The *Rhizobium* and calcium fertilizer application to peanut plant in dry land

A Farid Hemon\(^1\) and Sumarjan\(^1\)

\(^1\)Agroecotechnology, Faculty of Agriculture Mataram University, Jl. Majapahit No. 62 Mataram NTB, Indonesia

\*e-mail: faridhemon_1963@yahoo.com

ABSTRACT. One of the main problems of peanut planting in dry land is a lack of nitrogen and calcium availability. Efforts to increase the nitrogen and calcium availability for peanut farming in dry land is needed and one possible approach is by application of *Rhizobium* and calcium fertilizers. This research was conducted to determine the effect of the application of *Rhizobium* (Nodulin) and calcium fertilizers on growth and yield of peanut plants in dry land. Two experiments were conducted, one in a greenhouse using a completely randomized design and another experiment in the field using a randomized block design. The treatments were with the application of Nodulin, without Nodulin, with calcium fertilizer and without calcium fertilizer. The results showed that the application of calcium and Nodulin was able to increase the number of root nodules and peanut growth. Nodulin and calcium fertilizers increased the dry pod weight of 2.177 g per 6 m\(^2\) or 3.7 tons.ha\(^{-1}\). The application of Nodulin and calcium was also able to increase the nitrogen and calcium content of leaf tissue up to 18.9% and 20.9%, respectively.

Keywords: drought stress, calcium, *Rhizobium*, root nodule

1. Introduction
Peanut development is mostly directed at marginal land such as dry land. Planting peanuts in dry land often face drought stress due to limited availability of water in the root zone. Drought stress has become a major environmental factor that contributes to the decline in agricultural productivity including peanut plant [9].

Drought stress can affect plant growth, as shown by alteration in plant morphology and cell development, physiology and biochemistry [26]. Water deficit can reduce plant leaf area, reduce leaf biomass and dry pod of peanuts [4]. Plants that are exposed to drought stress have a decrease in leaf water potential, disrupt chlorophyll formation and reduce yield potential at harvest [2].

Besides the lack of water availability, dry land has very low availability of nitrogen nutrient at the levels that are insufficient for normal plant growth [1]. Nitrogen is one of the main macronutrient limiting plant production in dry land [13]. Nitrogen fertilizer such as urea is difficult to apply in dry land because urea is easily evaporated and leached so that it is not effective. In addition, water deficiency causes urea to be difficult to dissolve in groundwater, so it is difficult to be absorbed by plants. One way to increase nitrogen availability in dry land is through nitrogen fixation from the air. Inoculation of *Rhizobium* sp. On peanut seeds is one possible way to increase the availability of nitrogen in dry land [17]. Inoculation of peanuts with *Rhizobium* has led to considerable increases in nodulation, growth and productivity of peanut plants [20] [21] [16]. The effect of Nodulin application on peanut plants in dry land was not yet known. In this study, Rhizobium was inoculated on peanut seeds using Nodulin inoculums.

Beside the problem of nitrogen deficiency, low calcium level is also a serious problem in peanut cultivation in dry land. The result of the initial analysis showed that the soil calcium content in the dry
land of Lombok was 0.16% and it was categorized as very low [18]. Low calcium levels inhibit pod formation [12]. Calcium is found in every plant cell as calcium pectate in the cell walls of leaves and stems, so calcium will strengthen these parts. Calcium-deficient plants grow stunted because the new cells are small with a few numbers, stunted root development, and have weak stems because the cell walls are thin. Calcium is relatively immobile in plants, therefore it is not translocated from older parts to younger parts [25]. Adequate levels of soil calcium (Ca$$^{2+}$$) are crucial for the reproductive development of peanut [12]. Calcium also affects the size of peanut seeds [6]. Giving calcium to the soil increases the formation of plant root nodules. Calcium concentrations exceeding 2µM are necessary for optimum growth of *Rhizobium* in nutrient media [3]. The relative concentration of Ca at 10 mM is needed to maximize the penetration of *Rhizobium meliloti* on the roots of alfalfa plants [11]. Calcium is a regulator in the process of root nodule formation. The process of root nodule formation increases at certain calcium levels in root hair cells [5]. Therefore, this research was conducted to determine the effect of the application of *Rhizobium* (Nodulin) and calcium fertilizer on growth and yield of peanut plants in dry land.

2. Materials and methods

2.1. Test the effectiveness of Nodulin for root nodule and peanut growth

Nodulin is a root nodule inoculant product for legumes crop, containing *Rhizobium* sp., *Azospirillum* sp., *Bacillus* sp. Nodulin is produced by the Institute of Research and Development for Soil, Ministry of Agriculture, Republic of Indonesia. The experiment was carried out in the Greenhouse of the Faculty of Agriculture, Mataram University. This experiment used a completely randomized design, with the treatments: R1= Nodulin application, sterilized soil; R2= unsterilized soil without nodulin; R3= sterilized soil without Nodulin. Each treatment was repeated three times. Application of Nodulin was done by mixing Nodulin powder with peanut seeds that have been moistened with water. The dose of Nodulin was 10 g per kg of peanut seeds.

The soil used in this experiment was the top soil taken from previous peanut planting land of North Lombok Regency. All soil used in this experiment was sterilized, especially for the R1 and R3 treatments. Sterilization was done by spraying formalin to the soil placed inside a plastic bag. Sterilized or unsterilized soil (10 kg) was put in each polybag used in this experiment.

Peanut planting was done by inserting seeds into the planting hole of about 3 cm depth, and each planting hole contained 2 seeds. Before the planting, each planting hole was given insecticide Furadan 3G (0.5 g.polybag$$^{-1}$$). Weeding was carried out at the age of 30 days after planting (dap) and 60 dap, and followed by planting hoarding. Fertilization was carried out at earlier planting time by spreading over the soil surface with a dose of 2 g of TSP and KCl per polybag. Pest and disease control was carried out by spraying Bestfast insecticide with a concentration of 0.5 mL.L$$^{-1}$$ and Bestartop fungicide of 0.5 mL.L$$^{-1}$$.

Peanut planting was maintained under drought stress conditions (30-40% soil moisture content of field capacity). Drought stress treatment was given from seed germination stage (5 days old) until harvest. Plants experienced drought stress after 70% of leaf wilting symptoms occurred. Water was given back to field capacity a day after wilting [8]. Nitrogen content in leaf tissue was analyzed by the Kjeldahl method. Analysis of leaf nitrogen content was carried out at the age of 80 dap.

2.2. Effect of calcium with Nodulin application on root nodule formation and peanut growth in greenhouse

This experiment was carried out in the Greenhouse of the Faculty of Agriculture, Mataram University. This experiment used a completely randomized design, with the following treatments: K1 = application of calcium calcite (CaCO$$\_3$$) + Nodulin; K2 = application of calcium Gypsum (CaSO$$\_4$$2H$$\_2$$O) + Nodulin; K3 = application of calcium hydroxide (Ca(OH)$$\_2$$) + Nodulin; K4 = without application calcium + Nodulin; K5 = control (without calcium and nodulin application). Each treatment was repeated 3 times.
The soil used in this experiment came from used peanut rice fields (without sterilization) from North Lombok Regency. Calcium was given 2 (two) weeks before planting by mixing the soil surface with calcium at a dose of 300 kg per hectare (2.4 g per polybag). Nodulin treatment was done by mixing it with groundnut seeds that have been moistened with water. The dose of Nodulin was 10 g per kg of peanut seeds.

Peanut seeds were planted in polybags containing 10 kg of soil. Peanut planting was maintained under drought stress conditions (30-40% soil moisture content of field capacity). Peanut planting was done by inserting seeds into the planting hole of about 3 cm depth and each planting hole contains 2 seeds. Before the seeds were planted, each hole was given the insecticide Furadan 3G in the amount of 0.5 g per polybag. Weeding was carried out at the age of 30 dap and 60 dap, and followed by planting hoarding. Fertilization was carried out at planting time by spreading over the soil surface with a dose of 2 g TSP and KCl per polybag. Pest and disease control was carried out by spraying Bestfast insecticide with a concentration of 0.5 mL.L⁻¹ and Bestartop fungicide 0.5 mL.L⁻¹. Calcium levels in leaf tissue were analyzed by Shimadzu Atomic Absorption Spectrophotometer-AAS 6300. Analysis of leaf calcium content was carried out at the age of 80 dap.

2.3. Application of a combination of calcium (Gypsum) and Nodulin on the growth and yield of peanut in dry land

The experiment was conducted on dry land of farmers in Gumantar Village, Kayangan District, North Lombok Regency (1160 1’ 31.99” - 1160 29’ 35.76” east longitude and 80 12’ 37.44” - 80 28’ 49.58” south latitude). In this location, the average annual rainfall was 1,200 mm, distributed between December/January to April/May, the average temperature and humidity was 31°C and 84%, respectively.

This experiment used a randomized block design, and each treatment was repeated 3 times. The treatments tested were: 1). Nodulin-calcium Gypsum application; 2). Gypsum application-without Nodulin; 3). Nodulin application-without Gypsum, and 4. control (without Nodulin and Gypsum).

Nodulin was given by mixing it with groundnut seeds that have been moistened with water. The dose of Nodulin was 10 g per kg of peanut seeds. Calcium fertilizer used was Gypsum with a dose of 300 kg per hectare.

The experimental land was processed once until smooth and plotted was made in size 3 x 2 m². Gypsum was given 2 (two) weeks before planting, by mixing it evenly with the soil surface on the experimental plot. The soil was then given water so that calcium could dissolve in the soil.

Peanut planting was done by inserting seeds into the planting hole of about 3 cm depth and each planting hole contains 2 seeds. Before planting the seeds, each hole was given Furadan 3G 0.5 g per planting hole. Planting was done with a spacing of 20 x 40 cm.

At the age of 30 days after planting, the plants were weeded and followed by planting hoarding. Fertilization was carried out at planting time by spreading it over the experimental plot. The fertilizers used were 75 kg TSP and 50 kg KCl per hectare. Pest and disease control was carried out by spraying Bestfast insecticide with a concentration of 0.5 mL.L⁻¹ and Bestartop fungicide 0.5 mL.L⁻¹. Irrigation was carried out by an irrigation pump, which was carried out the day after planting and then irrigated every month.

3. Results and discussion

3.1. Test the effectiveness of Nodulin (source of Rhizobium) for root nodule growth and peanut growth

The application of Nodulin had a significant effect on the growth of root nodules and the growth of peanut plants (Tables 1 and 2). In the study, it was seen that the source of Rhizobium inoculum derived from Nodulin and soil taken from previous peanut plantation resulted in better root nodule growth than the sterile soil media.

Nodulin and soil taken from previous peanut planting were suspected to have an effective Rhizobium population compared to sterile soil media. Unsterilized soil taken from previous peanut plantations has Rhizobium and other microorganisms that are more varied than Nodulin. The
interaction of *Rhizobium* and other microorganisms in the soil helps *Rhizobium* to penetrate the peanut roots [23]. The success of the *Rhizobium*-legume symbiosis will increase the cooperation of nitrogen fixation in the soil ecosystem. The *Rhizobium*-legume symbiosis is the main source of nitrogen fixation in the soil system and can provide more than half of nitrogen fixation [24].

Root nodules are capable of free nitrogen fixation (Biological N\(_2\) fixation = BNF) and are an efficient nitrogen source. The success of the *Rhizobium*-legume symbiosis will increase BNF cooperation in the soil ecosystem. Peanuts have root nodules that are able to bind nitrogen (N) from the air through symbiosis with *Rhizobium* bacteria. Effective root nodules have the ability to nitrogen fixation and can meet the nitrogen requirement of 80-90% for plant growth [16].

In Table 2, it can be seen that the source of *Rhizobium* derived from Nodulin and soil taken from previous peanut plantation without sterilization resulted in a higher. Plant growth is influenced by the availability of nitrogen in the soil. The source of *Rhizobium* from Nodulin produced a dry weight of 7.5 g and was not significantly different from peanut grown in media taken from previous peanut plantations without sterilization.

**Table 1.** Average number of root nodules, fresh weight of root nodules (g), and dry weight of root nodules (g) at the age of 60 dap.

| Treatments | Number of root nodules | Fresh weight of root nodules | Dry weight of root nodules |
|------------|------------------------|------------------------------|---------------------------|
| R1         | 7.4 a                  | 0.8 a                        | 0.73 a                    |
| R2         | 10.2 a                 | 0.9 a                        | 0.75 a                    |
| R3         | 0.0 b                  | 0.0 b                        | 0.0 b                     |

Note: Numbers followed by the same letter in the same column have no significant difference on Duncan's 5% test. R1= Nodulin application; R2= without nodulin in media taken from previous peanut plantation sterilization; R3= sterilized media without Nodulin application

**Table 2.** Average plant height (cm), plant fresh weight (g) and plant dry weight (g) at the age of 60 dap.

| Treatments | Plant height | Plant fresh weight | Plant dry weight |
|------------|--------------|--------------------|------------------|
| R1         | 26.5 b       | 47.2 a             | 7.5 a            |
| R2         | 28.9 a       | 49.8 a             | 8.4 a            |
| R3         | 23.8 c       | 33.8 b             | 5.3 b            |

Note: Numbers followed by the same letter in the same column have no significant difference on Duncan's 5% test. R1= Nodulin application; R2= without nodulin in media taken from previous peanut plantation sterilization; R3= sterilized media without Nodulin application

Table 3 presents the effect of nodulin and without nodulin application on the weight of plant fresh and dry, the number of filled pods, and the weight of dry pods filled under drought stress. In Table 3, it can be seen that the application of Nodulin was able to increase the fresh and dry weight of peanut plants and produced more pods and heavier dry pods. Nodulin is a source of *Rhizobium* and it was able to increase N fixation for growth and yield of peanut plants. Nitrogen fixation can then be seen in leaf tissue N content. Nodulin application generated leaf tissue N content higher than without Nodulin application (Figure 1). *Rhizobium* inoculation of groundnut has led to considerable increases in nodulation, N content, growth and productivity [21].

**Table 3.** Average of plant fresh and dry weight (g), number of filled pods, dry pod weight (g) under drought stress at the age of 90 dap.

| Treatments | Plant fresh weight | Plant dry weight | Number of filled pods | Dry pod weight |
|------------|--------------------|-----------------|-----------------------|---------------|
| No Nodulin | 90.8 b             | 16.9 b          | 16.3 a                | 6.7 b         |
| Nodulin    | 127.7 a            | 24.5 a          | 22.3 b                | 20.4 a        |

Note: Numbers followed by the same letter in the same column have no significant different on Duncan's 5% test
Figure 1. Nitrogen content (%) of leaf tissue on Nodulin application under drought stress

3.2. Effect of various calcium and Nodulin application on root nodule growth and plant growth in greenhouse

In this experiment, we want to know the effect of calcium and Nodulin on the formation of root nodules and plant growth. As can be seen in Table 4, the application of Nodulin and calcium did not have a significant effect on the growth of root nodules and root length at the age of 60 dap, but there was a tendency that the application of Nodulin and calcium sulfate (CaSO4) or Gypsum produce heavier root nodules. In Table 5, the application of calcium with Nodulin tended to provide better plant growth than without calcium. Total Ca content of plant leaf tissue was higher than plants without Ca application. Application of calcium and Nodulin (source of Rhizobium) resulted in taller plants, more leaves and heavier plants dry weight.

*Rhizobium*-legume symbiosis is the main source of nitrogen fixation in soil systems and can provide more than half of nitrogen fixation [24]. Association of *Rhizobium* species with legumes is a renewable nitrogen source for agriculture [19]. Peanuts have root nodules that are able to bind nitrogen (N) from the air through symbiosis with rhizobium bacteria, so that the plant will receive nitrogen that is fixed by the root nodules.

| Nodulin and calcium application | Root length (cm) | Root nodule dry weight (g) | Root nodule fresh weight (g) |
|--------------------------------|-----------------|-----------------------------|-----------------------------|
| Nodulin - CaCO3                | 50.5            | 0.053                       | 0.127                       |
| Nodulin - CaSO4                | 48.8            | 0.068                       | 0.168                       |
| Nodulin - Ca(OH)2              | 45.3            | 0.047                       | 0.127                       |
| Nodulin - No Ca                | 42.1            | 0.055                       | 0.125                       |
| No Nodulin - No Ca             | 40.4            | 0.043                       | 0.118                       |

| Nodulin and calcium application | Plant height (cm) | Leaf number | Plant dry weight (g) | Ca- total of leaf tissue (%) |
|--------------------------------|------------------|-------------|----------------------|-------------------------------|
| Nodulin - CaCO3                | 33.3             | 32.0        | 8.11                 | 0.58 a                        |
| Nodulin - CaSO4                | 32.7             | 32.0        | 8.20                 | 0.52 a                        |
| Nodulin - Ca(OH)2              | 32.0             | 32.7        | 8.19                 | 0.56 a                        |
| Nodulin - No Ca                | 31.2             | 27.7        | 8.04                 | 0.47 b                        |
| No Nodulin - No Ca             | 30.5             | 26.0        | 7.01                 | 0.43 b                        |

Note: Numbers followed by the same letter in the same column have no significant different on Duncan’s 5% test.

Calcium fertilization is also needed for plant growth. Lack of calcium ions will interfere with root development, necrosis and wrinkled on leaves, discoloration of young leaves, chlorosis at the tips and edges (turns yellow) and this color spreads between the ends of the leaf bones, and leaf tissue in some places dead [22]; [13].
3.3. Application of Nodulin and calcium on peanut growth and yield in dry land
In this experiment, we want to know the effect of the combined application of *Rhizobium* (Nodulin) and calcium (Gypsum) on the growth and yield of peanuts in dry land. The results of the initial analysis showed that the soil calcium content in dry land of Lombok was 0.16%, and was categorized as very low [18].

The results in Table 6 showed that the combination of Nodulin and Gypsum application had significantly increased the plant height, the number of branches, the fresh pod weight and the dry pod weight per plot (per 6 m²). The application of Nodulin and calcium was able to increase the weight of wet pods to 4014.0 g per plot (or equivalent to 6.7 tons Ha⁻¹) and weight of dry pods per plot equivalent to 3.6 tons Ha⁻¹. Application of Nodulin and calcium in peanut cultivation was able to increase Nitrogen 1.95% (Figure 1) and Ca²⁺ ions in plant leaf tissue (Table 5). Adequate level of soil calcium (Ca²⁺) is crucial for the reproductive development of peanuts [12]. Calcium-deficient in peanuts inhibits pod formation, germination and seed vigor. Calcium also affects the size of peanut seeds [14].

Ca²⁺ plays an important role in controlling membrane structure and function [14]. Ca²⁺ binds to phospholipids to form bilayers on the cell wall and provide a compact structure to the cell membrane. Chloroplasts are also determined by the absorption of Ca²⁺. In plant cells with low concentrations of Ca²⁺, the function of the chloroplasts will not be ideal and photosynthetic electron transport is highly dependent on pH which is regulated by Ca²⁺ [15].

The Ca²⁺ element is a very important regulator for plant growth and development [7]; [10]. There is some damage caused by a lack of Ca²⁺ ions, namely root growth becomes stunted, leaves undergo necrosis and curls. Deficiency of Ca also causes weak plant, plant meristem tissue to be stunted, and even the fall of plant leaves [22]; [25]. Calcium affects the development of peanut pods and seeds. Research of Pathak (2010) showed that the application of gypsum increases the success of sperm and ovum fertilization, so that the ovule will develop perfectly.

| Treatments | Plant height at harvest time (cm) | Branch number at harvest time | Number of filled pods per plant | Weight of fresh pods per plot (g) | Dry pod weight per plot (g) |
|------------|---------------------------------|-----------------------------|--------------------------------|----------------------------------|-----------------------------|
| 1          | 82.4ab                          | 17.9a                       | 16.53                          | 4014.0a                          | 2177.3a                     |
| 2          | 87.1a                           | 16.6ab                      | 16.83                          | 3705.7b                          | 1164.2c                     |
| 3          | 71.7b                           | 14.7b                       | 17.13                          | 3654.7b                          | 1657.7b                     |
| 4          | 83.3ab                          | 5.3c                        | 17.13                          | 3299.7b                          | 1563.3b                     |

Note: Numbers followed by the same letter in the same column have no significant difference on Duncan's 5% test. Treatments: 1. Nodulin-calcium; 2. Calcium-no Nodulin; 3. Nodulin-no calcium, and 4. Control (no Nodulin-no calcium)

4. Conclusion
The application of calcium (Gypsum) and Nodulin was able to increase the root nodules and peanut plant growth, as well as increase the dry pod weight to 2.177 g per 6 m² or 3.7 tons ha⁻¹. The application of Nodulin and calcium was also able to increase the nitrogen and calcium content of leaf tissue up to 18.9% and 20.9%, respectively.

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