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Yield responses of *Macrotyloma axillare* (family *Fabaceae*) to combinations of doses of phosphorus and calcium

Respostas produtivas da leguminosa-macrotiloma a combinações de doses de fósforo e cálcio

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Abstract

The present study aimed to assess the effects of combinations of doses of phosphorus and calcium on the yield characteristics of *Macrotyloma axillare* access NO 279 (family *Fabaceae*) in a Red-Yellow Argisol with average P level (24 mg dm^{-3}). The experiment was carried out in greenhouse in Nova Odessa/SP, Brazil, from August to December 2015. A 5^2 fractional factorial design with 13 combinations for doses of phosphorus and calcium in mg dm^{-3}: 0-0; 0-40; 0-80; 15-20; 15-60; 30-0; 30-40; 30-80; 45-20; 45-60; 60-0; 60-40; 60-80, in a randomized complete block design with four replications. Two cuttings were made on the plants. The phosphorus x calcium interaction and the isolated doses of calcium were not significant for the parameters assessed. The production of dry biomass of the aerial part, the leaf area, the number of leaves and the number of branches of *Macrotyloma* showed significant responses to the isolated doses of phosphorus applied in the primary growth. In the secondary growth, only the number of branches and the dry biomass production of the dead material responded to the doses of phosphorus. Phosphorus was a modulator of dry biomass production of roots and of the production of nodules and the number of nodules of macrotyloma. The content of calcium present in the soil, together with the increase of the value of base saturation to 50%, was sufficient for the establishment and growth of the legume. Therefore, a supplemental dose of this nutrient was not required.
Key words: Fertilization. Forage. Legume. Plant nutrition.

Resumo

Objetivou-se avaliar os efeitos das combinações entre doses de fósforo e cálcio nas características produtivas da leguminosa-macrotiloma (*Macrotyloma axillare* acesso NO 279) num Argissolo Vermelho Amarelo com teor de fósforo médio (24 mg dm^{-3}). O experimento foi instalado em casa de vegetação, localizada em Nova Odessa/SP, Brasil, no período de agosto a dezembro de 2015. Utilizou-se o esquema fatorial 5^2 fracionado, com 13 combinações para as doses de fósforo e cálcio em mg dm^{-3}: 0-0; 0-40; 0-80; 15-20; 15-60; 30-0; 30-40; 30-80; 45-20; 45-60; 60-0; 60-40; 60-80, em delineamento experimental de blocos ao acaso, com quatro repetições. Foram realizados dois cortes nas plantas. A interação fósforo x cálcio e as doses isoladas de cálcio não foram significativas para os parâmetros avaliados. A produção de biomassa seca da parte aérea, a área foliar, o número de folhas e o número de

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ramificações da leguminosa-macrotiloma apresentaram significância para as doses isoladas de fósforo aplicadas no primeiro crescimento. No segundo crescimento apenas o número de ramificações e a produção de biomassa seca do material morto responderam as doses de fósforo. O fósforo foi modulador da produção de biomassa seca de raízes e de nódulos e do número de nódulos da leguminosa. O teor de cálcio presente no solo, aliado a elevação da saturação por bases para 50%, foi suficiente para a implantação e o crescimento da leguminosa-macrotiloma não sendo necessária a complementação desse nutriente.

Palavras-chave: Adubação. Forrageira. Leguminosa. Nutrição de plantas.

Introduction

The use of forage legumes in pasture systems can be a key tool for the sustainability and profitability of animal production. In the consortium between grass and legumes, the nitrogen supply provided by the legume increases forage (PIRHOFER-WALZL et al., 2012) and animal production (DEL PINO et al., 2016).

Despite the benefits of using legumes in consortium with grazing systems, many challenges must be overcome to grow these vegetables, such as low germination percentage, slow crop establishment and low persistence (GIMENES et al., 2017; MORAIS et al., 2017). Therefore, the choice of a plant with yield potential and characteristics consistent with the particularities of each region is essential for the successful implementation of a pasture.

Macrotyloma *[Macrotyloma axillare E. Mey (Verdc)]* access NO 279 *(*Family *Fabaceae*) is a forage legume for potential use in grazing systems. Access NO 279 was initially evaluated by Veasey et al. (1999) who classified it as promising variety for use in further studies due to its higher seed yield, greater vegetative growth and greater tolerance to pests and diseases.

Paiva et al. (2008) classified *Macrotyloma axillare* cv. Java as a variety growing on soils with lower fertility and very persistent under grazing and intercropping. However, despite requiring lower fertility conditions, forage legumes need phosphorus and calcium for their establishment, as these nutrients are essential to the growth of the aerial part and the root system of these plants.

Because phosphorus and calcium are essential for the growth of legumes and are found at low levels in tropical soils (DECHEN; NACHTIGALL, 2007), their supply is even more necessary for forage plants. Phosphorus and calcium uptake by plants occurs in a synergistic way, since the administration of calcium, through liming, favors the use of the phosphorus available in the soil (RAIJ, 2011).

Phosphorus supply increases the production of dry biomass by legumes (NOHONG; AKO, 2016), and in the consortium with grasses, the nutrient maintains minimal amounts of legumes, which provides positive interactions for the plants (NYFELER et al., 2011). Thus, calcium and phosphorus nutrients were considered important in the establishment and maintenance of the leguminous plant *Stylosanthes guianensis* cv. Mineirão (LOPES et al., 2011; LOPES et al., 2010).

Despite the importance of calcium and phosphorus for forage legumes, no studies have been conducted so far on the effect of the combined supply of these nutrients for legume macrotyloma*,* which may jeopardize its implementation and persistence in grazing systems. Therefore, the present study aimed to investigate the responses of *Macrotyloma axillare* access NO 279 to combinations of phosphorus and calcium doses in the implementation and growth, through the assessment of the yield parameters of the plant.

Material and Methods

The soil sample of the experiment was collected in ten points randomly distributed in *Urochloa brizantha* (syn *Brachiaria brizantha)* cv. Marandu at a depth of 0.20 cm. After being homogenized, dried and sifted, the sample was placed in pots with a capacity of 4.5 kg.

Two soil samples were analyzed for their chemical and physical properties. The chemical and physical soil analysis showed the following results: pH ($CaCl_2$) = 4.4; P- Resin (mg dm^{-3}) = 24; Organic matter (g dm^{-3}) = 25; K (mmolc dm^{-3}) = 2.8; Ca ($mmol_c$ dm^{-3}) = 17; Mg ($mmol_c$ dm^{-3}) = 8; H+Al ($mmol_c$ dm^{-3}) = 38; Al ($mmol_c$ dm^{-3}) = 3; SO_4 (mg dm^{-3}) = 3; Base Saturation ($mmol_c$ dm^{-3}) = 28; CEC (mmolc dm^{-3}) = 66; V (%) = 42; B (mg dm^{-3}) = 0.27; Cu (mg dm^{-3}) = 1.4; Fe (mg dm^{-3}) = 68; Mn (mg dm^{-3}) = 7.7; Zn (mg dm^{-3}) = 1.4; Clay = 278 g kg^{-1}; total sand = 560 g kg^{-1}; Silt = 162 g kg^{-1}. According to the results of the soil chemical analysis, the value of 50% of base saturation was determined for the liming (WERNER et al., 1996).

In the experiment, the treatments consisted of five combined doses of phosphorus (0, 15, 30, 45 and 60 mg dm-3 P2O5) and five doses of calcium (0, 20, 40, 60 and 80 mg dm^{-3} Ca) in polynomial regression with response surface analysis based on incomplete block design and 5^2 fractional factorial, according to Littell and Mott (1975). The treatments were conducted in greenhouse in a randomized complete block design, with four replications.

After soil incubation (30 days), thirty seeds of legume macrotyloma were sown, and fifteen days after germination, the seedlings were removed (thinning), so that only five plants per pot were left. Application of molybdenum and phosphorus and calcium doses was made on the day of sowing. After the treatments were applied, the other nutrients diluted in distilled water were supplied on alternate days, as follows: 71.21 mg pot $^{-1}$ of potassium (KCl), 116.60 mg pot $^{-1}$ of sulfur ($H_2S_3O_4$), 7.60 mg pot^{-1} of zinc ($ZnCl_2$), 4.10 mg pot $^{-1}$ of copper ($CuCl_2$) and 1.20 mg pot^{-1} of boron (H_3BO_3).

In the secondary growth, after application of the treatments, equal amounts of potassium, sulfur, zinc, copper, boron and molybdenum were applied, as well as 336.80 mg/pot^{-1} of magnesium ($MgCl_2$), on alternate days, to control water supply to the soil. It should be noted that magnesium was applied in the secondary growth in order to maintain the calcium: magnesium ratio, and in the primary growth, this nutrient was supplied through liming.

Two cuttings were made in the plants: the first 53 days after planting and the second 34 days after the first cut. The cutting height adopted for the plants was five centimeters from the soil surface, regardless of the presence of leaves and branches, thus allowing a residual aerial part for regrowth of the plants.

The count of leaves and branches per pot was carried out every seven days. The leaves were counted and marked with permanent marker, black, at the moment they were fully expanded, that is, at the moment when the stipule was open in the branch (FONTANELI et al., 2012) and the branches were counted and marked with a colored ribbon as soon as they emerged.

Evaluation of the leaf area per pot was made with the use of the LICOR model LI-3100 leaf area integrator, using the entire leaf as a measure of leaf area (FONTANELI et al., 2012).

After the cuttings, all the material was placed to dry in a forced air circulation oven at 65 ºC until reaching constant weight, and then weighed in a precision scale resulting in dry biomass. The production of dry biomass from the aerial part per pot was determined through the sum of the biomass of its components (leaflets, petioles and branches) in the primary and secondary growths. In turn, determination of dry root biomass per pot was made after root system washing at the end of the experimental period. The nodules present in the roots were collected with the aid of tweezers and separated for later computing.

All the results were given the recommended statistical treatment with surface analysis of response, and a level of 5% of significance was adopted. Initially, analysis of variance was performed for combinations of phosphorus and calcium doses, and when there was significance in the F test for the P x Ca interactions, polynomial regression (response surface) study was performed. When there was no significance for the interaction P x Ca, first and second degree regression studies were carried out for the effect of phosphorus and/or calcium doses alone. The SAS statistical program was used for data analysis.

Results and Discussion

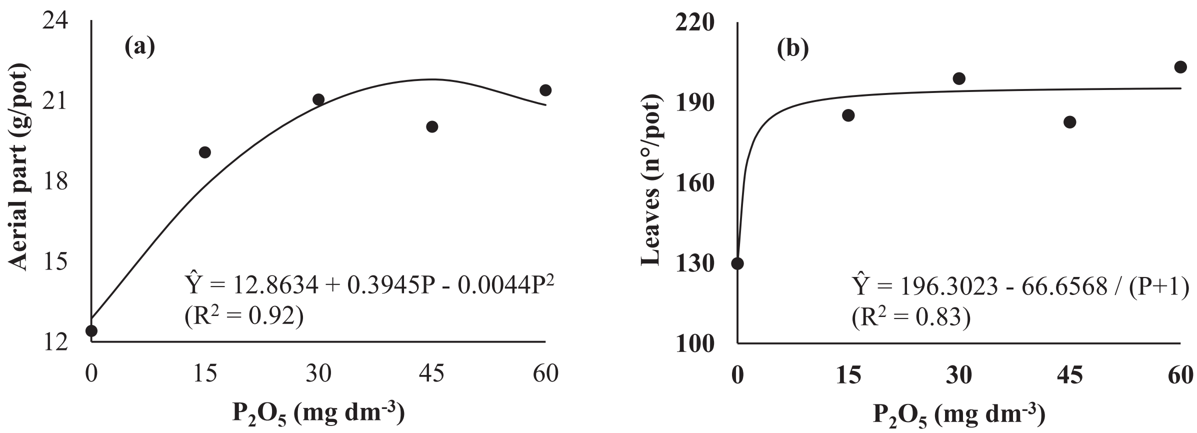
There was no phosphorus x calcium interaction for all the parameters assessed regardless of the growth periods (P> 0.05). Significant responses were also not observed for calcium doses at the time of the two growths (P> 0.05).

According to Raij (2011) responses to calcium doses alone have not been frequently recorded in the crops due to the supply of this nutrient via liming. As tropical soils have low calcium contents, the fact that the legume macrotyloma does not need an additional supply of this nutrient for its initial development is interesting, as it allows its use in most of these soils.

Leaf biomass production (P = 0.0001), number of leaves (P = 0.0001), leaf area (P = 0.0003) and number of branches (P = 0.0125) of legume macrotyloma showed significance for the doses of phosphorus alone applied in the primary growth. On the other hand, dry biomass of the dead material did not present responses to the phosphorus doses (P = 0.4123).

The production of dry biomass of legume macrotyloma increased with the supply of phosphorus, producing maximum yield with 45.22 mg dm^{-3} of P_2O_5 (Figure 1a). Regarding the number of leaves, this varied from 130 to 195 leaves after application of doses of phosphorus (Figure 1b). In low soil phosphorus, plants reduce leaf numbers and leaf expansion, prioritizing chlorophyll formation (AYUB et al., 2012; PRADO, 2008), resulting in lower growth.

Figure 1. Dry biomass of the aerial part (a) and number of leaves (b) in the primary growth of legume macrotyloma access NO 279 as a result of the supply of phosphorus.

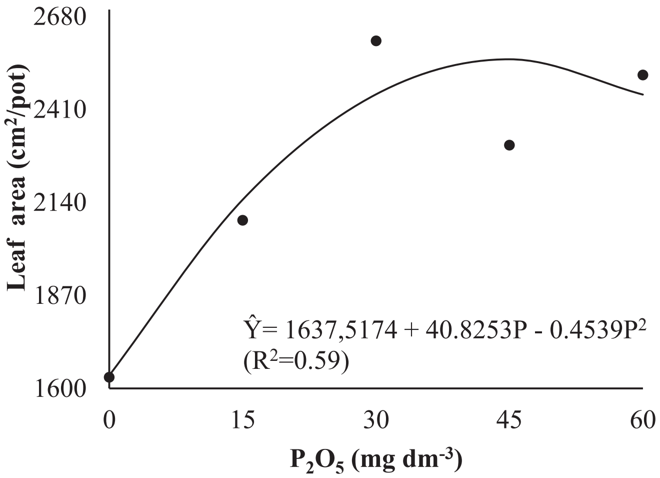


The effect of phosphorus on the production of dry biomass of legume macrotyloma demonstrates the need for supply of this nutrient in the establishment of forage legumes, even when the initial available P content in the soil (24 mg dm^{-3}) was considered of medium level (RAIJ, 2011). This may be due to the fact that most of the phosphorus present in the soil is retained (labile phosphorus), resulting in low soil absorption (DECHEN; NACHTIGALL, 2007), and low plant growth. Koufali et al. (2016) observed that legume growth was favored by the supply of phosphorus, which increased the dry biomass production of *Medicago sativa* (alfalfa).

In order to demonstrate the short-term effect of phosphorus deficiency, Høgh-Jensen et al. (2002) conducted experiments using different rates of relative phosphorus addition and found that the total withdrawal of this nutrient supply in the last three weeks of the experiment did not affect the total dry biomass yield of *Trifolium repens* (white clover) plants, demonstrating that a low supply of phosphorus generates adaptive responses. These authors also reported that even plants in a more advanced vegetative growth stage, which would tend to respond more slowly to phosphorus supply, responded rapidly to phosphorus removal.

In the present study, the maximum leaf area was verified in the primary growth of the legume macrotyloma with the supply of 44.97 mg dm^{-3} of P_2O_5 (Figure 2). This result is important, since the yield potential of forage plants may be related to the increase of the leaf area, which may intensify, to a certain extent, the accumulation of forage, since increased leaf area results in greater light interception and consequent increase in leaf production, thus having a positive effect on the production of dry biomass of the plants. In legumes, which have composite leaves, the leaflets expand due to variations in length and width, and the leaf area is the most appropriate term to express leaf elongation (LOPES de SÁ et al., 2015).

Figure 2. Leaf area in the primary growth of legume macrotyloma access NO 279 as a result of the supply of phosphorus.



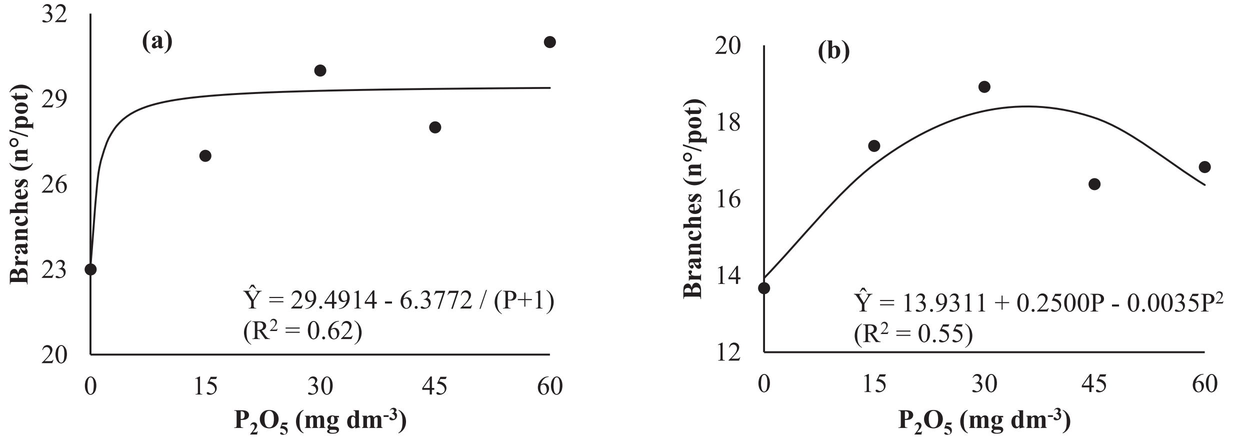
The relationship between leaf production and leaf area (Figures 1b and 2) showed that a greater number of leaves contributed to increase the leaf area. Thus, phosphorus supply is important, as this nutrient mobilizes the energy necessary to the development of leaf primordia (RAIJ, 2011), reflecting directly on the growth rates of leaflets, and, consequently, on larger leaf area. However different results were observed by Ayub et al., (2012) in their study with the supply of phosphorus to *Cyamopsis tetragonolobus* where increased leaf area resulted in an increased expansion rate rather than in a higher number of leaves.

In the secondary growth of legume macrotyloma, the number of branches (P = 0.0419) and the dry biomass of the dead material (P = 0.0102) showed significance for the doses of phosphorus alone applied. There were no significant differences for the doses of phosphorus applied in dry biomass of the aerial part (P = 0.3527), leaf area (P = 0.5488) and leaf number (P = 0.2282).

The number of branches varied from 23 to 31 as a result of the phosphorus supply in the primary growth (Figure 3a), and in the secondary growth, the number of branches had a higher value (19) at a dose of 35.71 mg dm^{-3} of P_2O_5 (Figure 3b). Branches are secondary structures essential to the development of forage legumes. They develop in the nodes of the main branch and maximize the biomass production of *Fabaceae,* as they substantially increase the photosynthetic surface (MAUAD et al., 2010).

The responses of the number of branches to phosphorus can be related to the fact that this nutrient participates in vital reactions of the plant, acting in growth areas and being one of the nutrients more readily redistributed in these areas (MALAVOLTA, 2006).

Figure 3. Number of branches in the primary growth (a) and secondary growth (b) of legume macrotyloma access NO 279 as a result of the supply of doses of phosphorus.



In the secondary growth of legume macrotyloma, a smaller number of branches were observed, a fact that can be explained by intraspecific competition, especially for light, resulting in less availability of photoassimilates for the development of the branches, which caused the plant to direct energy to the growth of its main branch, reducing the emergence of side branches (MARTINS et al., 1999; MAUAD et al., 2010). Therefore, after the cutting of the plant, priority was given to its development, with promotion of the elongation of its main branch for leaf emission.

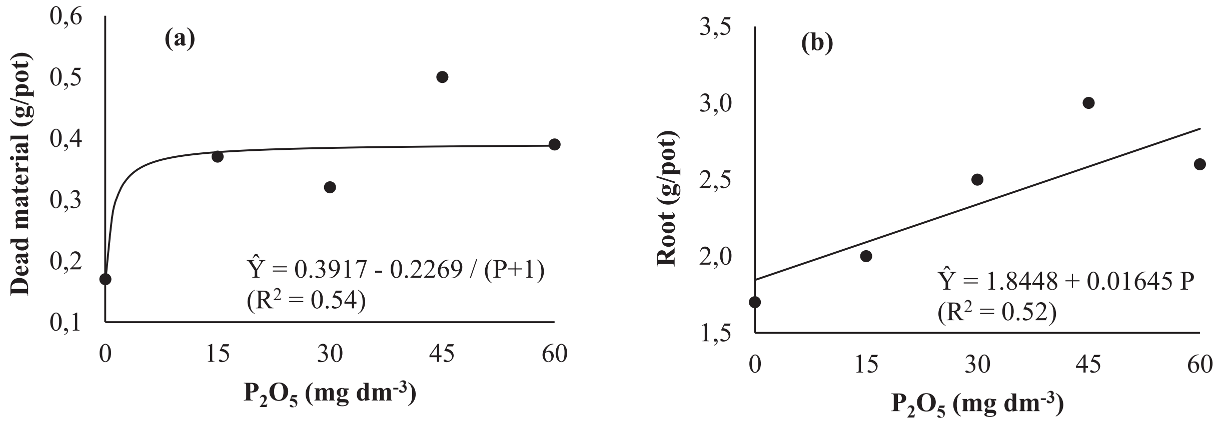
In addition to interfering with the structural responses of legume macrotyloma in the primary growth, phosphorus supply maximized the dynamics of leaf emergence. This fact may have influenced the accumulation of dry biomass of the dead material, since leaves age and undergo senescence (LEMAIRE et al., 2009). In addition, the greater number of expanded, residual leaves of the primary growth may have affected the accumulation of senescent material.

The accumulation of dry biomass of dead material in relation to phosphorus can also be related to a possible toxicity by manganese, caused by its excess in water supply and accumulation of water in the soil during the secondary growth. In flooded soils, some metal oxides, including manganese, are used as electron acceptors, causing the concentration of soluble manganese to increase beyond the levels accepted by the plants, which may result in crop toxicity (KHABAZ-SABERI;

RENGEL, 2010; SILVA et al., 2017). This is due to the fact that manganese is rapidly transported to the branches, causing, among other symptoms, necrotic scores between the new leaf veins, leaf chlorosis, wilting and poor formation of leaf limbs (LAVRES JUNIOR et al. 2008).

According to the inverse function, the dry matter biomass of legume macrotyloma ranged from 0.17 to 0.50 g/pot^{-1} as a result of phosphorus doses (Figure 4a). The production of dry biomass of roots increased with the supply of doses of phosphorus (Figure 4b).

Figure 4. Dry biomass of dead material in the secondary growth (a) and dry biomass of roots at the end of the experiment (b) of legume macrotyloma access NO 279 as a result of the supply of doses of phosphorus.



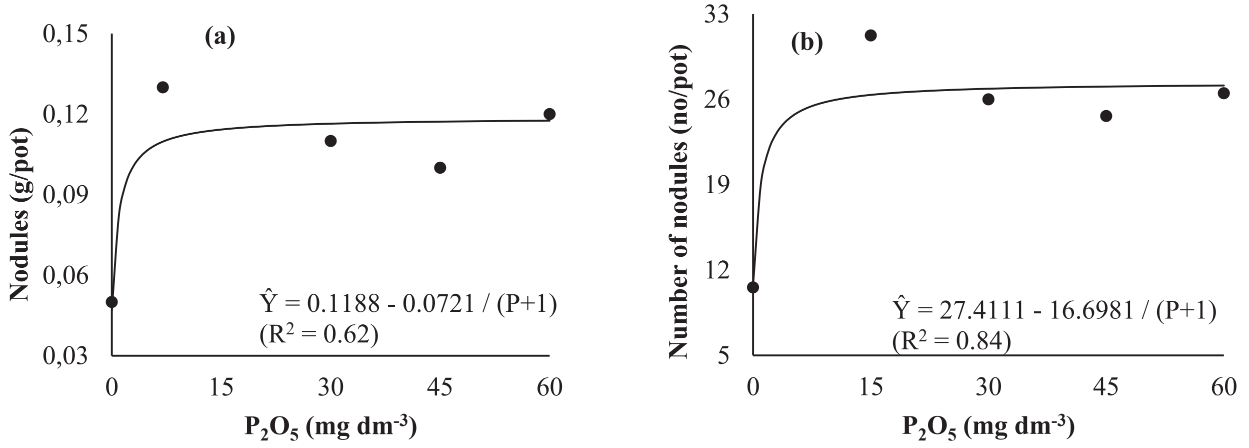
At the end of the experiment, the root system variables, dry root biomass (P = 0.0207), dry biomass of nodules (P =,0001) and number of nodules (P =,0001) showed responses only for the doses of phosphorus alone. According to Raij (2011), phosphorus in adequate amounts in the soil will stimulate root development, increasing early production and ensuring vigorous plant development.

In their assessment of the growth of roots of tropical legumes, *Crotalaria juncea* (crotalaria), *Mucuna cinereum* (gray mucuna), *Cajanus cajan* (pigeon pea) and *Dolichos lablab* (lablab) in response to phosphate fertilization, Fageria et al. (2016) also observed that biomass root dryness was significantly affected by doses of phosphorus, with the maximum values for growth of the root system observed with 100 mg kg^{-1} of P.

The production of dry biomass from nodules of legume macrotyloma varied from 0.05 to 0.13 g pot^{-1} as a result of the supply of phosphorus (Figure 5a). The number of nodules varied from 11 to 31 according to the phosphorus supply (Figure 5b). These increases in dry biomass of roots and nodules as a result of phosphorus doses corroborate the results of Nohong and Ako (2016) who reported that the supply of phosphorus is important for the development of the roots and nodules, as well as for biological nitrogen fixation (BNF).

The mean ratio between the nodule mass and the number of nodules per vessel was 0.0043 g nodule$^{-1.}$ It can be inferred that the effect of phosphorus on the production of dry biomass of nodules can be explained by the influence of this nutrient on the number of nodules of the legume. Thus, despite the apparent existence of differences in nodule size, comparison of Figures 5a and 5b revealed that the influence of this nutrient on the production of dry biomass can be related to the number of nodules present in the root system, and the higher the number of nodules the greater the dry biomass.

Figure 5. Dry biomass of nodules (a) and number of nodules (b) of legume macrotyloma access NO 279 as a result of the supply of doses of phosphorus at the end of the experimental period.



The fact that legumes have a higher requirement of phosphorus than grasses is mainly due to the costly process of BNF, which requires two molecules of adenosine triphosphate (ATP) for each electron transferred from the Fe-protein to the MoFe-protein, and N_2 reduction requires eight electrons, totaling 16 ATPs in the process (FAGAN et al., 2007; TAIZ et al., 2017). This has already been demonstrated by Høgh-Jensen et al. (2002) where BNF in white clover was reduced in situations of phosphorus supply below the optimum range. These authors also reported that the reduction of phosphorus supply paralyzed nodule growth and caused a substantial decline in nitrogenase activity.

Conclusions

1. The level of calcium in the soil combined with the increase of the soil base saturation to 50% was sufficient for the implementation and growth of legume macrotyloma. Therefore, supplementation of this nutrient was not necessary.

2. Implementation of legume macrotyloma requires the supply of phosphorus for its growth and establishment, even when the initial available phosphorus content in the soil is considered medium.

3. Phosphorus was the modulating nutrient of the production of dry biomass of the aerial part, roots, nodules, leaf area, number of leaves, branches and nodules of legume macrotyloma.

Acknowledgments

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