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Minimizing the effect of mineral nitrogen on biological nitrogen fixation in common bean by increasing nutrient levels

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Abstract

Although common bean (*Phaseolus vulgaris* L.) has good potential for N_2 fixation, some additional N provided through fertilizer usually is required for a maximum yield. In this study the suppressive effect of N on nodulation and N_2 fixation was evaluated in an unfertile soil under greenhouse conditions with different levels of soil fertility (low = no P, K and S additions; medium = 50, 63 and 10 mg kg^{-1} soil and high = 200, 256 and 40 mg kg^{-1} soil, respectively) and combined with 5, 15, 60 and 120 mg N kg^{-1} soil of ^{15}N-labelled urea. The overall average nodule number and weight increased under high fertility levels. At low N applications, nitrogen had a synergistic effect on N_2 fixation, by stimulating nodule formation, nitrogenase activity and plant growth. At high fertility and at the highest N rate (120 mg kg^{-1} soil), the stimulatory effect of N fertilizer on N_2 fixation was still observed, increasing the amounts of N_2 fixed from 88 up to 375 mg N plant^{-1}. These results indicate that a suitable balance of soil nutrients is essential to obtain high N_2 fixation rates and yield in common beans.

Introduction

Common bean is capable of fixing large amounts of atmospheric N_2 under certain conditions, but usually some mineral N is needed to achieve substantial yields under the prevailing cropping system in Latin America. However, nitrogen fertilization affects nodulation of bean plants and therefore the usually-recommended rates of 40–60 kg N ha^{-1} suppress N_2 fixation (Ruschel et al., 1979a; Graham, 1981). On the other hand, low rates have been shown to enhance nodule formation and function but are not sufficient to achieve maximum yields (Sistachs, 1970; Rosas and Bliss, 1987). While it is known that bean plants require high levels of P, K, S, Ca, Mg, Mo, Co and B (Bonetti et al., 1984; Franco and Day, 1980; Ruschel and Reuszer, 1973; Saito and Ruschel, 1978), production is restricted mostly to marginal soils where sufficient nutrients are not available. In soybean and tropical legumes, nodulation is especially sensitive to non-optimal levels of combined nutrients (Cassman et al., 1981; França et al., 1973; Gates and Wilson, 1974; Hernandez and Focht, 1985; Lynd and Ansman, 1989, 1990).

Gates and Muller (1979) observed that nodulation in soybean is affected by unbalanced nutritional conditions of N, P and S, and that N_2 fixation was not affected by mineral N only in presence of high levels of P and S. Lynd and Ansman (1989) found decreases in nodule number of peanut when K was applied alone, but not when P and Ca were added. A stimulatory effect of the high rate of 60 mg kg^{-1} of NH_4NO_3 on nodulation also has been reported in presence of P (Gates and Wilson, 1974).

This study was performed to determine whether applied nitrogen affects nodulation and plant biomass under limited and balanced soil nutrient situations. In addition, the amounts of N_2 fixed with increasing soil fertility were compared to determine if a nutritional balance for the plant growth could alleviate the detrimental effect of N fertilizer on the biological process.

Material and methods

Bean plants (*Phaseolus vulgaris* L.) were grown in a greenhouse in pots of 3 kg of an acidic dark oxissol collected from a 'cerrado' (savanah) region of Sao Paulo State (22°42'30" S latitude and 47°38'00" W longitude), Brazil. Seeds of cv. Carioca were provided by Dr. Eduardo Bulisani, Instituto Agronomico de Campinas – S. Paulo. Prior to sowing, seeds were surface sterilized with 0.5% HOCl for 5 min, rinsed several times with distilled water and inoculated with 0.10 mL (approximately 10^7 cells $seed^{-1}$) of a 3-day-old yeast mannitol broth culture of the *Rhizobium tropici* strain UMR-1899 (originally from Dr. P.H. Graham, University of Minnesota-MN, U.S.A.), known to be effective with common bean in tropical conditions (Vargas and Graham, 1989, Martinez-Romero *et al.*, 1991). Plants were thinned to two per pot at about 7 days after emergence (DAE).

Experimental pots were fertilized according to the usual agricultural recommendations to give a low, medium and a high level of phosphorus, potassium and sulphur (Table 1). ^{15}N-labeled urea (4.763 atom % ^{15}N) was added at planting at the rates of 15 and 60 mg N kg^{-1} soil, according to the low, medium and high N-rate treatments. An additional supply of 45 and 60 mg N kg^{-1} soil was added 3 weeks after germination for the treatments of 60 and 120 mg N kg^{-1} soil.

Chemical analysis of the soil gave the following values: pH ($CaCl_2$) – 5.5; O.M. – 3%; P – 10 $\mu g\ g^{-1}$ soil (resin); K – 0.18, Ca – 2.11, Mg – 0.94, H + Al – 1.34 meq 100 cm^{-3}, respectively. The soil was air dried and sieved (2 mm) and pH corrected to 6.0 with addition of 1.0 g of calcium carbonate per pot.

A randomized complete block design with four replicates was used at each sampling time (42 DAE and 90 DAE). The first sampling determined nodulation parameters – number, mass and acetylene reduction activity (ARA) – of the plants at the R_3 growth stage (42 DAE). For acetylene reduction activity (ARA) determinations gas samples were injected into a GC-65 Beckman gas chromatograph, with an H_2 flame ionization detector and N_2 as flow gas. At the second sampling at 90 DAE plant biomass (shoot plus pods) and total N, using a Perkin-Elmer autoanalyser were evaluated. ^{15}N enrichment of plant materials was determined for the two samplings, after Dumas combustion (Proksch, 1969) in an Atlas-Varian model CH-4 mass spectrometer. ^{15}N calculations were done as shown by Danso et al. (1983).

Table 1. Amounts of P, K and S added to the soil to obtain low, medium and high fertility levels and N-fertilizer rates used in this study

Fertility level	N*	P	K	S
	(mg kg^{-1} soil)			
Low	5	–	–	–
Low	15	–	–	–
Low	60	–	–	–
Low	120	–	–	–
Medium	5	50	63	10
Medium	15	50	63	10
Medium	60	50	63	10
Medium	120	50	63	10
High	5	200	256	40
High	15	200	256	40
High	60	200	256	40
High	120	200	256	40

* The application of 60 and 120 mg N kg^{-1} soil was split, at sowing and 3 weeks after germination, corresponding to 15 + 45 and 60 + 60 mg N kg^{-1} soil, respectively.

Results

Nodulation and plant growth

At 42 DAE, significant increases of nodule number, mass and acetylene reduction values were obtained by increasing soil fertility (Table 2). Nodule numbers increased significantly with increasing fertility from 66 (low fertility) to 168 (medium), and to 222 (high) nodules $plant^{-1}$.

Table 2. Nodule number, mass and acetylene reduction activity (ARA) of bean plants (42 DAE) grown under increasing soil fertility

	Fertility level		
	Low	Medium	High
Number (No. of plant^{-1})	66 C	168 B	222 A
Mass (mg plant^{-1})	39 B	111 A	108 A
ARA (μMol C_2H_4 pl^{-1} h^{-1})	6.4 B	14.5 A	17.5 A

* Capital letters may be used for comparisons among soil treatments.
Means followed the same letters do not differ statistically by Duncan's Multiple Range Test ($p < 0.05$).
Low = no P, K and S additions.
Medium = 50, 63 and 10 mg kg^{-1} soil of P, K and S
High = 200, 256 and 40 mg kg^{-1} soil of P, K and S

Although nodule mass nearly doubled from low – (39 mg plant^{-1}) to medium fertility (111 mg plant^{-1}), this trait as well as ARA was influenced less by additions of higher amounts of minerals. Nitrogenase activity was also increased by the P, K and S additions at 42 DAE, indicating that nodule function also depends on the nutritional status of the soil in order to obtain a substantial amount of fixed N_2.

Nitrogen showed a dual effect on nodulation (Fig. 1A, B, C and D). At low fertility, the addition of 60 mg N kg^{-1} soil was detrimental to nearly all nodulation traits (nodule number, mass and ARA, and to a less extent nodule size). However, this effect was less as the concentration of P, K and S in the soil was increased. Nodule number increased significantly from 41 (low) to 151 (medium) and 310 (high) nodules per plant. All nodulation parameters were affected greatly by the highest rate of N fertilizer (120 mg kg^{-1} soil). The synergistic effect of 15 mg N kg^{-1} soil observed at low fertility was also observed with higher N rates (60 mg N kg^{-1} soil) under higher soil fertility, giving the highest values for all nodulation parameters.

The high ARA rates obtained under the high fertility condition were comparable to ARA of soybean grown under similar conditions (Ruschel et al., 1979b). This was observed also in soybean by Gates and Muller (1979) where no detrimen-

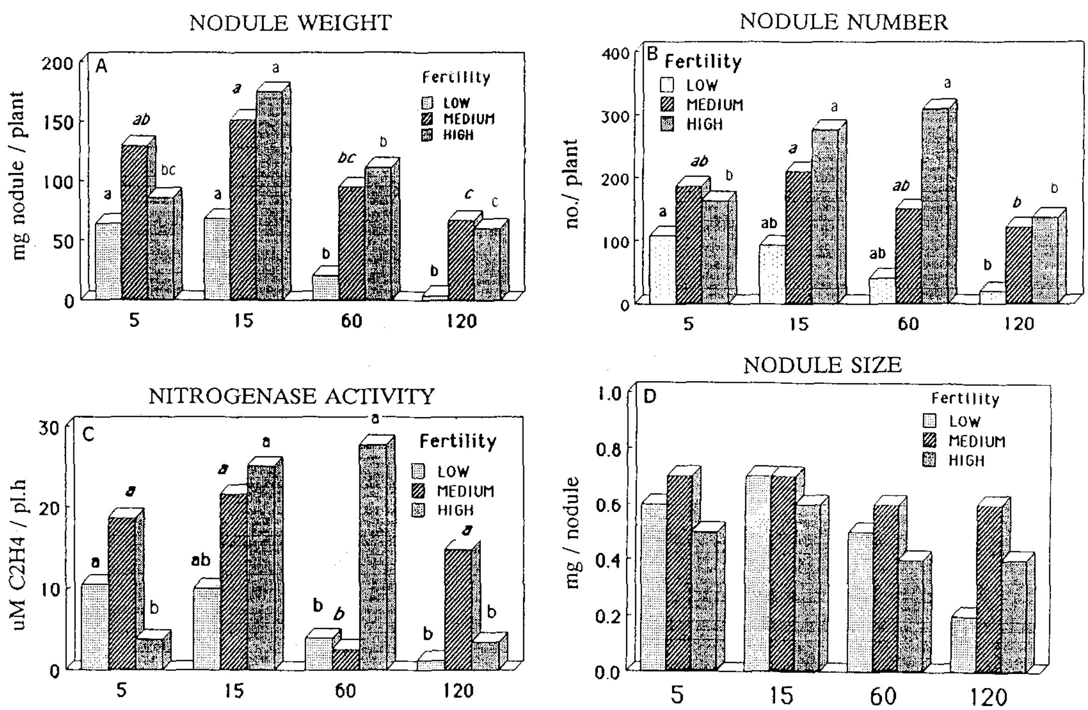


Fig. 1. Nodulation traits evaluated in N treatments (5, 15, 60 and 120 mg N kg^{-1}) of bean plants influenced by P, K and S additions: a) nodule weight (mg nodule/plant), b) nodule number (per plant), c) nitrogenase activity of nodules (μMol C_2H_4 plant^{-1} h^{-}) and d) average nodule size (mg nodule^{-1}). Comparisons are made only among N treatments from each soil.

tal effect of N fertilizer as high as 120 mg kg^{-1} was observed when high rates of P and S were used. Although not statistically different, high averages in nodule size (Fig. 1D) were obtained at low N and at high fertility. It appears that bean can still develop nodules at relatively high N concentrations when grown in fertile soil. Gates and Wilson (1974) have shown that 60 ppm N were less suppressive to nodulation in *Stylosanthes humilis* in the presence of medium and high rates of P fertilizer.

At 90 DAE, increases in dry matter and N content due to P, K and S additions were more consistent than those due to N application (Table 3). As P, K and S increased, dry matter increased from 6.1 g $plant^{-1}$ to 7.7 g $plant^{-1}$ (medium fertility) and to 10.7 g $plant^{-1}$ (high fertility). Total N followed the same pattern as dry matter, doubling the values with P, K and S additions (258.8 mg N $plant^{-1}$ in contrast to 127.3 mg N $plant^{-1}$ of the control treatment).

N_2-fixation and fertilizer use

The proportion of N derived from air (%Ndfa) was highly correlated to nodulation and total N (Fig. 2). The average proportion of N_2 fixed was 51.9% at low fertility and increased significantly to 66% and 64.7% ($p < 0.05$) at medium or high fertility, respectively. Although not statistically significant, overall highest N_2 fixation rates were observed with low inputs of N fertilizer (up to 15 mg N kg^{-1} soil) at medium soil fertility conditions.

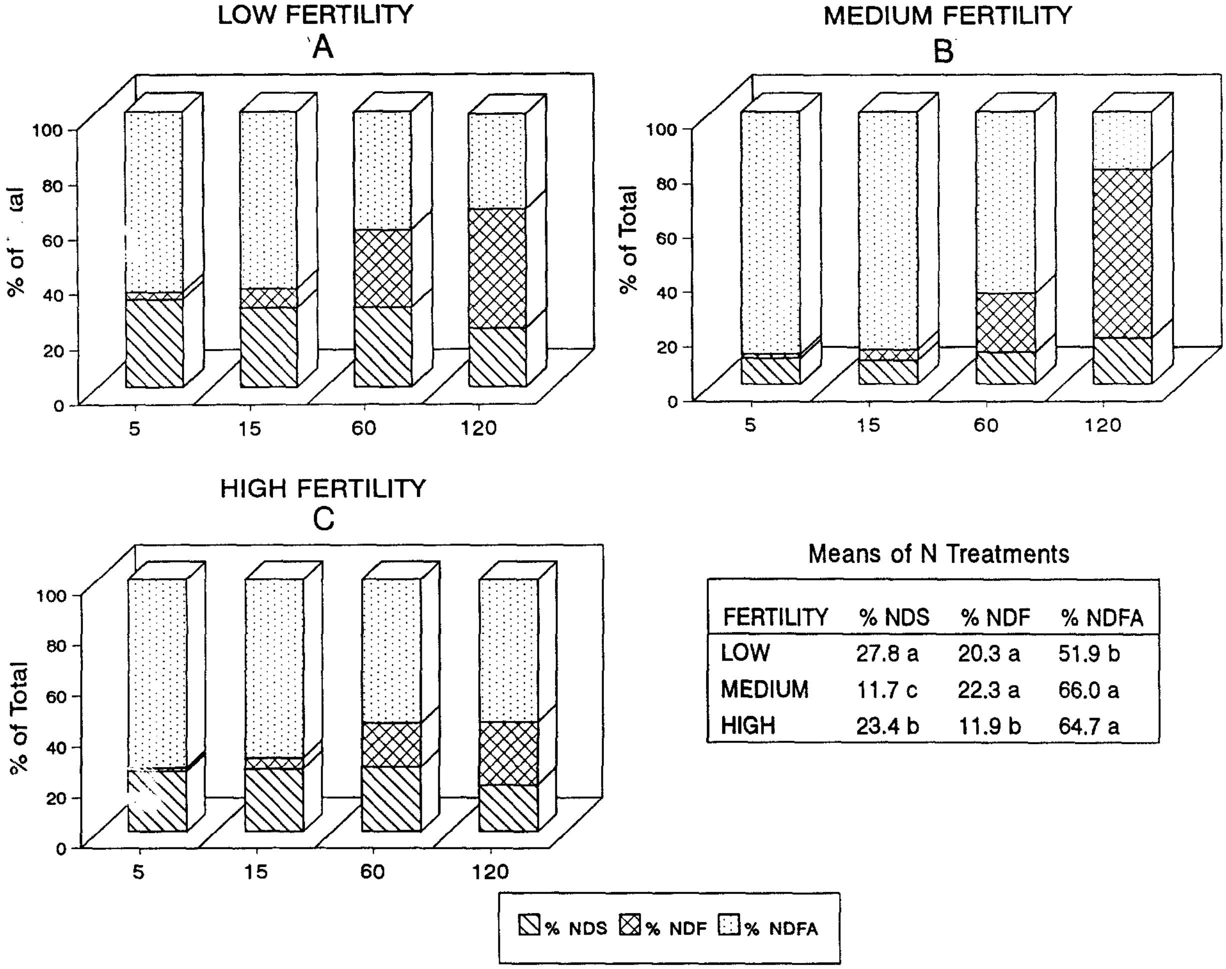
The uptake of N fertilizer by common bean was moderate at low fertility levels (Fig. 2), and was increased only with additions of 120 mg N kg^{-1} soil at higher soil fertility levels. The low response to N fertilizer indicates that soil factors other than nitrogen could limit bean plant growth and productivity in marginal soils. Soil nitrogen was also more available to the plant when P, K and S were present in high amounts.

In general, N fertilizer use averaged 35–50%, reaching the highest values of around 65% at an intermediate fertility (Table 4). The depressive effect of high levels of N on nodulation was alleviated at high fertility levels, as shown by the N_2 fixed values (Table 4). An estimation of the total N_2 fixed gave values corresponding to 312 and 375 mg N $plant^{-1}$, in treatments when 60 and 120 mg N kg^{-1} soil were added, in contrast to 128 and 88 mg N $plant^{-1}$ under low, and 187

Table 3. Dry matter and N content of bean plants at 42 DAE and 90 DAE grown under increasing levels of P, K and S and N-urea

N rate*	42 DAE			90 DAE		
	Low	Medium	High	Low	Medium	High
	Dry matter (g $plant^{-1}$)					
5	1.9	3.5	3.5	6.2	8.1	11.6
15	1.9	3.9	4.2	5.6	8.1	8.7
60	2.8	3.9	4.5	5.9	7.0	10.4
120	2.3	5.1	4.9	6.7	7.4	12.3
Mean	2.2 B	4.1 A	4.3 A	6.1 C	7.7 B	10.7 A
	Total nitrogen (mg N $plant^{-1}$)					
5	46.4	96.8	92.6	92.9	145.1	264.2
15	47.4	107.9	109.4	143.0	193.4	173.7
60	72.9	101.5	112.0	145.5	139.7	268.0
120	79.0	128.7	116.7	127.7	195.9	328.1
Mean	61.4 B	108.7 A	107.7 A	127.3 C	164.4 B	258.8 A

* mg N kg^{-1} soil
Low = no P, K and S additions.
Medium = 50, 63 and 10 mg kg^{-1} soil, respectively.
High = 200, 256 and 40 mg kg^{-1} soil, respectively.
Means followed by the same letters within each sampling time do not differ significantly by Duncan's Multiple Range Test ($p < 0.05$).



Means of N Treatments

FERTILITY	% NDS	% NDF	% NDFA
LOW	27.8 a	20.3 a	51.9 b
MEDIUM	11.7 c	22.3 a	66.0 a
HIGH	23.4 b	11.9 b	64.7 a

Fig. 2. Proportion (% of total) of N from soil, fertilizer and N_2 fixation in bean plants at 90 DAE, as function of N fertilizer additions (5, 15, 60 and 120 mg N kg^{-1}) and grown under three soil fertility levels. Mean data of N treatments for the soils are shown on the table inserted in the Figure.

and 87 mg N $plant^{-1}$ under medium fertility conditions (Table 5).

Discussion

Historically, selection in bean breeding programmes in Latin America has been for high yielding cultivars, that are responsive to N fertilizer. Nitrogen application presents a problem for N_2 fixation in common bean, since the recommended amounts of N fertilizer are capable of suppressing nodulation and nodule functioning in this crop (Graham, 1981; Ruschel and Saito, 1977; Silva et al., 1992). However, low amounts of mineral N can stimulate bean nodulation under certain soil conditions (Ruschel et al., 1979a; Vernichenko, 1984). Therefore, the ability of bean to fix enough N_2 for plant growth is a problem of great interest under Brazilian conditions.

Our data show that N_2 fixation can be expressed in a commercial bean cultivar, if inputs of N fertilizer required to increase plant growth are minimal and P, K and S are adequately supplied. By adding P, K and S and a low amount of N fertilizer (urea) substantial increases in nodulation and consequently high amounts of N_2 fixed were obtained with corresponding increases in the plant biomass. Several

Table 4. Effect of different levels of N, P, K, and S on percentage and amount of nitrogen derived from fertilizer (% NDFF and ANDFF, respectively), and efficiency of fertilizer utilization (EFU) by bean plants 90 days after germination

Fertility level*	% NDFF	ANDFF	% EFU
Low	20,3 A	54,1 B	38,0 A
5	2,6 c	4,1 c	32,0 a
15	7,0 c	20,0 c	44,1 a
60	28,3 b	80,9 b	44,9 a
120	43,4 a	111,0 a	30,8 a
Medium	22,3 A	80, A	41,0 A
5	1,5 c	4,4 c	29,2 b
15	4,0 b	15,7 c	34,8 b
60	21,6 b	60,0 b	33,4 b
120	62,2 a	240,1 a	66,7 a
High	11,9 B	67,9 AB	43,1 A
5	1,3 c	7,0 c	46,7 ab
15	4,2 c	14,3 c	31,8 b
60	17,1 b	90,1 b	50,0 a
120	24,8 a	160,2 a	44,5 ab
CV%	20	30	24

Means followed by the same letters do not differ statistically by Duncan Test ($p < 0.05$).
* Low = No P, K, and S addition.
Medium = 50, 63, and 10 mg kg^{-1} of P, K, and S, respectively.
High = 200, 256, and 40 mg kg^{-1} of P, K, and S, respectively.
N: 5, 15, 60 and 120 mg N kg^{-1}.

authors have noted the sensitivity of N_2 fixation to factors such as N fertilizer but others have cited that P and/or S may counteract this detrimental effect (Gates and Wilson, 1974; Gates and Muller, 1979). Under low P levels, nitrogen also can limit the expression of this trait in bean (Pereira and Bliss, 1987, 1989), and there are a few examples of low P tolerance among bean cultivars (Graham and Rosas, 1979). From the few reports of the effect of potassium on N_2 fixation, Thomas and Hungria (1988) have found that this element may contribute to the transport of fixed N_2 from nodules to reproductive organs of beans. P and K together may delay senescence of the soybean nodule, by increasing nodule number and nitrogenase activity (Kungl, 1987). These authors also found synergistic effects of mineral N on nodulation at rates up to 40 mg N kg^{-1} soil. Sulphur also is required to some extent in acidic soils, but can be supplemented partially as a companion ion from commercial P sources.

The high ARA values in this study obtained under a balanced nutritional situation indicate the potential of beans to express N_2 fixing ability in marginal soils, once adequate nutrients are available. Starter nitrogen should be included also in order to assure a high yield. The overall low values obtained for the N fertilizer use (%FUE) when no P, K and S were added, indicate that an additional factor may be limiting N uptake in bean plants under sub-optimum soil conditions. In contrast, as the nutrient level increased all three nitrogen sources became more available to the plant (Fig. 2). These findings indicate that when there are limitations such as P, K or S on the supply of nutrients, exogenous nitrogen can be deleterious to the biological system. When the required nutrients are available to the plant, it is able to utilize the available N sources. Altering soil fertility by adding balanced amounts of minerals or organic sources of P, K and S could enhance the efficiency of N_2 fixation in common beans as well as its ability for utilizing other sources of N from soil and fertilizer.

Table 5. Effect of different levels of N, P, K, and S on the percentage and amount of nitrogen derived from air (% NDFA and ANDFA, respectively) and from soil (% NDFS and ANDFA respectively) by bean plants 90 days after germination

Fertility level	% NDFA	ANDFA (mg)	% NDFS	ANDFS (mg)
Low	51,9 B	131,0 C	27,8 A	69,4 B
5	65,7 a	122,4 ab	31,7 a	58,5 ab
15	64,3 a	185,5 a	28,6 a	80,7 ab
60	42,9 b	128,3 ab	28,7 a	82,0 a
120	34,5 b	88,0 b	21,1 b	56,4 b
Medium	66,0 A	217,2 B	11,7 C	39,8 C
5	88,9 a	257,1 ab	9,5 b	28,4 b
15	87,3 a	337,4 a	8,7 b	33,8 b
60	66,8 b	187,1 b	11,6 ab	32,4 b
120	21,1 c	86,9 c	16,8 a	64,8 a
High	64,7 A	332,1 A	23,4 B	117,5 A
5	74,6 a	394,6 a	24,0 ab	126,8 a
15	70,7 a	246,7 b	25,1 a	86,5 b
60	57,0 b	312,1 ab	25,8 a	135,9 a
120	56,5 b	375,2 a	18,7 b	120,8 a
CV%	11	26	18	21

Means followed by the same letters do not differ statistically by Duncan Test ($p < 0.05$).
* Low = No P, K, and S addition.
Medium = 50, 63, and 10 mg kg^{-1} of P, K, and S, respectively.
High = 200, 256, and 40 mg kg^{-1} of P, K, and S, respectively.
N: 5, 15, 60 and 120 mg N kg^{-1}.

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