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CROP PRODUCTION

Inoculation of soybean seeds coated with osmoprotector in diferents soil pH's

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ABSTRACT. Inoculation with nitrogen-fixing bacteria (*Bradyrhizobium*) is an indispensable technology to increase the productivity of the soybean crop. The objective of this study was to evaluate the viability of the inoculation with *Bradyrizobium* bacteria associated with the osmoprotector coating on different pre-sowing days and the effect of different soil pH on the components of yield, nodulation and grain yield of soybean. Two soybean cultivars were used, sown in two seasons and submitted to soil pH of 5.3 and 6.5. The seeds were inoculated at different pre-sowin periods with *Bradyrizobium* bacteria in the presence or absence of osmoprotectants. In the cultivar NA 5909RG (pH 5.3), the seeds inoculated with *Bradyrhizobium* and osmoprotector, at four and seven days of pre-sowing, presented an increase of 10.8% and 8.3% of productivity, in relation to those without osmoprotector. The osmoprotector and the inoculation of *Bradyrhizobium* increases the number and dry mass of nodules in soil with more acidic pH. The soil of lower acidity is responsible for higher grain yield, compared to the higher acidity, when both were not inoculated. The variable with the greatest explanatory power and contribution in the variability in the data of the experiment is the grain yield.

Keywords: *Glycine max*; nitrogen biological fixation; early inoculation.

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Introduction

The expression of the productive potential of the soybean crop (*Glycine max* (L.) Merrill) depends on the conditions of the environment where the plants develop and on the correct management of fertilization. It is estimated that approximately 80 kg of N are required to produce 1,000 kg of soybeans (Hungria, Campo, & Mendes, 2007). The need can be met by the biological nitrogen fixation process (BNF) where bacteria of the *Bradyrhizobium* gender associate with the root system of the soybean plant, establishing a symbiosis, resulting in the formation of nodules (Zilli, Campo, & Hungria, 2010). The bacterium present in the nodule breaks the triple bond of the dinitrogen (N_2) and transforms it into forms assimilable by plants, nitrate (NO_3^-) and ammonium (NH_4^+) (Taiz & Zeiger, 2016).

However, the efficiency of the BNF is dependent on some biotic and abiotic factors. In relation to soil pH, the soybean plant usually develops in soils with a reference pH of 6.0, but the acidity will limit productivity to a low pH of 5.5 (CQFS, 2016). The soil acidity promotes a reduction in its development, especially the presence of manganese, iron and aluminum, which are toxic to most crops of economic interest (Souza, Novais, Alvarez, Hugo, & Villani, 2010). In addition, it acts negatively on the activities of the microsymbiont (rhizobia), which is the most sensitive part to the pH effect (Albareda, Rodríguez-Navarro, & Temprano, 2009), making it difficult to exchange signals between them and plant roots (Rufini et al., 2011), limiting its survival and multiplication, resulting in reduced nodulation (España, Cabrera-Bisbal, & López, 2006).

Therefore, considering the difficulties of survival of the bacteria, it is recommended that that the sowing is carried out no later than 24h after the inoculation so that the sufficient number of viable cells per soybean seed of 1,200,000 is achieved. Longer periods, in the order of weeks, can reduce bacterial survival by 70-90% of the value in 24h (Deaker, Hartley, & Gemell, 2012). However, the time spent on this operation reduces the efficiency of sowing, as well as the need for more manpower and equipment, which are not always available to farmers. This causes many producers to stop using technology, or to do it wrongly. This situation has increased the demand of the producers for technologies that aim at greater effectiveness of the process, as the obtaining of inoculated seeds directly from the industry, which havead vantages like better uniformity of the covering of the seeds, or seeds that can be inoculated days before sowing.

Considering the importance of seed treatment, an environment favorable to the survival of rhizobia and the need to make the inoculation process feasible, some measures are indispensable to guarantee higher levels of BNF and seed health. One of the measures is to increase the dose of the inoculant, ensuring a greater number of viable bacteria in the seed, especially in new areas or soils with high acidity, or to apply the inoculant in the seeding groove or by spraying, avoiding the direct contact of the cells with the fungicide (Campo, Araujo, Mostasso, & Hungria, 2010; Hungria, Nogueira, & Araujo, 2015).

Alternatively, the seed treatment can be done by using osmoprotectors as they have polymers that provide film formation by preventing direct contact of the inoculant with seed treatment and reducing the effects of relative humidity fluctuations (Deaker, Roughley, & Kennedy, 2007). They provide substrate for the survival of the bacteria during the period before symbiosis, and provide substances that act in the communication between seedlings and bacteria to accelerate the formation of the nodule in the roots of the plant and they provide greater uniformity of the inoculation, without harm the quality and the performance of the seeds (Pereira, Moreira, Oliveira, & Caldeira, 2010).

Therefore, the objective of this study was to evaluate the viability of the inoculation with *Bradyrizobium* bacteria associated with the osmoprotector coating on different pre-sowing days and the effect of different soil pH on the components of yield, nodulation and grain yield of soybean.

Material and methods

The study was conducted in the experimental area of the Department of Plant Science at the Universidade Federal de Santa Maria, at latitude 29° 43'2.81 "S, longitude 53° 43'58.28" W, and altitude of 116 meters. The local climate, according to the Köeppen classification, is of Cfa type (Peel, Finlayson, & McMahon, 2007).

The soil of the area is classified as Argisol Red dystrophic arenico (EMBRAPA, 2013). The chemical analysis after correction indicates that the soil presents as characteristics: soil with pH (water, 1:1) = 5.3; MO (%, m/v) = 2.9; Clay (%, m/v) = 27; P, P-Mehlich (mg dm^{-3}) = 2.1; K ($cmol_c$ dm^{-3}) = 0.031; H + Al ($cmol_c$ dm^{-3}) = 3.1; CTC (pH 7, $cmol_c$ dm^{-3}) = 10.9; Base saturation (%) = 71.8. Soil with pH (water, 1:1) = 6.5; MO (%, m/v) = 2.7; Clay (%, m/v) = 29; P, P-Mehlich (mg dm^{-3}) = 3.3; K ($cmol_c$ dm^{-3}) = 0.041; H + Al ($cmol_c$ dm^{-3}) = 2.5; CTC (pH 7, $cmol_c$ dm^{-3}) = 11.9; Base saturation (%) = 79.3.

Seeds were sown in the first and second seasons on October 19 and December 17, 2015, respectively. The purpose of the two-stage experiment was to evaluate different environments for the crop. Four experiments were conducted in a randomized block design with four replicates. Two with cultivar NA 5909RG (habit of indeterminate growth) and two with cultivar NS 6209RR (habit of determined growth).

The treatments were distributed in a bi-factorial model (2 x 7), the factors consisting of two soil pH (5.3 and 6.5) and seven seed treatments (TS: seed treatment only), TSB0: TS + inoculation with *Bradyrizobium* on the day of seeding, TSB4: TS + inoculation with *Bradyrizobium* four days before seeding; TSB11: TS + inoculation with *Bradyrizobium,* eleven days before seeding; TSBO0: TS + osmoprotector + inoculation with *Bradyrizobium* in TSBO4: TS + osmoprotector + inoculation with *Bradyrizobium* , four days before sowing, TSBO11: TS + osmoprotector + inoculation with *Bradyrizobium* , eleven days before sowing), for the first sowing season. For the second sowing season, the time between inoculation and sowing was changed in relation to the first season, being 0, 7, and 14 days prior to planting. Therefore, the treatments for the second season were described as: TSB0, TSB7, TSB14, TSBO0, TSBO7, and TSBO14. For sowing on December 17, 2015, data from the TSB7 and TSB14 treatments of NS 6209RR were not reported due to problems in the sowing machine, which resulted in problems in seed distribution, being observed only after germination.

The experimental unit consisted of 7.75 m long by 2.25 m wide, with five rows spaced 0.45 m, totaling 17.4 m^2 of total area and 6.75 m^2 of floor area. The fertilization was carried out from the result of the chemical analysis of the soil of the area, and the pH was corrected to 5.3 and 6.5. The macronutrients P_2O_5 and K_2O were supplied to the sowing furrow at the dose of 400 kg ha^{-1} of the formula 23-30, using fertilizer seeder. The seed density was 33 m^2. The management of insects, diseases and weeds was carried out according to their incidence through products recommended for the soybean crop (EMBRAPA, 2014).

In the chemical treatment of the seeds, the fungicide + insecticide mixture was used: Piraclostrobin 25g ia L^{-1} + Thiophanate methyl 225 g L^{-1} + Fipronil 250 g L^{-1} (Standak® Top) at the dose 2 mL kg^{-1} of seeds. To cover the seeds, 6 mL kg^{-1} of seeds of an osmoprotector (water, metabolic extract of bacteria, complex of sugars, and biopolymers) were used. The inoculation was carried out with a peaty inoculant at the dose of 4 g kg^{-1} of seeds, the base of the bacterium *Bradyrhizobium elkanii* (water, yeast extract, glycerol, potassium phosphate, magnesium sulphate, sodium chloride, and peat, containing a concentration of 5.5 x 10^9 CFU g^{-1} of N-fixing bacteria).

When the plants have reached the developmental stage R2 (full bloom, one flower open in the last two nodes of the stem with a fully developed leaf) according to phenological scale proposed by Fehr and Caviness (1977), the nodule count was performed d the Roots. Four plants were randomly collected from each experimental unit, taking care to maintain the pre-set volume of soil for each plant with dimensions 0.008 m^3 0.2 (L1) x 0.2 (L 2) x 0.2 (H) collected with the aid of the cutter. The number of nodules was determined by the direct count of nodules in the main and secondary roots of each plant (NN, plant^{-1}). After the nodules were washed, they were dried in a forced air oven at 65°C for 48 hours. After this period, the dry weight of nodules per plant (DMN, mg plant^{-1}) was determined. The aerial parts of these same plants were submitted to drying in forced air circulation at 65°C for 48 hours and after that the dry mass of the plant (DMP, g plant^{-1}) was measured.

Five plants were collected in sequence in the crop row at the full maturation of the crop (R8), from the beginning of the useful area of the experimental unit, to determine the number of vegetables per plant (NV, plant^{-1}). Also in R8, five meters of the three central rows of the experimental unit were collected, which represents its useful area. After the harvest, the samples were tracked and cleaned for the measurement and correction of grain moisture (base 13%), yield of grains (GY, kg ha^{-1}) and mass of one thousand grains (MTG, g).

The data were tested to meet the assumptions of the mathematical model and then submitted to analysis of variance (F test). The averages were submitted to complementary procedures according to the responses presented by the interaction. The averages separated by the Scott - Knott method clustering at a 5% error probability level, using statistical software Sisvar®.

The multivariate analysis was performed using the main components in the Genes® statistical software to indicate which were the variables observed in the study that explained the highest percentage of the total variation in the data. The choice of components was according to the 70% explanation of total variability criteria.

Results and discussion

In the two sowing seasons, for the cultivar NA 5909RG, in the analysis of variance there was a significant double interaction (treatments x pH) for the variables NN, DMN, DMP, NV, MTG, and GY, with the exception of NN and DMP in the second season. For the cultivar NS 6209RR, the significant double interaction was observed for the NN, DMN, and GY variables, in the first season and DMN and NV, in the second season. When there was no significant interaction, the averages between the levels of each factor were compared.

In both cultivars and soil pH, in the first sowing season, it was observed that the roots of the plants that contained nodules with smaller sizes (DMN and NN) were the ones that presented a significant difference between the treatments. The larger number of nodules of smaller sizes would allow a larger area of infected roots. The TSBO0 treatment presented the highest NN for all the situations evaluated, being the one that presented the highest DMN at pH 5.3 (Tables 1 and 2). This shows advantages of the treatments in which the osmoprotector is used, especially in the day of sowing, because in soils with low pH the presence of bacteria, like *Bradyrhizobium*, is limited, since the optimum rhizobium growth pH is between 6.0 and 7.0 (Jordan, 1984). In addition, the osmoprotector has a complex of sugars and provides substances that act in the gene expression for the formation of the nodule (Sugawara et al., 2006), which, respectively, help to maintain the rhizobia survival and accelerate the recognition of rhizobia and roots, as they act in the synthesis of nodulins, proteins that are important in the formation and maintenance of the nodule (Almaráz, Zhou, Souleimanov, & Smith, 2007). This may have been favorable in the soil with more acidic pH, since these hinder the exchange of signals between roots and bacteria, limiting their survival and multiplication (España et al., 2006).

Table 1. Number of nodules in R2 (NN, No. plant^{-1}), dry mass of nodules in R2 (DMN, mg plant^{-1}), dry mass of aerial part of plant in R2 (DMP, g plant^{-1}) as a function of the inoculation and soil pH for soybean cultivar NA 5909RG, for first and second sowing season. (Santa Maria, Rio Grande do Sul State, Brazil, crop season of 2015/16).

Cultivar NA 5909RG						
	NN		DMN (mg plant^{-1})		DMP (g plant^{-1})	
	pH		pH		pH	
Treatments	5.3	6.5	5.3	6.5	5.3	6.5
First sowing season						
TS	90.8 bA*	107.9 bA	574.7 bA	596.5 bA	12.4 cA	11.6 aA
TSB0	107.5 bB	139.0 aA	558.1 bB	657.0 aA	13.6 bA	14.4 aA
TSB4	124.5 bA	93.8 bB	480.2cA	482.7 cA	11.5 cA	13.4 aA
TSB11	98.6 bA	104.5 bA	435.7 cB	577.8 bA	11.1 cA	12.3 aA
TSBO0	178.2 aA	147.1 aB	752.6 aA	500.7 cB	16.7 aA	11.6 aB
TSBO4	111.0 bA	140.0 aA	501.6cA	521.7 cA	9.7 cB	12.9 aA
TSBO11	99.5 bA	124.8 aA	470.6 cB	554.5 cA	10.6 cA	10.2 aA
Average	119.3		547.0		12.2	
CV %	14.7		7.2		11.8	
Second sowing season						
TS	106.4	87.4	173.8 bA	187.9 aA	15.5	19.7
TSB0	97.1	103.9	192.6 bA	149.5 bA	16.5	19.6
TSB7	126.8	103.8	135.5 bB	211.0 aA	17.2	18.9
TSB14	105.2	110.8	250.0 aA	204.4 aA	16.0	23.0
TSBO0	115.0	92.3	235.7 aA	166.0 bB	18.7	17.7
TSBO7	115.4	69.2	158.5 bA	115.5 bA	17.1	19.5
TSBO14	134.0	100.1	230.5 aA	154.3 bB	16.2	19.6
Average	104.8		183.2		18.2	
CV %	22.7		20.8		11.6	

*Different upper-case letters in the row and lower-case letters in the column treatments differ statistically from one another by the Scott-Knott test (significant at 5% probability).

In the cultivar NS 6209 RR, at pH 6.5, TSBO0, did not differ from TSBO4 and TSB4, for NN and DMN variables. As for DMN, there was also no difference for TSB0. In NA 5909RG, the TSBO0 did not differ statistically from the other treatments using the osmoprotector and TSB0 (Tables 1 and 2). In the treatment of seeds, a fungicide was used and its application can reduce the *Bradyrhizobium* population due to its active principle, pH and solvents used in the formulations (Annapurna, 2005). Therefore, decreasing the number of viable cells to carry out the symbiosis with the roots of the plants and consequently the nodulation, probably occurring with greater intensity in the treatments inoculated days before sowing by the longer time of contact with the bacteria.

Table 2. Number of nodules in R2 (NN, No. plant^{-1}), dry mass of nodules in R2 (DMN, mg plant^{-1}) and dry mass of aerial part of plant in R2 (DMP, g plant^{-1}) for cultivar NS 6209RR as a function of the moment of inoculation of the soybean seeds, for first and second sowing season. (Santa Maria, Rio Grande do Sul State, Brazil, crop season of 2015/16).

Cultivar NS 6209RR						
	NN		DMN (mg plant^{-1})		DMP (g plant^{-1})	
	pH		pH		pH	
Treatments	5.3	6.5	5.3	6.5		
First sowing season						
TS	131.1 bA*	107.5 bA	557.5 bA	488.5 bA	14.9 a	
TSB0	154.8 Aa	123.5 bA	569.3 bA	566.7 aA	16.8 a	
TSB4	126.5 bA	146.1 aA	522.5 bA	608.2 aA	15.4 a	
TSB11	112.8 bA	101.7 bA	432.6 cA	455.3 bA	13.7 b	
TSBO0	166.6 aA	158.7 aA	658.6 aA	632.7 aA	12.9 b	
TSBO4	94.4 bB	150.2 aA	445.0 cB	676.4 aA	13.8 b	
TSBO11	131.1 bA	107.5 bA	557.5 bA	488.5 bA	14.4 b	
Average	130.6		543.6		14.5	
CV %	14.5		10.5		10.6	
Second sowing season						
TS	105.7	90.1	190.8 bA	164.1 aA	18.4	16.2
TSB0	98.6	124.0	163.1 bA	177.2 aA	20.4	17.2
TSBO0	115.3	112.6	138.6 bA	179.2 aA	15.8	17.5
TSBO7	89.7	86.7	211.3 bA	189.1 aA	19.2	18.4
TSBO14	156.3	95.3	323.1 aA	204.6 aB	17.2	19.4
Average	107.4		194.1		17,9	
CV %	27.7		22.2		12.4	

*Different upper-case letters in the row and lower-case letters in the column treatments differ statistically from one another by the Scott-Knott test (significant at 5% probability).

For both cultivars, sown in the second season, in soil with pH 6.5, the plants presented higher DMP than those sown in soil with pH 5.3 (Table 3). This may have occurred because the soybean plant is sensitive to soil acidity, which influences the reduction of its development (CQFS, 2016), directly interfering with DMP.

In an experiment conducted by Zilli et al. (2010), with inoculation in pre-sowing and without fungicide use in the treatment of seeds also obtained reduction in the number and dry mass of nodules, not occurring reduction in standard inoculation (performed on sowing day). For Araújo et al. (2017), in work using seed treatment together with the larger osmoprotector, NNs were observed in the presence of osmoprotector. In view of this, it can be seen that the osmoprotector may have acted in a way to avoid the direct contact of the bacteria with the fungicide, since the presence of polymers in its constitution minimizes the risks of phytotoxicity caused by the treatment of seeds, through the formation of a film (Pires, Bragantini, & Costa, 2004), allowing a greater survival of the bacteria (Hartley, Gemell, & Deaker, 2012; Marks et al., 2013) and consequently greater nodulation.

Table 3. Dry mass of aerial part of plant in R2 (DMP, g plant^{-1}), number of vegetables (NV, No. plant^{-1}) and mass of a thousand grains (MTG, g) for the cultivar NS 6209RR sown in the first season and dry mass of aerial part of plant in R2 (DMP, g plant^{-1}) for soybean cultivar NA 5909RG sown in the second season, as a function of soil pH. (Santa Maria, Rio Grande do Sul State, Brazil, crop season of 2015/16).

pH	NS 6209RR			NA 5909RG
	DMP (g plant^{-1})	NV	MTG	DMP (g plant^{-1})
5.3	13.6 b*	87.4 b	150.7 a	16.7 b
6.5	15.5 a	102.0 a	144.1 b	19.1 a

*Averages not followed by the same letter in the column differ statistically from one another by the Scott-Knott test (significant at 5% probability).

In soil with pH 5.3, for cultivar NA 5909RG, in the seeds inoculated on the day of sowing with the use of osmoprotector, presented plants with higher DMP inferring that the larger the NN and DMN, the greater the possibility of obtaining plants with greater development of aerial part (Table 1). Behavior that probably occurred due to the greater number of bacteria that carried out symbiosis with the roots of the plant and, consequently, was the supply of N for its development (Santos et al., 2013).

Among the main components of productivity for the soybean crop are the number of vegetables and the dry mass of grains per plant (Dalchiavon & Carvalho, 2012). In the cultivar NA 5909RG, in soil with pH 6.5, in the two sowing times, the NV did not differ as a function of the treatments applied. It can be explained by the fact that soils with higher pH have their *Bradhiryzobium* population already established, that is, the nodulation of the roots of the plants is not only dependent on the inoculation. In relation to the absence of significant differences between inoculated and non-inoculated seed Golo, Kappes, Carvalho, and Yamashita (2009) also did not find significant differences, because in the area of the experiment soybean was already being cultivated, where inoculations of the rhizobia were carried out.

At pH 5.3, in the first season, for the same cultivar, the plants that presented the highest NV were those that contained osmoprotector in the treatment. In the second season, higher NV were found in the plants where the seeds were only inoculated and in those with osmoprotector, except for the treatment where the seeds were inoculated 14 DAS (Table 4). However, when populations of established rhizobia are already present, there may be competition between these and inoculated ones. In general, those already established no longer have the genotypes of the bacteria that are inoculated due to genetic alterations that occur (Melchiorre et al., 2011), eventually competing with the rhizobia that were inoculated and of better quality (López-García et al., 2009). Therefore, the NN may have been lower, but the bacteria that perform the symbiosis were more efficient in BNF. However, for the cultivar NS 6209RR, in the first sowing season there was a significant difference only for the main pH factor, indicating a higher NV in the plants cultivated in soil with higher pH (Table 3), which is related to the greater development of the plant in pH taller.

The MTG showed a significant interaction between the treatments of the different pH and inoculation moments only in cultivar NA 5909RG, both in the first and in the second sowing season. At pH 6.5, in the first season, when inoculation was carried out on sowing day, higher MTG was obtained and, for second season, higher averages were obtained in treatments TSB7 and TSB14 (Table 4). It can be observed, for the first season, that the treatments achieved greater MTG were the same as contained higher DMN. In a study by Pereira, Monteiro, Botin, Manhaguanha, and Braulino (2016), no significant difference was found in MTG, however, the authors verified a relationship between MTG and DMN. At pH 5.3, in the first season, inoculations performed up to seven days obtained a higher MTG independent of osmoprotector use, however, in the treatment where the osmoprotector was used, MTG remained higher up to 11 DAS (Table 4).

As for productivity, in both cultivars, it is possible to observe that at pH 6.5 when not inoculated the productivity was higher when compared to the same treatment at pH 5.3, for the first sowing season. The GY difference was 15.6% for NA 5909RG and 10.3% for NS 6209RR. Also, in the first sowing season, in both pH, for cultivar NA 5909RG, GY was superior when the seeds were inoculated on the day of sowing, using the osmoprotector (TSBO0). However, at pH 5.3, this productivity did not differ from TSBO4 treatment. These were also the treatments that were already presenting superiority over the others, for the variables NN, DMP, NV, MTG. For the second sowing season, at pH 5.3, the same trend is observed as in the first season. It is also observed that TSBO4 and TSBO7, treatments of the first and second season respectively, allowed increases of 10.8% and 8.3% of productivity to the treatments performed on the same days without the use of osmoprotector (Table 4).

Table 4. Number of vegetables (NV, No. plant^{-1}), mass of a thousand grains (MTG, g) and grain yield (GY, kg ha^{-1}) as a function of inoculation time and soil pH for soybean cultivar NA 5909RG, for the first and second sowing season. (Santa Maria, Rio Grande do Sul State, Brazil, crop season of 2015/16).

	Cultivar NA 5909RG					
	NV		MTG (g)		GY (kg ha^{-1})	
	pH		pH		pH	
Treatments	5.3	6.5	5.3	6.5	5.3	6.5
First sowing season						
TS	61.3 bA*	69.7 aA	149.3 bB	155.7 bA	3934 cB	4662 bA
TSB0	58.5 bA	77.5 aA	155.3 aB	162.7 aA	4399 bA	4515 cA
TSB4	60.4 bB	77.6 aA	160.9 aA	156.7 bA	4066 cB	4340 cA
TSB11	55.2 bB	75.6 aA	151.1 bB	158.6 bA	4296 bA	4205 dA
TSBO0	80.6 Aa	70.2 aA	156.1 aB	165.4 aA	4495 aB	4883 aA
TSBO4	73.1 Aa	76.0 aA	157.7 aA	153.9 bA	4561 aA	3654 eB
TSBO11	80.3 Aa	53.7 aB	154.8 aA	156.0 bA	4261 bA	4011 dB
Average	69.1		156.7		4306.5	
CV %	17.3		2.28		3.2	
Second sowing season						
TS	175.5 bB	313.6 aA	149.0 aA	141.2 bB	3664 bA	3529 bA
TSB0	236.7 aA	278.2 aA	151.8 aA	143.4 bB	3646 bA	3785 aA
TSB7	270.3 aA	276.5 aA	147.3 aA	147.7 aA	3481 bB	3738 aA
TSB14	282.3 aA	230.0 aA	147.6 aA	148.5 aA	3546 bB	3847 aA
TSBO0	271.7 aA	232.2 aA	144.9 aA	142.2 bA	3868 aA	3826 aA
TSBO7	261.5 aA	230.7 aA	145.8 aA	145.1 bA	3797 aA	3573 bA
TSBO14	213.0 bA	258.0 aA	148.1 aA	143.0 bB	3629 bA	3466 bA
Average	252.1		146.1		3655.7	
CV %	17.0		1.8		4.0	

*Different upper case letters in the row and lower case letters in the column treatments differ statistically from one another by the Scott-Knott test (significant at 5% probability).

It should be emphasized that in the absence or presence of low amounts of the micro-symbionts that can nodule and fix the N_2 and there is toxicity caused by the fungicide being used, there may be a reduction in root nodulation and, consequently, in the efficiency of BNF, culminating in reduction of grain yield due to the availability of N in insufficient quantities. In a study by Zilli et al. (2010), results allowed to anticipate the inoculation within five days before sowing without damaging crop productivity, but without the use of fungicides in seed treatment.

In this sense, it was observed that the osmoprotector proved to be an alternative for inoculation up to four and seven days prior to sowing in soils with more acidic pH, facilitating the sowing logistics of the producer, and also for inoculations carried out on sowing day to less acidic pH, without losing productivity through the use of chemical seed treatment. Conceição et al. (2014), studying the chemical treatment of seeds with fungicide, insecticide, micronutrient and polymer observed a greater protection of the seeds and seedlings in the field, but without significant effect on productivity.

At pH 6.5, no significant difference was observed between inoculated treatments and inoculated on the day of sowing with addition of osmoprotector, those being the ones that presented higher productivity than the others (Table 4). Possibly by the population of *Bradyrizobium* already present in the area where the experiment was installed.

For NS 6209RR at pH 6.5, at the first sowing season, no significant difference in productivity was obtained when treatments were performed only with seed treatment, inoculated at day and at 11 DAS and inoculated at 4 and 11 DAS with addition of osmoprotector. At pH 5.3 the productivity was higher with the inoculation on the day of sowing and at 4 DAS with the use of osmoprotector. In the second season, a significant difference was observed between the yields, being higher in the treatments where there was no inoculation or when the inoculation was performed at 14 DAS with osmoprotector use (Table 5).

Table 5. Number of vegetables (NV, plant^{-1}), mass of a thousand grains (MTG, g) and grain yield (GY, kg ha^{-1}) as a function of inoculation time and soil pH for soybean cultivar NS 6209RR, for the first sowing season. (Santa Maria, Rio Grande do Sul State, Brazil, crop season of 2015/16).

Cultivar NS 6209RR						
	NV		MTG (g)		GY (kg ha^{-1})	
	pH		pH		pH	
Treatments	5.3	6.5	5.3	6.5	5.3	6.5
First sowing season						
TS	85.3	88.8	146.6	144.2	4292 bB*	4787 aA
TSB0	80.6	83.7	151.7	142.1	4720 aA	4600 aA
TSB4	89.5	107.7	154.2	145.3	4378 bA	4091 bA
TSB11	109.0	109.7	148.7	147.4	4363 bA	4489 aA
TSBO0	82.6	97.0	152.9	140.8	4046 bA	4077 bA
TSBO4	84.5	115.3	150.9	143.3	4573 aA	4377 aA
TSBO11	80.6	112.3	150.4	145.7	4276 bA	4515 aA
Average	94.8		147.5		4396.55	
CV %	22.5		2.94		5.18	
Second sowing season						
TS	263.2 aA	202.2 aB	129.9	127.6	3506.60 a	
TSB0	218.3 bA	210.0 aA	124.4	127.9	3462.30 a	
TSBO0	190.2 bA	185.0 aA	127.3	123.7	3372.13 b	
TSBO7	256.5 aA	204.0 aB	128.6	124.8	3300.57 b	
TSBO14	199.6 bA	211.5 aA	125.3	126.0	3531.34 a	
Average	214.06		126.5		3434.59	
CV %	12.30		2.32		3.83	

*Different upper-case letters in the row and lower-case letters in the column treatments differ statistically from one another by the Scott-Knott test (significant at 5% probability).

Regarding the multivariate analysis, it was opted to work with the first main component (CP), since it allowed explaining more than 77% of the variance contained in the original variables. When evaluating the eigenvectors associated with each of these components, the variable with the highest weight was the GY, being the main variable that explains the existing variability of the data of the experiment. The main variable that explains this retention is the grain yield, with 0.99 for the cultivar NA 5909RG in the first and second sowing season. For NS 6209RR the values were 0.98 and 0.97 for the first and second seasons, respectively. The lowest contribution variable in the total variability of the experiment was the DMP (Table 6).

Table 6. Weights in the first two main components of the variables number of nodules in R2 (NN), dry mass of nodules in R2 (DMN), dry mass of aerial part of plant in R2 (DMP), number of vegetables (NV) (GM) and grain yield (GY) as a function of inoculation time and soil pH for soybean cultivars NA 5909RG and NS 6209RR, for the first and second sowing seasons.

	First sowing season		Second sowing season	
	NA 5909RG	NS 6209RR	NA 5909RG	NS 6209RR
NN	0.3481	0.1803	0.9117	1.2535
DMN	6.3790	8.4665	7.1744	15.6536
DMP	0.0011	0.0024	0.0037	0.0009
NV	0.0658	0.2581	4.9285	5.1962
MTG	0.0084	0.0168	0.0231	0.0196
GY	93.1973	91.0755	86.9583	77.8759
Set of associate deigen vectors				
1st Component	93.1973	91.0755	86.9583	77.8759
NN	0.0124	-0.0569	0.0170	0.0924
DMN	0.0388	-0.1667	0.0711	0.2203
DMP	0.0006	0.0023	0.0007	-0.0001
NV	0.0105	-0.0160	-0.0404	-0.0246
MTG	0.0067	0.0010	0.0005	0.0008
GY	0.9991	0.9842	0.9965	0.9707

Conclusion

Seeds inoculated at four and seven days prior to sowing, using osmoprotector and *Bradyrhizobium,* present 10.8% and 8.3% higher yields than the treatments without osmoprotector, for cultivar NA 5909RG, in soil with pH 5.3.

The osmoprotector, together with *Bradyrhizobium*, can be used in soils with low pH, increasing the number and the dry mass of nodules and yield of grains.

The analysis of main components is efficient, highlighting the importance of maintaining the variable grain yield, as well as the elimination of the dry mass of the aerial part, to explain the total variability in the data of the experiment.

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