COSTA, N.L., et al. Effect of arbuscular mycorrhizal inoculation and phosphorus fertilization on forage yield and chemical composition of *Codariocalyx gyroides.* **PUBVET**, Londrina, V. 8, N. 20, Ed. 269, Art. 1792, Outubro, 2014.

PUBVET, Publicações em Medicina Veterinária e Zootecnia.

Effect of arbuscular mycorrhizal inoculation and phosphorus fertilization on forage yield and chemical composition of *Codariocalyx gyroides*

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Abstract

The effects of three arbuscular mycorrhizae (AM) fungi species (*Acaulospora muricata*, *Scutellospora heterogama* and *Gigaspora margarita*) and two levels of P (0 and 22 mg P/kg) on dry matter (DM) yield, nodulation, and N and P uptake of *Ccodariocalyx gyroides* CIAT-3001 were evaluated under greenhouse conditions, utilizing a Yellow Latosol (Oxisol), clayey, previously sterilized. All parameters measured were increased by AM inoculation and P fertilization. There are differences among the species of mycorrhizal fungi in their ability to stimulate growth depending on the P level applied. However, the mycorrhizal effects were improved by the phosphate addition. In the absence of phosphate fertilization, no significative differences were found among AM fungi species,

however, AM inoculation increased the DM yields about 58 to 127% in relation to control. In presence of P, plants inoculated with *G. margarita* or *S. heterogama* recorded the higher DM yields. AM inoculation significantly improved (P=0.05) the P contents. In the presence of phosphate, maximum P concentrations occurred with the inoculation of *.A. muricata* and *S. heterogama*, while without P addition the fungi more effectives were *G.* margarita and A*. muricata*. In relation to P uptake, the higher values were observed with the inoculation of *S. heterogama* and *G. margarita* in the presence of P addition, however there were no differences among mycorrhizal fungi without phosphate fertilization. P application slightly increased colonization rates. The highest percentage was obtained with the inoculation of *S. heterogama* and *A. muricata*, irrespective of P level. AM inoculation had a significant (P=0.05) positive effect on the nodulation (number and weight of nodules) and N content. The level of response was greater with P fertilization. These data showed that mycorrhizal inoculation of *C. gyroides* with *S. heterogama* or *G. margarita* when combined with a sufficiently low application of soluble phosphate had potential as an economic way to increase the productivity of pastures on P-deficient soils.

Index terms: dry matter, nitrogen, phosphorus, root colonization

Efeitos da inoculação de micorrizas arbusculares e fertilização fosfatada sobre a produção e composição química da forragem de *Codariocalyx gyroides*

Resumo

Os efeitos da inoculação de micorrizas arbusculares - MA (*Acaulospora muricata, Scutellospora heterogama* e *Gigaspora margarita*) e de níveis de P (0 e 22 mg P/kg de solo) sobro o rendimento de matéria seca (MS), nodulação e absorção de nitrogênio (N) e fósforo (P) de *Codariocalyx gyroides* CIAT-3001 foram avaliados em casa de vegetação, utilizando-se um Latossolo Amarelo, argiloso, previamente esterilizado. Todos os parâmetros avaliados foram

incrementados pela inoculação de MA e adubação fosfatada. Existem diferenças entre as espécies de MA em sua capacidade de estimular o crescimento da leguminosa, em função do nível de P aplicado. No entanto, os efeitos de micorrização foram maximizados com a adição de P. Na ausência de adubação fosfatada, não houve diferenças significativas (P>0,05) entre as espécies de MA, no entanto, a inoculação promoveu acréscimos entre 58 e 127% no rendimento de MS, em relação ao tratamento controle. Na presença de P, as plantas inoculadas com *G. margarita* ou *S. heterogama* apresentaram os maiores rendimentos de MS. A inoculação de MA afetou significativamente (P<0,05) os teores de P. Na presença de fosfato, as concentrações máximas de P ocorreram com a inoculação de *A. muricata* e *S. heterogama*, enquanto que na ausência de P os fungos mais efetivos foram *G. margarita* e *A. muricata*. Em relação à absorção de P, os valores mais elevados foram observados com a inoculação de *S. heterogama* e *G. margarita* na presença de P, não ocorrendo diferenças (P>0,05) entre os fungos micorrízicos sem adubação fosfatada. A aplicação de P aumentou ligeiramente as taxas de colonização. Os maiores percentuais foram obtidos com a inoculação de *S. heterogama* e *A. muricata*, independentemente do nível de P. A inoculação de MA proporcionou efeito positivo e significativo (P<0,05) sobre a nodulação (número e peso seco de nódulos) e conteúdo de N, sendo o nível de resposta maior com a adição de P. A inoculação de *C. gyroides* com *S. heterogama* ou *G. margarita*, combinada com a aplicação de doses moderadas de fosfato solúvel, potencializa a produtividade das pastagens em solos com baixos teores de P.

Termos para indexação: matéria seca, nitrogênio, fósforo, colonização de raízes

Introduction

There are several factors which determine the low animal performance in the tropics. Among these factors, inadequate plant nutrition is the most limiting, followed by the low natural fertility and high acidity of the soils in

these regions. Phosphorus deficiency is probably the major limitation to the growth of forage legumes, which have a high P requirement for establishment, optimum growth, nodule formation, and nitrogen fixation (Costa et al., 2012). However, P fertilization practices are restricted by poor infrastructure for P fertilizer distribution and the high P fixing capacity of tropical soils. A possible mechanism for maximizing fertilizer efficiency is the improved use of the mycorrhizal plant symbiosis (Howeler and Sierverding, 1982). In this symbiotic association the fungus uses carbohydrates produced by the plant and benefits the plant with increased uptake of P and others nutrients through external hyphae extending from the root surface into the soil (Mosse, 1981).

The Southeast Asian shrub legume*, Codariocalyx gyroides* is well adapted to acid, low fertility and poor drainage soils. Has high forage potential yield and appropriate chemical composition: crude protein (15.11%), neutral detergent fiber (47.91%), acid detergent fiber (31.78%), ether extract (2.74) and ash (7.22%) (Nasrullah et al., 2003).

The present study assessed the effects of three arbuscular mycorrhizae (AM) fungi species (*Acaulospora muricata*, *Scutellospora heterogama* and *Gigaspora margarita*) and two levels of P (0 and 22 mg P/kg) on dry matter (DM) yield, nodulation, and N and P uptake of *Codariocalyx gyroides* CIAT-3001.

Material and Methods

The trial was conducted under greenhouse conditions using a P-deficient (2 mg/kg available P by NH_4F + HCl extraction) Yellow Latosol (Oxisol), clayey soil of pH 5.5. The soil was sterilized at 110^{o}C for one hour each day for three days and reinoculated with a soil microbial suspension free of mycorrhizal fungi spores.

The treatments were arranged in a 4 x 2 factorial, complete randomized blocks, with three replications. Each experimental unit was represented by a pot with a 3.0 kg dry soil capacity. For treatments with P, triple superphosphate at the rate of 22 mg P/kg was added to the soil and mixed

thoroughly. Seeds for sowing were inoculated with specific *Rhizobium* (cowpea group). Hydric control was achieved daily by weighing the pots and keeping soil at 80% field moisture capacity.

Six days after emergence, seedlings were thinned to three plants per pot. At 130 days after thinning, the plants were cut at soil level and oven dried at 65^{o}C for 72 hours. N and P concentrations of above-ground DM were determined. The nodules were detached from the roots, cleaned, oven dried at 65^{o}C for 72 hours, counted, and weighted. AM root colonization was determined by the gridline intersect method (Giovannetti and Mosse, 1970) after root segments were stained (Phillips and Hayman, 1970).

Results and Discussion

All parameters measured were increased by AM inoculation and P fertilization. There are differences among the species of mycorrhizal fungi in their ability to stimulate growth depending on the P level applied. However, the mycorrhizal effects were improved by the phosphate addition. In the absence of phosphate fertilization, no significative differences were found among AM fungi species, however, AM inoculation increased the DM yields about 58 to 127% in relation to control. In presence of P, plants inoculated with *G. margarita* or *S. heterogama* recorded the higher DM yields (Table 1). According to Abbott and Robson (1977) and Shockley et al. (2004) small amounts of P applied with AM inoculation give greater benefit to the plant than either inoculation or P application alone, because the P concentration in tropical soil may be so low that AM cannot develop extensively. Plants that did not receive mycorrhizal treatments showed a positive response (104%) to phosphate fertilization compared to the control.

Table 1. Effect of AM inoculation and phosphate fertilization on dry matter (DM) yields, P contents and uptake and AM colonization of *Codariocalyx gyroides* CIAT-3001.

Treatments	DM yield	Phosphorus		AM colonization
	(g/pot)	%	mg/pot	%
Control	3.54 d	0.098 e	3.47 e	--
S. heterogama (M_1)	8.04 bc	0.116 d	9.32 cd	52
G. margarita (M_2)	7.33 bc	0.152 bc	11.14 cd	48
A. muricata (M_3)	5.61 cd	0.145 c	8.13 d	59
M_1 + 22 mg P/kg	11.73 a	0.160 abc	18.77 a	56
M_2 + 22 mg P/kg	12.48 a	0.149 c	18.60 ab	53
M_3 + 22 mg P/kg	9.11 b	0.171 a	15.58 b	64
22 mg P/kg	7.23 bc	0.168 ab	12.14 c	--

- Means followed by the same letter in each column are not significantly differed at 5% probability by Duncan´s test

AM inoculation significantly improved (P=0.05) the P contents. In the presence of phosphate, maximum P concentrations occurred with the inoculation of *A. muricata* and *S. heterogama*, while without P addition the fungi more effectives were *G.* margarita and A*. muricata*. In relation to P uptake, the higher values were observed with the inoculation of *S. heterogama* and *G. margarita* in the presence of P addition, however there were no differences among mycorrhizal fungi without phosphate fertilization. P application slightly increased colonization rates (Table 1). The highest percentage was obtained with the inoculation of *S. heterogama* and *A. muricata*, irrespective of P level. Similarly, Manjunath and Bagyaraj (1984) and Costa et al. (1987) reported that mycorrhizal infection of pigeon pea and leucaena, respectively, was not inhibited by the application of 22 kg P/ha. Carneiro et al. (2011) showed that P fertilization and AM inoculation positively influence the forage production and cumulative quantities, especially CP, P, K, Ca, and Mg, of *Stylosanthes guianensis* cv. Mineirão. Inoculation with *Glomus clarum* in soil under natural microbial condition and low phosphorus supply, promotes increased legume participation in the pasture, however the AM inoculation inhibits the participation of legume at high levels of phosphorus.

Data on N contents and uptake and nodulation are presented in Table 2. AM inoculation had a significant (P=0.05) positive effect on the nodulation (number and weight of nodules) and N content. The level of response was greater with P fertilization. In relation to N content, the fungus more effective was *A. muricata*, independently of P level. The decline in N content for inoculated plants may due to dilution effect resulting from higher DM accumulation. In the absence of phosphate, plants inoculated with *S. heterogama* and *G. margarita* recorded higher N uptake, while with P addition no significative differences (P=0.05) were found among AM fungi species. Mycorrhizal inoculation significantly increased nodulation compared with the uninoculated plants, mainly in the presence of P addition. In general, AM fungi species showed a similar performance. Nodulation by *Rhizobium* depends on adequate mycorrhization or available P supply, therefore AM can have important effects on nodulation and N-fixation in forage legumes (Jalonen et al., 2013). According to Munns and Mosse (1980) the main effect of AM on nodulation and N uptake is undoubtedly P-mediated, it is also known to aid other processes involved in nodulation and N-fixation such as supply of photosynthates, trace elements or plant hormones.

Table 2. Effect of AM inoculation and phosphate fertilization on N contents and uptake and nodulation of *Codariocalyx gyroides* CIAT-3001.

Treatments	Nitrogen		Nodulation	
	%	mg/pot	number[1]	dry weight (mg/pot)
Control	2.48 cd	87.8 e	6.5 d	0.397 f
S. heterogama (M_1)	2.06 e	165.6 c	10.2 c	0.648 e
G. margarita (M_2)	2.34 de	171.5 cd	12.8 bc	0.755 de
A. muricata (M_3)	2.72 bc	152.6 d	16.0 b	0.819 de
M_1 + 22 mg P/kg	2.19 de	256.9 ab	22.4 a	1.136 bc
M_2 + 22 mg P/kg	2.22 de	277.0 a	20.7 a	1.278 a
M_3 + 22 mg P/kg	3.10 a	282.4 a	19.5 ab	1.161 ab
22 mg P/kg	2.87 ab	207.5 bc	13.7 bc	0.946 cd

- Means followed by the same letter in each column are not significantly differed at 5% probability by Duncan´s test

1 - Values analyzed after square root of (X + 1) transformation.

Conclusion

These data showed that mycorrhizal inoculation of *Codariocalyx gyroides* with *S. heterogama* or *G. margarita* when combined with a sufficiently low application of soluble phosphate had potential as an economic way to increase the productivity of pastures on P-deficient soils.

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