Nodulation and agronomic performance of soybean grown in different seeding periods and glyphosate formulations

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ABSTRACT

The aimed to evaluate the influence of different glyphosate formulations in nodulation, agronomic performance and RR soybean seeds yield, grown in two sowing periods. The experimental design was the casualized blocks with treats disposed in four replicates, being organized in factorial scheme, two seeding periods x five glyphosate formulations. The measured characters were number of nodules per plant, number of active nodules, number of inactive nodules, height of the first legume insertion, plant height, number of legumes per plant, number of seeds per plant, mass of thousand seeds and yield of seeds per plant. The isopropylamine salt influences nodular activity, number of vegetables and seeds per plant, as well as seed yield. The sowing of soybeans at the recommended time propitiates smaller modifications face to glyphosate formulations.

Keywords: *Glycine max L., Roundup Ready, EPSPS,* production seeds.

INTRODUCTION

Soybean is the crop with main grain exportation in Brazil. The 2017/2018 crop season revealed a production of 118 million tons of grains and a cultivated area of 35 million hectares (Matsumoto et al., 2017 Companhia Nacional de Abastecimento [CONAB], 2018; Frota et al., 2020; Ferreira et al., 2020; Hanyu, Costa, Cecon, & Matsuo, 2020; Soares, Sediyama, & Matsuo, 2020), being surpassed only by United States. The evolution of production and yield of soybean is linked to the insertion of RR event (*Roundup Ready*) being destined to the control of weeds in no-till sowing system (Nepomuceno, Varela, Alves, & Martins, 2012).

Glyphosate is a systemic herbicide of broad spectrum, inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSP), responsible for catalyze the condensation of phosphoenolpyruvate, essential step in the shikimic acid biosynthesis (Berlvald et al., 2010; Malty, Siqueira, & Moreira, 2006), thus, glyphosate is commonly used in RR soybean genotypes (Merotto-Junior, Wagner, & Meneguzi, 2015). However, even though RR soybean is resistant to this molecule, there are cases where farmers verify toxicity effects after post-emergence spraying, resulting in leaf chlorosis (Basso, Santi, Lamego, & Girotto 2011), nutritional unbalance (Cavalieri, Velini, Silva, São-José, & Andrade, 2012) and negative effect in soil microbiota (Dallmann, Scheneider, & Kuhn, 2010). These effects can be caused by the utilization of non-recommended doses of the product, inadequate formulation for post-emergence use, or the addiction of other non-recommended products in the spraying volume (Santos, Ferreira, Oliveira, Silva, & Fialho, 2007a).

Currently, in the market plenty glyphosate formulations are available, although, not all vegetal organisms react similarly to the different glyphosate formulations, case of the non-target organisms (Nörnberg et al., 2008). Even though formulations have the same action mechanism, they may be constituted of different salts, varying its concentrations (Santos, Ferreira, Reis, Silva, Fialho, & Freita, 2007b).

There are many negative impacts of glyphosate in the soil microbiota, among the affected organisms are soil endosymbiotic microorganisms and bacteria that fixates nitrogen (Reis et al., 2010; Bohm & Rombaldi, 2010), which, in symbiosis with soybean roots, promotes the atmospheric nitrogen transformation to the

plant. The negative effect depends on the bacteria strain, especially the species *Bradhyrizobium japonicum* an *B. elkanii* (Oliveira-Junior et al., 2008), if present in soybean fields, but commonly occurs the use of *Bradhyrizobium* tolerant to some glyphosate formulations (Chagas, Reis, Santos, Erasmo, & Chagas, 2013).

The negative effects in symbiotic bacteria, caused by glyphosate spraying, may occur, resulting in the decrease of nodules formed in soybean roots, being essentials to the assimilate and assist nitrogen to the plant. Glyphosate can induce, direct or indirectly, the lack of cationic micronutrients (Fe, Zn and Mn) in soybean with RR event. However, besides being found these effects in nodulation and agronomic performance (Dvoranen, Oliveira-Junior, Constantin, Cavalieri, & Blainski, 2008), the results found are contradicting, since did not expressed effects of glyphosate formulations in these situations. In this manner, this study aimed to evaluate the influence of different glyphosate formulations in nodulation, agronomic performance and RR soybean seeds yield, grown in two sowing periods.

MATERIAL AND METHODS

The study was carried out in Capão do Leão-RS (31°52' S e 52°21' O) six meters of altitude, at the Federal University of Pelotas. The climate is classified, according Köppen, as Cfa type, with 2000 mm of precipitation within the year. The soil is classified the soil as SolodicPlanosol, belonging to the Pelotas mapping unit (Streck et al., 2008).

The climate data of air temperature, solar radiation, air relative humidity and rainfall were measured in meteorological station, where the average maximum and minimum temperatures, average solar radiation, air relative humidity and rainfall accumulated in the extent of the experiment was of 27 °C and 16 °C, 435 Cal cm2 d-1, 88% and 548 mm, respectively (Figure 1).
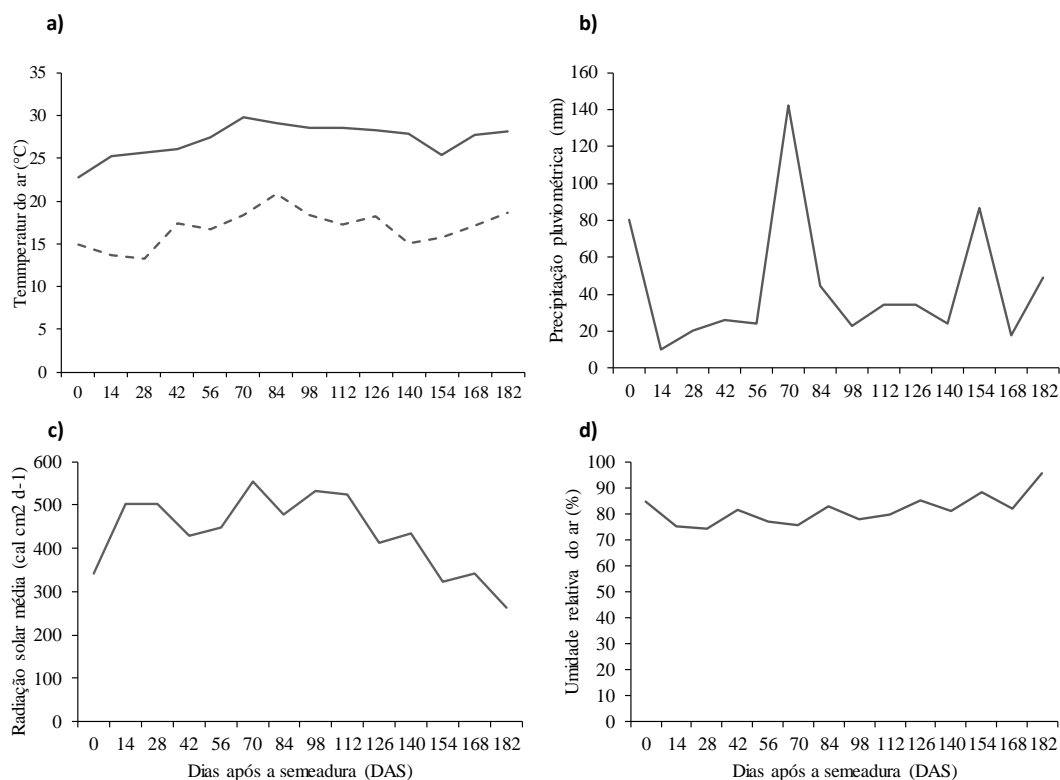
Figure 1 – Maximum (-) and minimum (----) air temperature (a), rainfall (-) (b), solar radiation (c), air relative humidity (-). Source: Agroclimatological Station of Pelotas-RS (*Campus* Capão do Leão), 2017-2018.

The experiment was distributed in experimental units of three meters of length and five seeding lines spaced in 0.45 meters. It was used, as utile area, the three central rows, seeking to minimize the border effect. The experimental design was the casualized blocks with treats disposed in four replicates, being organized in factorial scheme, two seeding periods x five glyphosate formulations.

The management and crop traits were equally executed for both seeding seasons. The seeding for period I was realized day 25/12/2017 and for period II 07/12/2017, using the sowing system based on 400 kg ha^{-1} of base fertilizing, formulation NPK 5-20-20, basing on soil analysis and recommendations of the Chemical

Commission of Soil Fertility (CQF-RS/SC, 2004).

The sowing density was of nine plants per meter after paring, or 200 thousand plants ha^{-1}. In the area, there was no cultivation of soybean in previous crop seasons, the seeds inoculation was realized in the same day with peaty inoculate with *Bradyrhizobium japonicum*-Semia5079 and 5080, proportion of three times the recommended dose for seeds treatment (6 g kg^{-1} of seeds). The cultural traits for pests, weeds and diseases control were realized according crop recommendations.

The harvesting was carried out with the same procedures for both periods, where period I: 06/04/2018, and period II: 27/04/2018, where it was all manual when plants achieved R8 stage, which is full maturing (Fehr & Caviness, 1977), with 16-18% moisture. For harvesting, it was considered as utile area the three central lines of the plot, each with two meters long not considering the first and last 0.5 m of each line. For evaluation of yield compounds, it was randomly collected 10 plants within the utile area and the rest of the utile area was harvested. The seeds were placed to dry in kiln of forced air, with 41ºC until humidity stabilization at 12%. The seeds processing was carried out according recommendations for soybean seeds, being the seeds stored in dry and cold chamber, with air humidity and temperature controlled.

It was used the variety Nidera NA 5909 RR, and the herbicides used were composed of different salts (glyphosate formulations), recommended for RR soybean in postemergence, weeds control and desiccate in pre-seeding. The dosages applied in field and herbicides characteristics for the experiment are represented in table 1.

Table 1. Different active ingredient, formulations and glyphosate doses.

Nome	Concentration	Formulation type	Dose
Glyphosate	Isopropylamine salt 480 g L^{-1} (355 g L^{-1} acid equivalent)	Soluble Liquid (SL)	3 L ha^{-1}
Glyphosate	Dimethyl amine salt 608 g L^{-1}(480 g L^{-1} acid equivalent)	Soluble Liquid (SL)	2,25 L ha^{-1}
Glyphosate	Potassium salt 588 g L^{-1}(480 g/L acid equivalent)	Soluble Liquid (SL)	2,25 L ha^{-1}
Glyphosate	Ammonia salt 715 g Kg^{-1} (650 g/kg acid equivalent)	Wettable grain (WG)	1,5 Kg ha^{-1}

It were realized two a glyphosate spraying during the plant cycle during V4 and V7 vegetative stages (Fehr et al., 1971). The first spraying (V4) was realized day 24/11/2017 and second spraying (V7) took placed day 08/12/2017, for seeding period of October. For the December seeding, the first spraying (V4) of glyphosate was 05/01/2018 and second (V7), day 19/01/2018. The sprayings were assisted by a costal sprayer with constant pressure by a compressed carbonic gas (CO_2), equipped with 5 flat fan nozzles in the spraying bar, spaced by 0.5 meters and spraying volume of 120 L ha^{-1}. The measured characterswere:

Number of nodules per plant (nodp)

The counting of nodules was realized from the extraction of roots from the soil. It were extracted five plants in the same line in each experimental unit, taken to lab and counted the number of nodules from the main and secondary roots (number).

Number of active nodules (NODA)

The counting of active nodules occurred from the total nodules. The nodules suffered a transversal cut with scalpel blade. It was considered active nodules the ones that presented rosaceous color or reddish (Pommeresche & Hansen, 2017) (number).

Number of inactive nodules (NODI)

The counting of inactive nodules was similar to the actives, although, in this time the brownish and greenish colored nodules were considered inactive (number).

Height of the first legume insertion (HFL)

The first legume insertion was measured with ruler when the plant was still on soil, being measured from the top of the soil to the first legume. It were evaluated ten random plants (cm).

Plant Height (PH)

The plants height was verified with a ruler when the plant was still on field, measuring the top of the plant to the soil. It were measured 10 plants in each experimental unit (cm).

Number of legumes per plant (NLP)

It was removed the legumes from the plants and counted, first sorting out the legumes with one seed, two seeds and three seeds, and the subtotal corresponding to the number of total legumes (number).

Number of seeds per plant (NSP)

Determined from the separation of legumes with one, two and three seeds. After counting, the subtotal of seeds of each legumes category were summed composing the total seeds (number).

Mass of thousand seeds (MTS)

Determined from the mass of eight samples of 100 seeds collected from the utile area of each experimental unit and manually counted (grams).

Yield of seeds per plant (YSP)

It was assessed the number of seeds per plant and multiplied by the mass of seeds, previously, the humidity was corrected to 13% (grams).

The data was submitted to variance analysis by F test at 5% probability, where it was verified its presuppositions. Next, it was realized a diagnosis of the interaction between seeding period x glyphosate formulations, at 5% of probability. When the interaction was significant it was derived to simple effects the factors of seeding period and glyphosate formulations. The variables that did not present interaction were disjoint to main effects using complementary analysis of Duncan, at 5% probability, for the factor seeding period and glyphosate formulations.

RESULTS AND DISCUSSION

The variance analysis revealed significance for the interaction seeding period x glyphosate formulation for the variables of NODA, HFL, YSP, NLP and NSP. In the other hand, did not revealed significance for the variables NODT and NODI, being verified only simple effect as significant. In addition, the thousand seeds mass did not revealed significance for interaction neither isolate.

Since the variance analysis revealed significance, it was inferred that the variation factors execute distinct effects on the analyzed variables (Table 2). In this manner, the interactions between seeding period (E) and glyphosate formulation (FG) generates different results from the variables of compounds of agronomic performance, being necessary to disjoint the simple effects in order to obtain results that better explain the variables behavior.

Soybean is a crop that requires plenty of nutrients from the soil in order to develop, among them the most required is nitrogen, which, through symbiosis of nitrogen fixing bacteria and the roots, create nodules that are responsible for assimilate this nutrient, partially supplying all the nitrogen demand for grains production (Brandelero, Peixoto, & Ralisch,2009). The glyphosate has negative effects in nodulation, such effects can be caused by adjuvants or type of saltin formulations (Reddy & Zablotowicz, 2003). In this context, it is observed that there was significant difference between the variables of the main effects (Table 3), NOP and NODI demonstrating that the treatment isopropylamine salt was the most harmful treatment, presenting lower NODP as well larger NODI, same as the potassium salt treatment. Although, potassium salt obtained larger NODP than the rest of treats.

Certain glyphosate formulations may damage the symbiotic activity of nodules, depending of the bacteria strain present in the soil, some may present larger susceptibility to determined glyphosate salt, harming the performance of normal nodules (Jacques et al., 2010). A higher soybean nodulation in roots have a larger advantage to recover from determined stresses and present a higher yield, due to its larger nitrogen assimilation (Zilli et al., 2008).

The number of active nodules with the effect of different glyphosate formulations in each sowing period (Table 4) demonstrate that in the October sowing period, the treatment without glyphosate obtained the larger NODA in relation to the rest, while in December, presented the lower NODA. Therefore, the treatment ammonia salt present larger NODP in October than in December, and the others treatments did not differ within sowing periods. The fact that October showed larger NODP may be attributed to longer vegetative period in the month, which presented 16 days of difference in the vegetative stage than in December, favoring a larger nodules formation until the beginning of reproductive period.

Due to glyphosate formulations in each sowing period, the glyphosate treatment with isopropylamine salt

had effect of lowering the NODA in the October sowing period. In December, the potassium salt treatment occasioned lower effect, presenting larger number of active nodules, while in the absence of glyphosate, demonstrated lower mean of NODA.

Table 2. Summary of variance analysis with mean square of Number of nodules per plant (NODP), Number of active nodules (NODA), Number of inactive nodules (NODI), Height of the first legume insertion (HFL), Plant Height (PH), Number of legumes per plant (NLP), Number of seeds per plant (NSP), Mass of thousand seeds (MTS), Yield of seeds per plant (YSP)in relation to sowing period (E) and Gliphosate formulations (FG). Capão do Leão, RS, UFPel, 2018.

SV	DF	Mean Square				
		NODP	NODA	NODI	HFL	PH
Sowingperiod (E)	1	48392.8*	20683.5*	5339.3*	40.45NS	2940.4*
Glyphosate formulations (FG)	4	5927.1*	3975.6*	229.6*	133.4*	387.3 ^{ns}
E * FG	4	2351.7^{ns}	2710.6*	57.9^{ns}	113.1*	817.6*
Blocks	3	1480.1	1011.6	75.1	148.8	2044.7
Residue	183	1034.95	850.43	49.43	37.3	203.8
CV (%)		13.5	25.1	22.05	21.2	12.3
		TSM	YSP	NLP	NSP	
Sowingperiod (E)	1	12.7 ^{ns}	14536.7*	102842.7*	502182*	
Glyphosate formulations (FG)	4	82.8 ^{ns}	589.07*	3456.84*	16783.8*	
E * FG	4	35.9 ^{ns}	758.7*	3284.9*	13361.8*	
Blocks	3	310.6	832.5	2166.7	12985.8	
Residue	183	63.3	70.1	417.2	1912.2	
CV (%)		5.12	26.6	25.7	25.4	

* and ^{ns} – significant and non-significant at 5% probability, respectively; CV – coefficient of variation.

Table 3. Mean results of main effects for the variables of number of nodules per plant (NODP) and inactive nodules (NODI), due to glyphosate treatments, independently of sowing periods. Capão do Leão, RS, UFPel, 2018.

Glyphosate formulations	Number of nodules (NODP)	Inactive nodules (NODII)
Glyphosate absence*	83.5 b^{1}	8.5 c
Isopropylamine salt 36%	73 c	12.9 ab
Dimethyl amine salt 48%	81 b	10.6 bc
Potassium salt 48%	109.5 a	14.1 a
Ammonia salt 64%	82.4 b	9.4 c
CV (%)	23.7	25.42

^{1}Means followed by the same letter in column did not differ at 5% probability by Duncan test.

The nodules activity is conditioned to plenty factors, such as environmental conditions and management (Bergamin, Venturoso, Souza, & Vitorino, 2013). It can be influenced some treatments to achieve different behaviors between sowing periods. According Chagas et al. (2013), the soybean plant can present variations in different evaluation stages for number of nodules after glyphosate spraying, independent of the glyphosate formulation, but at 45 to 60 days after spraying had reductions in nodules number, where it have a tendency to reduce with glyphosate spraying (Zablotowicz & Reddy, 2007).

The HFL presented significant difference only for the treatment dimethyl amine glyphosate, which presented higher values in December, and was also significant for ammonium salt, which obtained higher HFL in December. The HFL means are within the favorable height of the cultivars in the market, being the ideal height above 15cm (Carvalho et al., 2010), the lower height was presented by the treatment with absence of glyphosate spraying, with 26 and 27cm for October and December, respectively. According to

Ludwing et al (2010), the HFL can be influenced by genetic characteristics as well the type o nutritional management and herbicide spraying management.

For PH, the sowing period of October obtained higher averages in relation to the treatments sown in the period of December, but did not differ statistically. Only the treatments with potassium salt differed, presenting higher height for the October period in relation to December, and for the treatment with ammonium salt that had the same significance. This may be due to the greater number of days of vegetative stage presented by the sown plants of the October season compared to those of the December season. The height of the plant is influenced by the sowing period, when lower heights are obtained in plants of later times in comparison to the recommendation (Cruz, Peixoto, & Martins, 2010).

In relation to the height of the plant influenced by the application of glyphosates, the treatment with absence of glyphosate and ammonium salt obtained the highest averages in the period of October. Although, in the period of December the treatment of potassium salt obtained the lowest average. The glyphosate-free treatment did not differ from the highest averages at the time of December, evidencing that the treatments with glyphosate applications had none or little negative effect in comparison to absence of glyphosate. Albrecht et al. (2011) did not show lower height of the plant when glyphosate was applied in vegetative stages and from two different doses.

For the NLP, it can be observed that the October sowing period had, in general, a higher number of vegetables per plant compared to the means of December sowing period, which obtained lower averages (Table 4). This may be due to the fact that in October the vegetative stage was higher in comparison to December, as happened for the characters NODP and PH. There was a significant difference for the treatments in relation to the sowing period. At the time of October the treatment of dimethyl amine was the least affected and obtained a larger number of legumes, being still superior to the treatment with absence of glyphosate. Melhorança-Filho, Martins, Pereira and Espinosa (2010) reports that soybean plants were submitted to different doses of glyphosate and did not differ from the control for the NLP.

For the period of December, the treatment with absence of glyphosate obtained a higher average number of vegetables compared to the other treatments, which may be because the second application or sequential application was performed near the reproductive stage. Zadinello, Chaves, Santos and Bassegio, (2010) reported a decrease in the yield of soybean plants when glyphosate was applied at initial reproductive stages. It is also noticed that the treatment with isopropylamine salt was the most affected in the two sowing periods in comparison to the other treatments, presenting a lower mean of NLP.

For the NSP (Table 4), the same tendency of the number of vegetables was observed, being the greatest averages for the sowing period of October in comparison of the time of December. The treatments composed of dimethyl amine and ammonium salt showed less influence in the period of October, although, for December the treatment of absence of glyphosate showed a better result than glyphosate, which may impair the agronomic performance. As consequence, YSP showed the same tendency of the NLP and NSP (Table 4).

The YSP from the sowing period of October was higher in all treatments compared to the sowing period of December. At October, the effect of glyphosate on the treatments was not verified, because the treatment with absence of glyphosate obtained a lower yield of seeds in comparison to the treatments of ammonium salt and dimethyl amine. At December, the effect of the sequential application of glyphosate was verified, because the treatment with absence of glyphosate was superior to the other treatments for the yield of seeds. This may have happened due to the shorter vegetative stage that presented the sowing season of December, when there was a difference of 16 days of vegetative stage. The sequential application of glyphosate was closer to the reproductive stage, which may have caused a decrease in yield.

It is also observed that the treatment with isopropylamine salt was the treatment that had the greatest negative effect on YSP, NLP and NODP. Reports such as Santos et al., (2007a) show glyphosate based on isopropylamine salt has a greater negative effect and that high doses of this formulation may impair nodulation and other plant characteristics that affect final yield. In contrast, Correia and Durigan (2007) did not evidenced injuries in glyphosate-resistant soybean plants when glyphosates based on isopropylamine salt and aminoacid salt were applied in vegetative stages.

The correlation estimates aimed to reveal the magnitude and the direction of the associations between the evaluated characters that were submitted to the applications of different salts of glyphosate. The linear correlation performed for eight characters revealed 36 associations, where 21 are significant (Table 5). In relation to NODT positive correlation with NODI, NODA, YSP, NLP and NSP, on the other hand were not negative for any character. Thus, there is a relationship between the number of nodules and yield, as affirmed by Fagan et al. (2007), due to the gain and assimilation of nitrogen.

Table 4. Interaction of the characters Number of active nodules (NODA), Height of the first legume insertion (HFL), Plant Height (PH), Number of legumes per plant (NLP), Number of seeds per plant (NSP), Yield of seeds per plant (YSP) in relation to the treatments of glyphosate formulations in each sowing period, Capão do Leão, RS, UFPel, 2018.

Glyphosateformulations	NODA		HFL (cm)		PH (cm)	
	2° FortnightofOctober	1° FortnightofDecember	2° FortnightofOctober	1° FortnightofDecember	2° FortnightofOctober	1° FortnightofDecember
Glyphosateabsence*	99 aA	55 bB	26.4 bA	27.7 bcA	120.4 aA	113.9 abA
Isopropylamine salt	72 bA	69 bA	28.9 abA	30.95 aA	119.4 aA	117.4 aA
Dimethyl amine salt	76 bA	59 bA	26.9 bB	30.5 abA	112.5 bA	116.4 aA
Potassium salt	97 aA	89 aA	30.4 aA	29.9 aA	118.7 abA	107.1 bB
Ammonia salt	88 abA	57 bB	29.1 aA	26.2 cB	120.7 aA	108 bB
CV%: 24.1						

Glyphosateformulations	NLP		NSP		YSP (g)	
	2° FortnightofOctober	1° FortnightofDecember	2° FortnightofOctober	1° FortnightofDecember	2° FortnightofOctober	1° FortnightofDecember
Glyphosateabsence*	94 bA[1]	75 aB	205 bcA	168 aB	35.0 cA	30.6 aB
Isopropylamine salt	81 cA	57 bB	176 dA	122 bB	31.2 dA	22.3 bB
Dimethyl amine salt	109 aA	55 bB	239 aA	118 cB	44.4 aA	21.7 bB
Potassium salt	92 bA	58 bB	201 cA	124 bcB	38.7 bA	22.7 bB
Ammonia salt	105 aA	64 bB	224 abA	141 bB	39.8 bA	27.2 aB
CV%: 25.7						

[1]Means followed by the same lowercase letter in colunm, and uppercase letter in line do not differ at 5% of probability.

Table 5. Linear correlation between the variables Number of nodules per plant (NODP), Number of active nodules (NODA), Height of the first legume insertion (HFL), Plant Height (PH), Number of legumes per plant (NLP), Number of seeds per plant (NSP), Mass of thousand seeds (MTS), Yield of seeds per plant (YSP). Capão do Leão, RS, UFPel, 2018.

Variáveis	NODI	NODA	HFL	PH	TSM	YSP	NLP	NSP
NODT[1]	0.57*	0.97*	0.09[NS]	0.05[NS]	0.08[NS]	0.169*	0.23*	0.22*
NODI		0.37*	0.07[NS]	0.07[NS]	0.09[NS]	0.438*	0.34*	0.38*
NODA			0.08[NS]	0.02[NS]	0.17[NS]	0.071[NS]	0.17*	0.14[NS]
HFL				0.19*	0.29[NS]	-0.15*	-0.2*	-0.24*
PH					0.36*	0.28*	0.22*	0.20*
TSM						0.11[NS]	0.08[NS]	0.04[NS]
YSP							0.89*	0.90*
NLP								0.98*

[1]* and [NS] significant and non-significant at 5 % of probability, respectively.

The magnitude of active nodules revealed a positive correlation coefficient only with NLP, meanwhile did not present a positive correlation coefficient with the other characters. High yields can be achieved with a larger number of legumes, as shown by Melo and Zilli (2009), the highest yield in cowpea with higher nodular activity. For the insertion height of the first legume, a positive correlation coefficient was found with PL, and negative with YSP, and NLP. The height of the plant was associated with positive correlation with TSM, YSP, NLP and NSP, on the other hand it did not show a negative correlation coefficient with the other characters. However, there is a positive relationship between plant height and height of insertion of the first legume (Vieira, 2007).

In this work, the highest yields were from plants with larger statures, this being determined by the genetic fraction. In the soybean crop the use of varieties with lower insertion of the first legume and greater height of the plant represents genotypes models, to achieve higher yields as Rigon et al. (2012) states. The TSM showed a positive correlation coefficient only with the PH. The YSP showed positive correlation coefficient with NLP and NSP, as well as NLP obtained positive correlation coefficient with NSP. According to Andrade et al., (2016), the NSP is linked to the NLP.

CONCLUSIONS

The isopropylamine salt influences nodular activity, number of vegetables and seeds per plant, as well as seed yield.

The sowing of soybeans at the recommended time propitiates smaller modifications face to glyphosate formulations.

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