International Journal of Science, Environment and Technology, Vol. 7, No 1, 2018, 207 – 218

ISSN 2278-3687 (O)
2277-663X (P)

PEANUT PHYSIOLOGAL CHARACTERS WITH DIFFERENT NITROGEN FERTILIZATION SOURCES

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Abstract: Peanuts (*Arachis hypogea*) are a major commodity bean in Indonesia, since they are an important source of vegetable protein. This study was conducted to determine the optimal N source for peanut cultivation by determining the content of chlorophyll and nitrate reductase (NR) and the grain yield and biomass of peanuts.The research used a 2 × 4 factorial design that was repeated three times.The first factor was sources of N (NO_3- (potassium nitrate) and NH_4^+ (ammonium sulfate), and second factor was N fertilizers, namely 0, 50, 100, and 150 kg N/ha. Parameters in this research were the chlorophyll content, NR activity, grain yield, and peanut straw stover yield. The leaf chlorophyll content of peanuts with an NO_3^- N source higher than those with NH_4^+ N sources. The NR of peanuts with an NO_3^-N source higher than those with NH_4^+ N sources. The results of peanut seeds with an NO_3^-N source higher than those with NH_4^+ N sources. The peanut biomass increased with increasing doses of nitrogen. It can be concluded that peanut plants prefer NO_3^- to NH_4^+; this preference is indicated by the higher chlorophyll content, NR activity, weight of pods and seeds, and the biomass with NO_3^- N than with NH_4^+ N fertilization. The effect of the N rate on chlorophyll content, NR activity, seed yield, and peanut straw is linear. There is a positive correlation between chlorophyll content and NR activity, and between NR activity and the peanut seed yield.
Keywords: Peanut, Nitrogen, Chlorophyll, Nitrate reductase, Yield.

Introduction

Peanuts (*Arachis hypogea*) are a major commodity bean in Indonesia, since they are an important source of vegetable protein. They facilitate food diversification and support national food security. As they are protein crops, peanuts require considerable amounts of nitrogen. Olajide and Igbeka (2003) reported that peanuts are a legume crop capable of nitrogen (N) fixation in air. Peanuts are also called "earthnuts," and are a valuable protein source for humans and as feed for livestock.

N is the main nutrient for crop production. N deficiency has three potential effects, namely: (1) a decrease in leaf area and in leaf N content preservation for photosynthesis, (2) a decrease in managing the leaf area and lowering the leaf N content, or (3) a combination of both responses (Flecther *et al.,* 2013). N is a constituent element of plants, forming nucleic

*Received Jan 17, 2018 * Published Feb 2, 2018 * www.ijset.net*

acids, amino acids, and proteins. N is one of the most expensive nutrients to supply, and commercial fertilizer is the main cost in plant production. With sufficient N supply, N uptake largely depends on the level of plant growth through internal regulation (Gastal and Lemaire, 2002). Short roots are able to absorb 70% of the N allocated to the leaves while long roots can only absorb about 35% N. Growth is influenced by root uptake and accumulation of N allocation. Although long roots can only absorb a small amount of N, N allocation to older leaves is higher in plants with long roots. N is needed to support the growth of leaves. When root growth rate is low, N is supplied from other parts of the plant, such as old leaves that are almost yellowing (Schoene and Yeager, 2007).

N is absorbed by plant roots and translocated primarily in the form of nitrate (NO_3^-), ammonium (NH_4^+), and amino acids, and is mostly available as NO_3^-. However, in some soils, such as those of late-successional temperate and boreal forest environments, NH_4^+ is the major or even the sole inorganic N source (Brito and Kronzucker, 2005). These forms of inorganic N, NH_4^+ and NO_3-, are readily available to plants but they have different reactions in soil. NH_4^+ is retained by cationic exchange in clay and organic matter, and it is therefore mobile and easily absorbed by the roots. NH_4^+ in solution can undergo nitrification and is found in high concentrations. Plants absorb N based and their favorite properties. Information on the effects of the source of N fertilizers on peanuts is still very limited. This study was conducted to determine the optimal N source for peanut cultivation by determining the content of chlorophyll and nitrate reductase (NR) and the grain yield and biomass of peanuts.

Materials and Methods

The study was conducted in the laboratory of Ecology and Plant Production, Faculty of Agriculture and Animal Husbandry, Diponegoro University, during the dry season (August to December) in 2014. The research station is located at 6° 58' 0" SL 110° 25' 0" EL, and has Oxisol soil with a clay texture, a soil pH of 6.7, 0.12% total N content, 0.10% total P, 0.22% total K, and 0.99% C. Kidang peanut varieties were used, and the study was carried out using 24 experimental plots measuring 1.5 × 1 m^2, with a spacing of 30 × 20 cm so that each plot contained 16 plants. The research used a 2 × 4 factorial design that was repeated three times.

Factor I : sources of N (NO_3- (potassium nitrate) and NH_4^+ (ammonium sulfate)

Factor II : N fertilizers, namely 0, 50, 100, and 150 kg N/ha.

Each plant was fertilized with N, P, and K with appropriate treatment doses of N and P fertilizer at 25 kg P_2O_5 per hectare and K at 25 kg KCl per hectare. Micronutrients were administered in the form of approximately 5 ml of liquid fertilizer dissolved in 1 L of water and sprayed onto the plants 1 wk after planting.

The chlorophyll content of the fifth leaf from the tip of the plant, NR activity, grain yield, and peanut straw stover yield were determined. Chlorophyll content was measured in fresh leaf samples. Leaf samples (0.5 g) were homogenized with acetone (90% v/v), filtered, and made up to a final volume of 50 mL. Chlorophyll concentration was calculated based on the absorbance of the extract measured by a spectrophotometer 645 UV/Vis and 666 UV/Vis (Kumar et al, 2012). Analysis of NR activity was performed according to the methods used by Hartiko (1989). The third leaves of soybean plant shoots were harvested between 9 and 10 a.m. and used for further observation. The leaves were washed with distilled water and finely sliced, and 200 mg of the leaf sample was added to 5 ml NaH_2PO_4 and $NaHPO_4$ buffer solutions at pH 7.5 in dark tubes that were covered and maintained for 24 h, after which the buffer solution was replaced with 5 mL of fresh buffer solution. Next, 0.1 mL of 5 M $NaNO_3$ was added to every dark tube. The time of addition of $NaNO_3$ was designated the 0-incubation point, and samples were incubated for 2 h. Meanwhile, another test tube was filled with 0.2 mL 1% sulfanilamide reagent, which was dissolved in 3N HCl and 0.2 mL 0.02% napthylethylendiamide. Then 0.1 mL of the filtrate incubated for 2 h was placed in a test tube containing the sulfanilamide reagent, HCl, and a napthylethylendiamide solution. The test tubes were agitated to mix the filtrate and accelerate the reaction, and allowed to stand for 15 min; this resulted in the reduction of NO_2 with dye reagents to bring up the pink color. Next, 2.5 ml distilled water was added to the test tube as a color diluent. The absorbance of this solution was measured at a wavelength of 540 nm.

Peanut seeds were removed from their skins and weighed. Stover is peanut straw, the peanut crop yield after the nut is removed.

Data analysis

The data were analyzed using ANOVA. If the ANOVA revealed a difference, the analysis was followed by Duncan's multiple range test as described by Steel and Torrie (1965). The effect of N fertilizer on chlorophyll, NR, grain yield, and stover was assessed using a linear regression (Steel and Torrie, 1990). The relationship between chlorophyll and NR, chlorophyll and grain yield, or NR with grain yield was determined using a regression-correlation (Steel and Torrie, 1965).

Results

The results of the ANOVA indicate that the N source has a significant impact on chlorophyll content, NR, pods, seeds, and biomass yield of peanuts, and the dose of the N fertilizer significantly affects plant height, chlorophyll content, NR, and the yields of pods, seeds, and biomass of peanuts. The interaction between the N source and N fertilizer dose significantly effected chlorophyll content, NR, pods, seeds, and biomass yield of peanuts (Table 1).

The effect of N source

The leaf chlorophyll content of peanuts with an NO_3^- N source is 46.69% higher than those with NH_4^+ N sources. The NR of peanuts with an NO_3^-N source is 14.71% higher than those with NH_4^+ N sources. The results of peanut seeds with an NO_3^-N source is 16.23% higher than those with NH_4^+ N sources. The biomass of peanuts with NO_3^-N sources is 32.71% higher than that of peanuts with NH_4^+ N sources.

The effect of N dosages

The chlorophyll content of peanut leaves increased with increasing N doses of 50–150 kg N per ha was 43.1%–164.6% higher than when sources of N were not provided. NR activity with increasing N doses increased by 15.8% (50 kg N/ha) to 91.1% (150 kg N/ha) compared to that of peanuts cultivated without nitrogen. The N content of peanut seeds and biomass increased by 20.4%–125.9% with increasing N (50–150 kg N per ha), while biomass increased from 18.2%–155.6%.

The effect of interaction

Chlorophyll content, NR, and grain and biomass yield increased with increasing doses of N fertilizer containing either NO_3^- N or NH_4^+N. Fertilizer containing NO_3^- N sources of 0–100 kg and 150 kg N per ha increased the leaf chlorophyll content of peanuts by 42.5%–164.2%, while NH_4^+-N increased the leaf chlorophyll content by 51.3%–70.0%. Increasing fertilizer doses of NO_3^- N increased NR activity by 20.9% (50 kg N per ha) to 117.1% (150 kg N per ha). NR activity increased by 10.6%–65.7% when NH_4^+N fertilizers were applied at doses of 50–150 kg per ha. NO_3^- N applied at increasing doses of 50–150 kg N per ha increased seed yield by 15.4%–129.8%, while NH_4^+ N increased the seed yield from 26.5% to 28.9%, respectively. The peanut biomass increased with increasing doses of nitrogen of 50–150 kg N per ha, i.e. 28.0%–219.4% with NO_3^- N, while NH_4^+-N sources increased biomass from 8.3% to 32.7%.

Table 1. The plant height, chlorophyll content, NR, and the yields of pods, seeds, and biomass of peanuts affect by N sources and N dosage

	Plant height (cm)	chlorophyll conten(mg/g)	NR	the yields of pods	Seeds	Biomass of peanuts
Nitrate 0	32.0 d	1.69 e	39.7 e	416.7 e	1501.7 de	2400.0 d
50	39.0 c	2.41 d	47.9 cd	577.0 d	1733.3 d	3071.7 cd
100	46.7 b	3.87 b	64.0 b	817.3 bc	2333.3 c	4203.3 bc
150	57.3 a	4.47 a	86.1 a	1219.3 a	3451.0 a	7666.7 a
Ammonium 0	25.0 e	1.19 e	41.5 de	370.0 e	1253.3 e	2400.0 d
50	27.7 e	1.71 e	45.9 cde	560.0 d	1585.0 d	2600.0 d
100	34.0 d	2.43 d	51.2 c	777.7 c	2150.0 c	3466.7 bcd
150	41.0 c	3.15 c	68.7 b	895.0 b	2771.3 b	4600.0 b
Nitrate	43.75 a	3.11 a	59.43 a	757.58a	2254.83a	4335.4 a
Ammonium	31.92 b	2.12 b	51.81 b	650.67b	1939.92b	3266.7 b
0	28.5 d	1.44 d	40.6 d	393.3 d	1377.5 d	2400.0 c
50	33.3 c	2.06 c	46.9 c	568.5 c	1659.2 c	2835.8 c
100	40.3 b	3.15 b	57.6 b	797.5 b	2241.7 b	3835.0 b
150	49.2 a	3.81 a	77.4 a	1057.2 a	3111.2 a	6133.3 a
ANOVA:						
NSource (S)	0.0001*	0.0001*	0.0003*	0.0003*	0.0003*	0.0007*
NDosage (D)	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
S*D	0.0056*	0.0500*	0.0028*	0.0007*	0.0529*	0.0030*

Discussion

NO_3^-fertilizer N sources had more effect than NH_4^+ fertilizer on growth (chlorophyll, NR, seeds, and peanut straw) (Fig. 1). These results are similar to those of Zaman et al (2015), who observed an increase in plant dry matter due to higher N levels, resulting in more efficient plant growth and higher yields (Khaliq *et al.,* 2008). However, these results are different from the theory of Muhammad *et al*., (2007), who reported that various sources of N fertilizer did not significantly affect oil and seed yield of canola (*Brassica napus L*). Adequate N is essential for normal plant growth and development, as it is an integral component of protein and is essential to the structure and function of chloroplasts (Stefanelli *et al.,* 2010).

Plants absorb N in the form ofNO_3^- or NH_4^+. N in the form of NO_3^- is highly soluble in water and is very mobile, and is therefore easily dissolved in the soil beyond the reach of plant roots. NH_4^+ N is not easily lost through leaching and denitrification. NH_4^+ uptake by roots reduces the uptake of Ca^{2+}, Mg^{2+}, and K^+, and increases the uptake of $H_2PO_3^-$, SO_4^{2-}, and Cl^- (Samuel and Ebenezer, 2014). N is an essential ingredient for plant growth and protein formation, as well as the synthesis of nucleic acids, which is related to growth as represented by plant height.

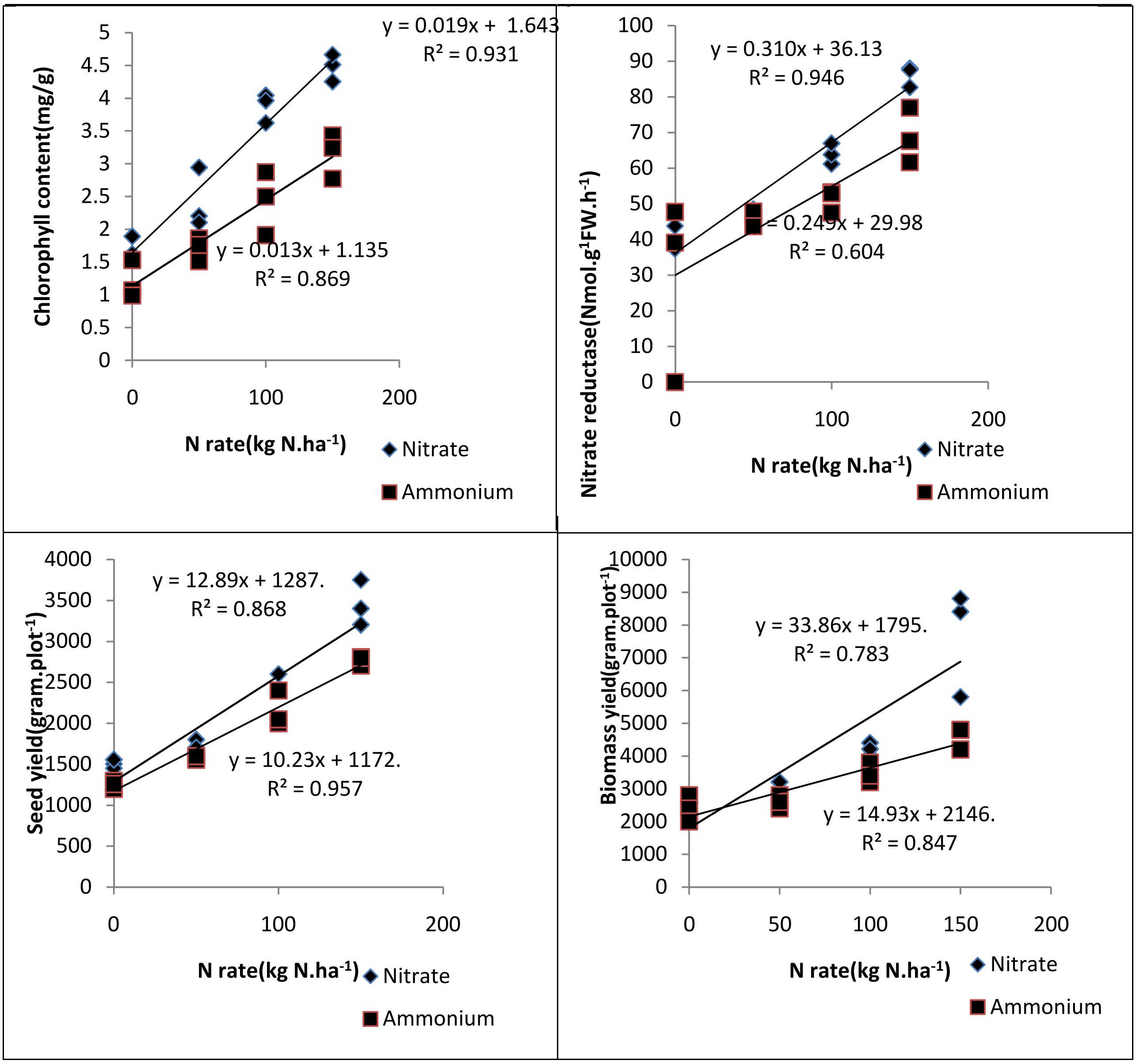

Fig. 1. N dose response of the source of N as nitrate and ammonium in chlorophyll content (A), nitrate reductase (B), seed yield (C), biomass (D) ofpeanuts (*Arachis hypogeal*).

Adequate N is associated with vegetative growth and efficient utilization of resources, which increases the productivity of plants, resulting in higher peanut seed yield. This is in accordance with the results of Suprayogi *et al*., (2011) who observed that the amount of N during the grain filling stage determined the seed yield because most of the N would be mobilized from vegetative organs.

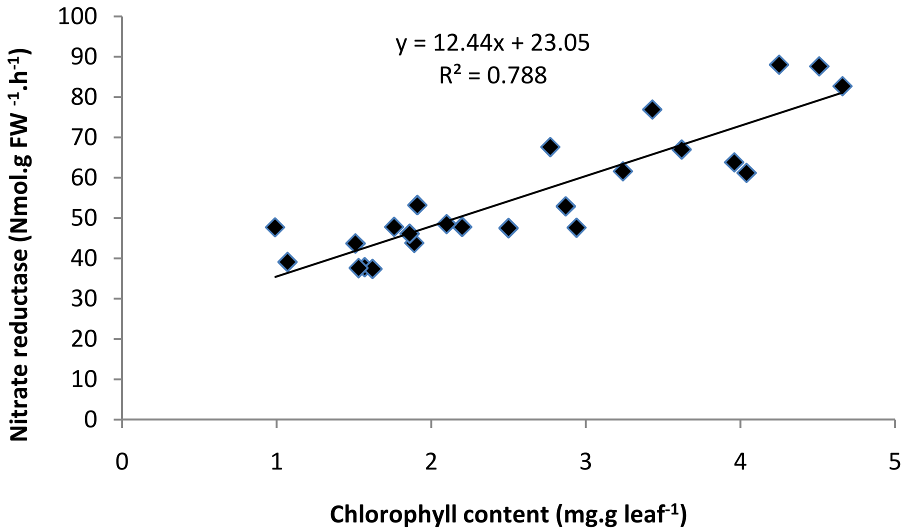

Fig. 2. The relationship between the chlorophyll content and nitrate reductase in nitrogen source of nitrate and ammonium.

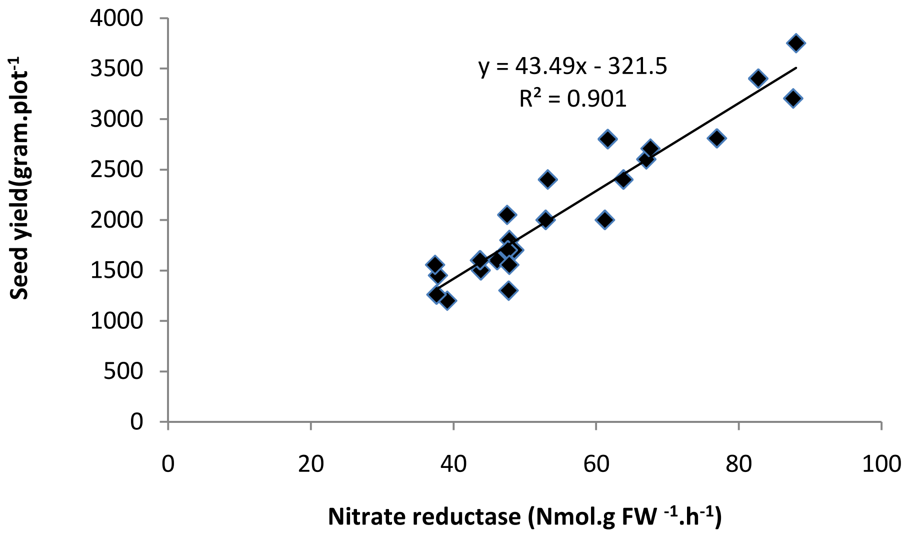

Fig. 3. The relationship between nitrate reductase and peanut seeds yield in nitrogen source in the form of nitrate and ammonium

N boosts vegetative growth and increases flower buds. The application of N and the use of low plant density during cultivation increased plant height and flower yield (Qasim*et al.,* 2005). The growth rate of plants increased significantly with increasing doses of N fertilizer (Ali and Hameed (2011); Qamar *et al.,* 2012). Crop production is the result of photosynthesis and respiration and the translocation of assimilates to plant dry matter. Increased production is directly proportional to increased relative growth and the net result of photosynthesis. The results of the present study are similar to those of Wajid *et al.,* (2007), who showed that an optimal N supply is crucial for the optimal production of dry matter and other aspects of plant growth. It facilitates high-efficiency conversion of the dry matter into economic results, and leads to high harvest index values. According to Masclaux-Daubresse (2010), crop production is determined by plant organs, age, environmental conditions, climate, and cultivation.

Regression analysis showed that the effect of NO_3^-N fertilizer on leaf chlorophyll content of peanuts (Figure 1) is expressed by the model equation Y = 0.019x + 1,643 (R^2 = 0.931). The model shows that an increase of one unit of NO_3^-N fertilizer results in an increase in chlorophyll content of 0.019 units. The coefficient of determination (R^2) demonstrates a 93.1% variation in the chlorophyll content (Y) caused by NO_3^-N fertilization.

Regression analysis revealed that the effect of NH_4^+N fertilizer on the chlorophyll content of peanut leaves is expressed by the model equation Y = 0.013x + 1,135 (R^2 = 0.869). The model shows that an increase of one unit of NH_4^+-N fertilizer results in a 1,135-unit increase in chlorophyll. R^2 reveals that NH_4^+-N fertilization causes an 86.9% variation in chlorophyll content (Y).The regression analysis of NO_3^- N fertilizer on peanut NR activity (Figure 1) is expressed by the model equation Y = 0.310x + 36.13 (R^2 = 0.946). The model showed that each increase of one unit of NO_3^- N fertilizer results in a 0.310 unit increase in NR activity. R^2 indicates that a 94.6% variation in NR (Y) results from NO_3^- N fertilization.

The regression analysis of NH_4^+N fertilizer on NR activity of peanut leaves is expressed by the model equation of Y = 0.249x + 29.98 (R^2 = 0.604). The model shows that an increase of one unit of NH_4^+-N fertilizer increases NR activity by 0.249 units. R^2 shows that a 60.4% variation in NR (Y) is caused by NH_4^+-N fertilization (Figure 1).

The regression analysis of NO_3^- N fertilizer on peanut yield is expressed by the model equation Y = 12.89x + 1287 (R^2 = 0.868). The model showed that each increase of one unit of NO_3--N fertilizer results in an increased grain yield of 12.89 units. R^2 showed that 86.8% of the variations in grain yield (Y) are caused by NO_3^- N fertilization (Figure 1).

The regression analysis NH_4^+N fertilizer for peanut seed yield is expressed by the model equation Y = 10.23x + 1172 (R^2 = 0.957). The model showed that each increase of one unit NH_4^+-N fertilizer results in an increase in peanut seed yield of 10.23 units. R^2 showed that 95.7% of the variations in peanut seed yield (Y) are caused by NH_4^+N fertilizer (Figure 1).

The regression analysis result of NO_3^-N fertilizer on stover yield of peanut straw is expressed by the model equation Y = 33.86x + 1795 (R^2 = 0.783). The model showed that each increase of one unit of NO_3--N fertilizerresulted in an increase in the stover yield of peanut straw of 33.86 units. R^2 showed that 78.3% of the variations in peanut seed yield (Y) are caused by NO_3^-N fertilizer (Figure 1).

The regression analysis of NH_4^+N fertilizer on stover yield of peanut straw is expressed by the model equation Y = 14.93x + 2146 (R^2 = 0847). The model showed that each increase of one unit of NH_4^+-N fertilizer results in an increase of the stover yield of peanut straw of 14.93 units. R^2 showed that 84.7% of the variations in the stover yield of peanut straw (Y) are caused by NH_4^+N fertilizer (Figure 1).

Increasing doses of N fertilization improved NR activity in the leaves. N nutrients in the soil are taken mainly in the form of NO_3^- and NH_4^+, and of these forms, NO_3^- is the form most commonly taken (Marschner, 1995). N fertilizer increases NR, and it proves that most of the NH_4^+ released by urea in the soil is transformed into NO_3^-. An increase in the levels of NO_3^- in the leaf tissue further increases NR. This is in accordance with the results of a study by Munzarova *et al.,* (2006), who stated that the higher the substrate content of NO_3- in the tissue, the more the NR rate increases (Darjanto *et al.,* 2011). NR catalysis is controlled by substrate availability and the level and activity of NR. NR capacity is determined by the overall level of plant metabolism, including metabolism sensors and signal transduction pathways. NR, which is located at the intersection of two energy pathways (nitrate assimilation and carbon fixation), controls the response to environmental changes that affect photosynthesis (Baroniya *et al*., 2014).

The relationship between chlorophyll content and NR

Chlorophyll content is positively correlated with NR (r = 0.888), which is expressed by the model equation Y = 12.44x + 23.05 (R^2 = 0.788, $p < 0.05$). The model showed that an increase of one unit of chlorophyll results in an increase of 43.49 units of NR. R^2 showed that 90.1% of the variations in NR (Y) are caused by chlorophyll content (Figure 2).

The relationship between NR and peanut seed yield was linear, with the formula Y = 43.49x - 321.5 (R^2 = 0.901, $p < 0.05$), indicating a positive correlation (r = 0.949) (Figure 3). The model indicates that each increase of one unit of NR activity results in an increase of seed yield of 43.49 units. R^2 showed that 90.1% of the variations in the seed yield (Y) are caused by nitrate reductase activity.

NR activity has a positive correlation with production, dry weight, and crop power yield. At the beginning of the N fixation process, NO_3^- is reduced to NO_2^- by the enzyme NR. NO_2^- formed in the cytosol nodule is then transported to the roots or leaves to be reduced to NH_4^+. These reactions require electrons from water (H_2O) and are catalyzed by NR (Camacho-Cristobal, 2002). Ammonium or NH_4^+, which is generated quickly, is converted into amide groups of the amino acids glutamine and asparagine (Yu and Zhang, 2012).

Conclusion

From the results of this study, it can be concluded that peanut plants prefer NO_3^- to NH_4^+; this preference is indicated by the higher chlorophyll content, NR activity, weight of pods and seeds, and the biomass with NO_3^- N than with NH_4^+ N fertilization. The effect of the N rate on chlorophyll content, NR activity, seed yield, and peanut straw is linear. There is a positive correlation between chlorophyll content and NR activity, and between NR activity and the peanut seed yield.

Acknowledgement

The authors wish to acknowledge the financial support from the Diponegoro University. We would like to thank the Department of Agroecotechnology, Faculty of Agriculture and Animal Science, Diponegoro University, Semarang, Indonesia for the research facility.

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