

## NODULATION AND GROWTH OF SOYBEAN PLANTS AS A FUNCTION OF COATING THE SEEDS WITH PHOSPHOROUS

### *NODULAÇÃO E CRESCIMENTO DE PLANTAS DE SOJA EM FUNÇÃO DO RECOBRIMENTO DAS SEMENTES COM FÓSFORO*

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**ABSTRACT:** The increase of phosphorous concentration in soybean seed enhances the subsequent potential plant yield. The objective was to evaluate the effect of coating the seeds with phosphorous on the nodulation and growth of soybean plants. Seeds from two lots of cultivar Pioneer – P98R31 were treated with monobasic phosphate sodium in doses of 0.0; 0.2; 0.4; 0.8 and 1.2 g hg<sup>-1</sup> of seeds and, with the inoculant ‘turfo Microxisto’ using 3.0 millions cells of bacteria (*Bradyrhizobium japonicum*) by seed. The experiment was carried out in a totally randomized block design with five replications in a factorial scheme of 2x2x5+4, consisting of seeds with different phosphorus content, two levels of phosphorus fertilizer in the soil, five levels of P used in the coating of seeds, and four controls. After treatments, the seeds were sowed in pots with 3.0 dm<sup>3</sup> of red yellow Latosol, which received P doses of 200 and 400 mg kg<sup>-1</sup> having triple superphosphate as the source. In the stage R2 of plant development, the P and N concentration in the third trifoliolate was evaluated. In the initial stage of strings formation (R3), the plant height, whole shoot, root and the dry matter of viable nodules were evaluated. Independently of the endogenous P concentration in the seed, coating soybean seeds with P did not increase nodulation and growth of plants in soil with higher high P availability, whereas in soil with less P available, the coating of seeds increased the nodulation and growth of soybean plants.

**KEYWORDS:** *Glycine max.* Soil. Nodule. Nitrogen.

## INTRODUCTION

Phosphorus has several functions in plants as a structural element, being vital for the energy metabolism of cells as an intermediate in metabolic pathways (SENGIK, 2003). The absence of phosphorus is more detrimental to the productivity of soybean plants than any other macronutrient, resulting in losses of up to 94% if phosphorus is not available to the plants in adequate amounts. This occurs because the phosphorus deficiency has a negative impact on the biological fixation of nitrogen, which is a process that requires energy (ALMEIDA et al., 2000).

One of the problems with phosphorus is retention and its availability in soil (NOVAIS et al., 2007), which reduces the availability of this nutrient for the plants and can compromise the productivity. Localized application of phosphate fertilizers in soybean crop has many advantages (LOPES, 2001): to be the initial phosphorous source for seedling development; less contact between soil and phosphorus, associated to the faster use by the seedling, resulting in low phosphorus retention to soil colloids; and placing the phosphorus in an available position for the small root system of the early seedling development. Zelonka et al. (2005) concluded that coating seeds of barley with

phosphorus increased the grain production of the subsequent plant, from 3% to 91%. Trigo et al. (1997) found that increasing the concentration of phosphorus in soybean seeds, from 0.58% to 1.10% resulted in a yield increase of 37% in soil with phosphorus fertilization, and of 20% in soil without phosphorus fertilization. Araújo et al. (2002) found that bean seeds with high phosphorus content can result in plants with increased shoot growth, nodulation and nitrogen accumulation, particularly under low levels of phosphorus in the soil.

This work aimed to study the effect of phosphorus, via coating the seeds in the nodulation and growth of soybean plants.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse of the Fitotecnia Department of the Universidade Federal de Viçosa, in Viçosa – MG, on the second half of 2010. Two soybean seeds lots of the cultivar Pioneer – P98R31 were used, which were harvested in 2009/2010 by Pioneer Sementes – Planaltina – DF. Those lots had seeds with phosphorus endogenous concentrations of 4.41 g kg<sup>-1</sup> (S1) and 5.38 g kg<sup>-1</sup> (S2).

The seeds of each lot were submitted to the P coating, using monobasic sodium phosphate as the

P source. The seeds were treated in this sequence: Derosal Plus fungicide (carbendazim + thiram) at a dose of 200 mL 100 kg<sup>-1</sup> of seeds; monobasic sodium phosphate (0.0, 0.2, 0.4, 0.6, 0.8 and 1.2 g hg<sup>-1</sup> of seeds) diluted in 2 mL of deionized water and applied to the seeds; peat inoculant in a dose of 3.0 million cells per seed of *Bradyrhizobium japonicum* bacterium. After treatment, the seeds were sown in plastic pots containing 3.0 dm<sup>3</sup> of red yellow Latosol, which were fertilized with two P levels, having triple superphosphate as source (200 and 400 mg kg<sup>-1</sup> of soil), providing two levels of P availability, lower (P1) and higher (P2). The fertilization with other nutrients was performed according to Novais et al. (1991).

Four seeds were sown in each pot at a depth of 3 cm. After emergence, two seedlings were kept per pot. Irrigation was performed with deionized water to maintain the water content of the soil within 2/3 of field capacity.

The experiment was conducted in a randomized block design with five replications in a factorial 2x2x5+4, which refers to seeds with two different phosphorus contents at two levels of phosphorus fertilizer in the soil and five levels of P used in the coating of seeds, and four controls, which resulted in 24 treatments. The controls are T1, T2, T3 and T4, which refer to S2 seeds (high phosphorus content) sown in P2 soil (highest P dose); S1 seeds (lower P content) sown in P2 soil, S2 seeds sown in P1 soil (low dose of phosphorus) and S1 seeds sown in P1 soil, respectively. The witnesses were not coated with phosphorus and inoculant.

During the R2 stage of plant development, i.e. plentiful flowering, the third trifoliolate leaves of the plants were harvested, the samples were washed in deionized water, dried in oven at 60 °C until constant mass, and ground. Nitrogen concentration was determined by Kjeldahl's method after digestion in sulfur acid, the concentration of phosphorus via nitric-perchloric digestion and the quantification by spectrophotometry (MALAVOLTA et al., 1997).

During the R3 stage, which corresponds to the stage of early pod formation, the plants were harvested, placed in paper bags, kept at 65 °C for 72 hours in an oven with forced air and weighed. The nodules were removed from the root system, placed in paper bags and taken to the oven with forced air circulation, kept at 65 °C for 72 hours, and weighed. The plant height was measured with the aid of a ruler.

Data were subjected to variance analysis and the averages obtained for the different

phosphorus levels in the seed and in the soil, both with only two factors, were compared using the F test ( $p < 0.05$ ). Data were subjected to regression analysis considering the P levels in the coating of the seeds as the independent variable. The analysis was performed using the SAS statistical program (SAS INSTITUTE, 2002).

## RESULTS AND DISCUSSION

The interaction between the content of phosphorus in seeds (S1 and S2) and the doses of phosphorus in soil (P1 and P2) was not significant for the SDM, RDM, PL, NDM and NC (Table 1), at a dose of 0.8 g hg<sup>-1</sup> of monobasic sodium phosphate used in the seed coating. There was no difference between the characteristics evaluated in S1 and S2 seeds by the F test at 5% of probability. However, the characteristics evaluated between P1 and P2 soils, showed a significant difference between P levels in soil, with P2 soil showing that the SDM, RDM, PL, PC, NDM and NC were 131.90, 66.09, 38.11, 71.63, 189.6 and 50.69% higher than in the P1 soil, respectively.

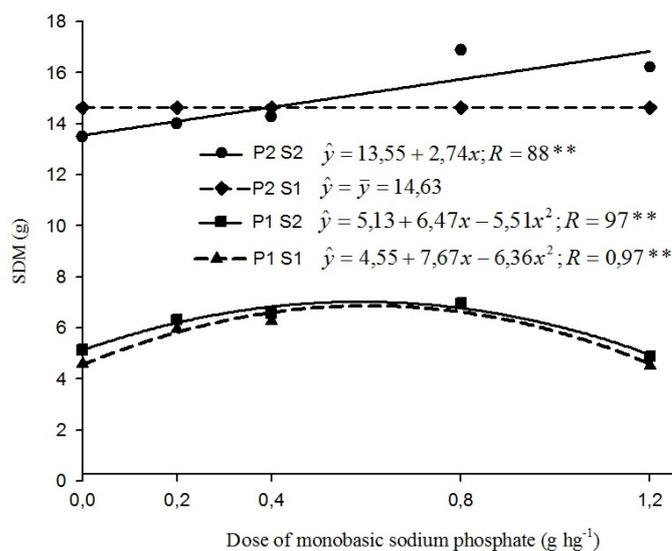
The results presented in Table 1 show that the dose of 0.8 g hg<sup>-1</sup> of monobasic sodium phosphate in coating seeds gives the best results with respect to increased nodulation and growth of soybean plants. The same results were found by Salum et al. (2008), which studying the effect of phosphorus content in bean seeds and the phosphorus content of the soil in chemical and physiological characteristics, did not find a significant difference between seed lots with different levels of phosphorus. Those results differ of several other studies (TRIGO et al., 1997; TEIXEIRA, 1995; TEIXEIRA et al. 1999), which can be explained by the small difference between the P contents of the lots used to create this experiment, since in other studies this difference was more expressive.

There was a linear increase in the shoot dry matter (SDM) of the plants in response to the P dose in the soil from seeds with higher endogenous phosphorus, subjected to phosphorus coating and sown in soil with high P level (P2) (Figure 1). As for the seeds with lower levels of endogenous P (S1) there was no effect of the levels used in the seed coating. At P1 soil level, there was a quadratic response for the two lots. For S2 seeds the maximum SDM (7.01 g) was obtained with the dose of 0.58 g hg<sup>-1</sup>, and for S1 seeds, the maximum SDM (6.86 g) was obtained with the dose of 0.61 g hg<sup>-1</sup> (Figure 1).

**Table 1.** Shoot dry matter (SDM), root dry matter (RDM), plant length (PL), phosphorus content in the third trifoliolate (PC), viable nodules dry matter (NDM) and nitrogen content in the third trifoliolate leaf of plant (NC), as a function of the 0.8 g hg<sup>-1</sup> dose of monobasic sodium phosphate on seeds coating for lots with higher (S2) and lower (S1) endogenous phosphorus content in soils with more (P2) and less (P1) phosphate fertilizer.

Endogenous P content in the seed	Dose of P in the soil		
	P1	P2	Mean
<b>Shoot dry matter (g)</b>			
S1	6.88	15.26	11.07 a
S2	6.98	16.89	11.93 a
Mean	6.93 B	16.07 A	
<b>Root dry matter (g)</b>			
S1	3.54	5.92	4.73 a
S2	3.62	5.98	4.80 a
Mean	3.58 B	5.95 A	
<b>Plant height (cm)</b>			
S1	50.40	69.80	60.10 a
S2	51.40	70.80	61.10 a
Mean	50.90 B	70.30 A	
<b>PC (dag kg<sup>-1</sup>)</b>			
S1	0.094	0.160	0.127 a
S2	0.092	0.160	0.127 a
Mean	0.093 B	0.160 A	
<b>NDM (g)</b>			
S1	0.241	0.716	0.478 a
S2	0.293	0.806	0.549 a
Mean	0.267 B	0.7609 A	
<b>NC (dag kg<sup>-1</sup>)</b>			
S1	2.583	3.988	3.285 a
S2	2.627	3.863	3.245 a
Mean	2.605 B	3.925 A	

S1 = 0,441 dag kg<sup>-1</sup> of P in the seed; S2 = 0,538 dag kg<sup>-1</sup> of P in the seed; P1 = 200 mg kg<sup>-1</sup> of P in the soil; P2 = 400 mg kg<sup>-1</sup> of P in the soil; Means followed by the same capital letter on the line and small letter on the column do not differ by the F test ( $P < 0,05$ ).

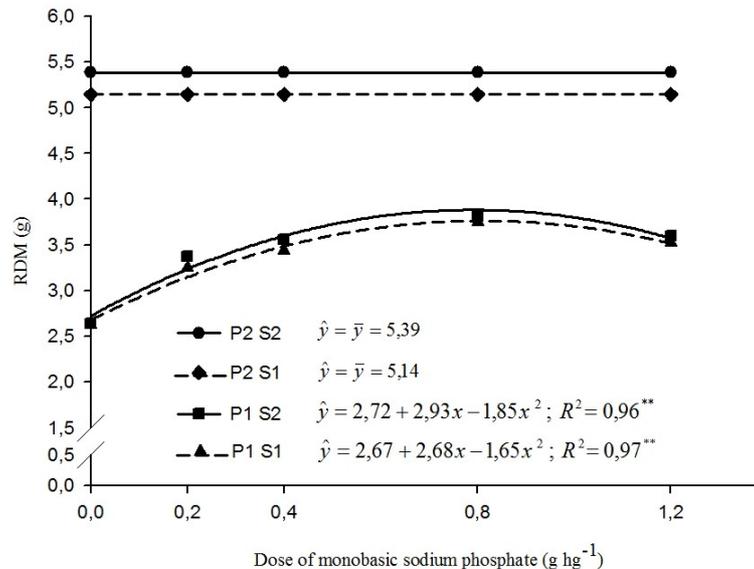


**Figure 1.** Shoot dry matter (SDM) per plant under monobasic sodium phosphate doses on seed lots with higher (S2) and lower (S1) endogenous phosphorus content for soils with more (P2) and less (P1) phosphate fertilizer.

According to MEIS et al. (2003), the increase of endogenous P in the seeds was responsible for the significant increase in shoot dry matter in soybean plants at 21 days after emergence, due to increased nutrient translocation to the shoot, which contributed for the improved biomass.

There was no effect of P levels in seed coating on the root dry matter (RDM) for two lots of seeds in P2 soil. In P1 soil there was a quadratic effect of the phosphorus applied in the coating for

the two lots, with maximum RDM (3.88 g) obtained with the dose of the  $0.79 \text{ g hg}^{-1}$  for S2 seeds and maximum RDM (3.76 g) obtained with the dose of  $0.81 \text{ g hg}^{-1}$  for S1 seeds (Figure 2). According to DREW et al. (1978), phosphorus promotes rapid formation and growth of the root system. Furthermore, the coating of the seeds provides a phosphorus availability to the root system in the early seedling development (LOPES, 2001).



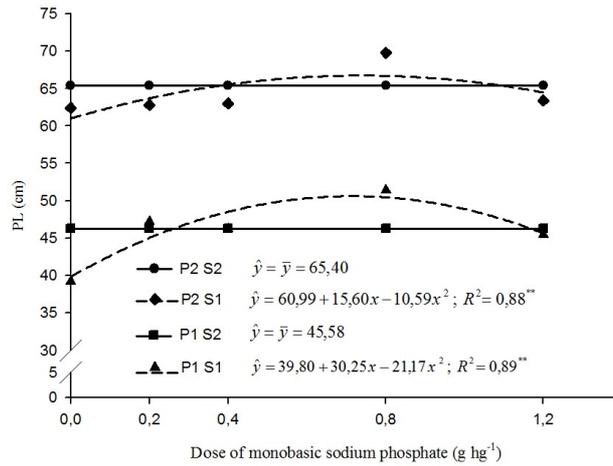
**Figure 2.** Root dry matter (RDM) per plant under monobasic sodium phosphate doses on seed lots with higher (S2) and lower (S1) endogenous phosphorus content for soils with more (P2) and less (P1) phosphate fertilizer.

The P levels effect in the plant height (PL) in P2 soil and in P1 soil was significant only for S1 seeds; The maximum PL was obtained with the dose of  $0.74 \text{ g hg}^{-1}$  in P2 soil (66.73 cm) and with a dose of  $0.71 \text{ g hg}^{-1}$  in P1 soil (50.61 cm) (Figure 3). TRIGO et al. (1997) found similar results.

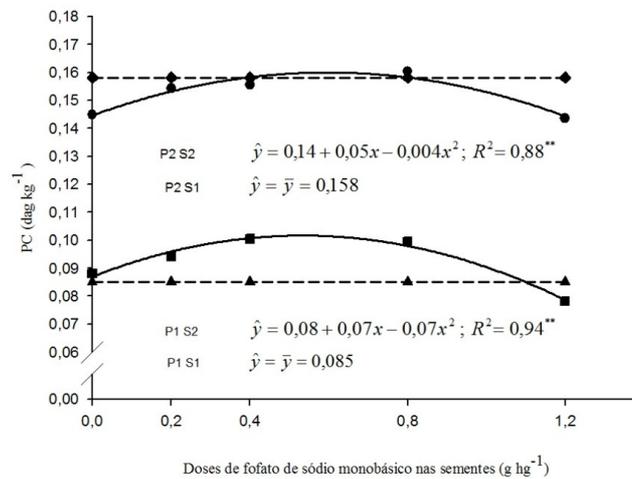
The effect of the phosphorus doses used in coating on the phosphorus concentration in the third trifoliolate leaf of the plant (PC) was significant only for S2 seeds in P2 and P1 soil. The maximum PC ( $0.16 \text{ m}^2 \text{ kg}^{-1}$ ) was obtained with the dose of  $0.59 \text{ g hg}^{-1}$  for P2 soil and for P1 soil, the maximum PC ( $0.10 \text{ m}^2 \text{ kg}^{-1}$ ) was obtained with the dose of  $0.55 \text{ g hg}^{-1}$  (Figure 4). Increased levels of P in soil and seeds resulted in high P availability and provided increments in the amount of P in shoots, a fact noted in several studies (SILVA et al., 2002).

Data of viable nodules dry matter (NDM) show that there are effects of P doses used in the coating of S1 seeds in P2 soil, where the maximum NDM (0.69 g) was obtained with a dose of  $0.58 \text{ g hg}^{-1}$ . For the P1 soil, the dose effect was significant

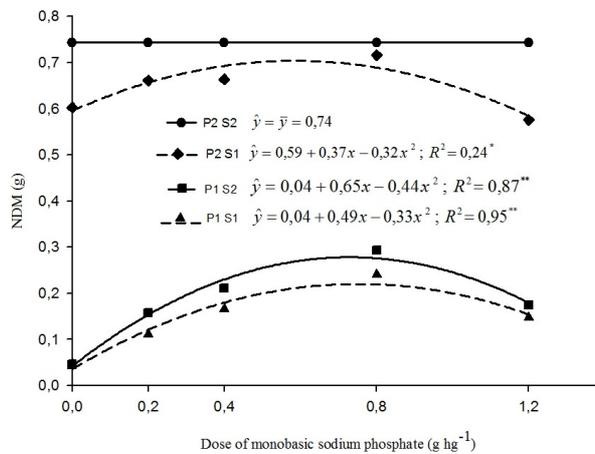
for S2 seeds that obtained maximum NDM (0.27 g) with the dose of  $0.72 \text{ g hg}^{-1}$ , and for S1 seeds that obtained maximum NDM (0.22 g) with the dose of  $0.75 \text{ g hg}^{-1}$  (Figure 5). According to Teixeira et al. (1999), bean plants from seeds with high endogenous P produced the highest number and dry matter from nodules and were less responsive to the P supply in soil when compared with plants grown from seeds with low P levels. This fact can be explained due the greater increase of P in seeds promoting a faster and greater root development, resulting in increased nodulation (TEIXEIRA, 2002; ARAÚJO et al. 2002).



**Figure 3.** Plant length (PL) per plant under monobasic sodium phosphate doses on seed lots with higher (S2) and lower (S1) endogenous phosphorus content for soils with more (P2) and less (P1) phosphate fertilizer.



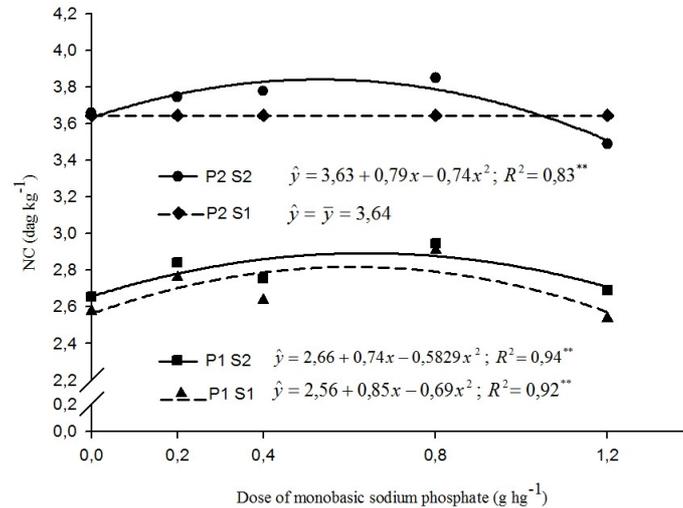
**Figure 4.** Phosphorus content in the third trifoliate leaf of the plant (PC) per plant under monobasic sodium phosphate doses on seed lots with higher (S2) and lower (S1) endogenous phosphorus content for soils with more (P2) and less (P1) phosphate fertilizer.



**Figure 5.** Viable nodules dry matter (NDM) per plant under monobasic sodium phosphate doses on seed lots with higher (S2) and lower (S1) endogenous phosphorus content for soils with more (P2) and less (P1) phosphate fertilizer.

The data of nitrogen concentration in the third trifoliolate leaf (NC) show that there was a significant effect of coating the seeds with phosphorus in P2 soil only for S2 seeds. The maximum NC ( $3.84 \text{ dag kg}^{-1}$ ) was obtained with the dose of  $0.53 \text{ g hg}^{-1}$ . For the P1 soil, there was an effect of coating S2 and S1 seeds, in which the

maximum NC ( $2.89$  and  $2.81 \text{ dag kg}^{-1}$ ) was obtained with the dose of  $0.63$  and  $0.60 \text{ g hg}^{-1}$ , respectively (Figure 6). According to ARAÚJO et al. (2002), bean seeds with higher P content can become plants with increased shoot growth, nodulation and N accumulation during the vegetative stage of growth, particularly at low doses of P in the soil.



**Figure 6.** Nitrogen content in the third trifoliolate leaf of plant (NC) per plant as a function of monobasic sodium phosphate doses on seed lots with higher (S2) and lower (S1) endogenous phosphorus content for soils with more (P2) and less (P1) phosphate fertilizer.

Comparing the treatments P2S2, P2S1, P1S2 and P1S1 for the dose of  $0.0 \text{ g hg}^{-1}$  of monobasic sodium phosphate in seeds and their respective controls, T1, T2, T3 and T4, (Table 2) using the F test with 5% of probability, we can see that there is a significant difference in treatments P2S2 and P2S1 for all evaluated characteristics when compared to their witnesses. Between treatments P1S2 and P1S1 and their witnesses, there was no significant difference in AP only. These results demonstrate the importance of inoculation for the biological nitrogen fixation in the soybean. According to Finoto et al. (2004), the total use of nitrogen fertilizers makes economically unfeasible the soybean cultivation, because the success of a culture is related to the efficiency of the association with the nitrogen-fixing bacteria.

In a recent work, Peske et al. (2009), studying the performance of soybean seeds coated with phosphorus in different doses of phosphorus in soil. They found that the seeds coated with P had an increased productivity, depending on the sources and doses of P used, and the dose of 21 g of phytin per kg of seed increased productivity of soybeans by 14% of the total weight of seeds.

As for the characteristics in which there was significant effect of coating the seeds with

phosphorus, there was an increase in the nodulation and growth of the plants. The recommended dose for the phosphorus coating of the seeds remained between  $0.6$  to  $0.8 \text{ g hg}^{-1}$ .

According to Thomson et al. (1992), the increased yield in plants coming from seeds with high concentration of phosphorus, both via endogenous and exogenous, could be attributed to the greater growth of roots and shoots, as well as to the higher nodulation. Plants originating from seeds with high increase of P respond better to the initial metabolic demand, making them less dependent on the existing levels of P in the soil at this stage. Seeds with a higher amount of endogenous P may be important for the plant establishment in soil with low availability of this nutrient. However, it does not mean that these plants are exempt of a correct fertilization of the soil, because, according to the results, the effects of P in soil are more eminent than in seeds.

**Table 2.** Shoot dry matter (SDM), root dry matter (RDM), viable nodules dry matter (NDM), plant length (PL), phosphorus content in the third trifoliolate (PC), and nitrogen content in the third trifoliolate leaf of the plant (NC), as a function of the dose of 0.0 g hg<sup>-1</sup> of monobasic sodium phosphate on seeds coating for lots with higher (S2) and lower (S1) endogenous phosphorus content for soils with more (P2) and less (P1) phosphate fertilizer, compared with their respective controls T1, T2, T3 and T4.

Treatment	SDM -----g-----	RDM -- mg--	NDM --cm--	PL -dag g <sup>-1</sup> -	PC -dag g <sup>-1</sup> -	NC -dag g <sup>-1</sup> -
P2S2	15,01 a	5,39 a	740,0 a	64,28 a	0,152 a	3,714 a
T1	9,20 b	3,27 b	11,3 b	51,00 b	0,075 b	2,308 b
P2S1	14,71 a	5,15 a	643,4 a	65,40 a	0,158 a	3,645 a
T2	9,49 b	3,07 b	119,0 b	49,80 b	0,075 b	2,223 b
P1S2	5,98 a	3,31 a	14,6 a	45,58 a	0,092 a	2,627 a
T3	2,99 b	1,65 b	5,6 b	36,60 a	0,054 b	1,328 b
P1S1	5,64 a	3,40 a	13,6 a	43,24 a	0,085 a	2,583 a
T4	2,76 b	1,52 b	4,7 b	35,20 a	0,051 b	1,228 b

P2S2 (S2 seeds in the P2 soil); P2S1 (S1 seeds in the P2 soil); P1S2 (S2 seeds in the P1 soil) and P1S1 (S1 seeds in the P1 soil). These treatments do not received a dose of monobasic sodium phosphate and inoculation; T1 (S2 seeds sown in P2 soil); T2 (S1 seeds sown in P2 soil); T3 (S2 seeds sown in P1 soil) e T4 (S1 seeds sown in P1 soil). These witnesses do not received a dose of monobasic sodium phosphate and inoculation; Means followed by the same letter on the column do not differ by the F test (P < 0,05).

## CONCLUSION

The seed coating with phosphorus did not increase nodulation and growth of soybean plants in

soil with larger availability of phosphorus. Otherwise, in soil with less availability of phosphorus, seed coating with phosphorus increased nodulation and growth of soybean plants.

**RESUMO:** O incremento da concentração de fósforo na semente de soja aumenta o potencial de rendimento da planta subsequente. O objetivo do trabalho foi estudar o efeito do fósforo, via recobrimento das sementes, na nodulação e no crescimento das plantas de soja. Utilizaram-se sementes de soja de dois lotes, cultivar Pioneer P98R31, com diferentes teores de fósforo. As sementes foram recobertas com fosfato de sódio monobásico nas doses de 0,0; 0,2; 0,4; 0,8 e 1,2 g hg<sup>-1</sup> de sementes e, em seguida, com inoculante turfoso com 3,0 milhões de células de bactéria (*Bradyrhizobium japonicum*) por semente. O experimento foi instalado em delineamento de blocos ao acaso com cinco repetições, em esquema fatorial 2x2x5 + 4, combinando os dois níveis de P nas sementes, dois níveis de fertilizante no solo, cinco níveis de P usados no recobrimento das sementes, além de quatro controles. Após os tratamentos, as sementes foram semeadas em vasos plásticos contendo 3,0 dm<sup>3</sup> de Latossolo Vermelho Amarelo, sendo adicionado fósforo nas doses de 200 e 400 mg kg<sup>-1</sup> como superfosfato triplo. No estágio R2 de desenvolvimento das plantas, foi avaliado o teor de fósforo e nitrogênio no terceiro trifólio, e no estágio R3, a altura de plantas, a matéria seca da parte aérea, da raiz e dos nódulos. Independente da concentração original de fósforo na semente, o recobrimento das sementes com fósforo não aumentou a nodulação e o crescimento das plantas de soja sob maior disponibilidade de fósforo no solo. Já sob menor disponibilidade de fósforo no solo, o recobrimento das sementes aumentou a nodulação e o crescimento das plantas de soja.

**PALAVRAS-CHAVE:** *Glycine max.* Solo. Nódulos, Nitrogênio.

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