Sumarno S. 2015. Status kacang tanah di Indonesia. Monograf Balitkabi No. 13. Malang: Balai Penelitian Tanaman Aneka Kacang dan Umbi. Balitkabi. hal 29–38. http://balitkabi.litbang.pertanian.go.id.

Thorin E, Lindmark J, Nordlander E, Odlare M, Dahlquist E, Kastensson J, Leksell N, Pettersson CM. 2012. Performance optimization of the Växtkraft biogas production plant. *Applied Energy*, vol 97: 503–508. doi: https://doi.org/10.1016/j.apenergy.2012.03.007.

Xu M, Xian Y, Wu J, Gu Y, Yang G, Zhang X, Peng H, Yu X, Xiao Y, Li L. 2019. Effect of biogas slurry addition on soil properties, yields, and bacterial composition in the rice-rape rotation ecosystem over 3 years. *Journal of Soils and Sediments*, vol 19(5): 2534–2542. doi: https://doi.org/10.1007/s11368-019-02258-x.

Yusuf M, Sulistyawati E, Suhaya Y. 2014. Distribusi Biomassa di Atas dan Bawah Permukaan dari Surian (*Toona sinensis* Roem). *Jurnal Matematika dan Sains*, vol 19(2): 69–75.

Zhang M, Gao B, Chen J, Li Y. 2015. Effects of graphene on seed germination and seedling growth. *Journal of Nanoparticle Research*, vol 17(2): 1–8. doi: https://doi.org/10.1007/s11051-015-2885-9.

Zhang J, Li W, Lee J, Loh KC, Dai Y, Tong YW. 2017. Enhancement of biogas production in anaerobic co-digestion of food waste and waste activated sludge by biological co-pretreatment. *Energy*, vol 137: 479–486. doi: https://doi.org/10.1016/j.energy.2017.02.163.