Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 22 (2) : 2020 : 273-282

ISSN-0972-3005

CHANGES IN SOYBEAN PRODUCTIVITY UNDER DIFFERENT DURATIONS OF PRETREATED SEED INOCULATED WITH BRADYRHIZOBIUM ELKANII

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(Received 28 December, 2019; accepted 13 February, 2020)

Key words : *Nitrogen, Soil fertility, Soil microbiology, Modern sustainable agriculture, Dryland production, Integrated seed treatments.*

Abstract – This work aimed to establish the contribution on soybean productivity of seed inoculated with *Bradyrhizobium elkanii* applied at different days of pretreatment before planting, under field conditions in regions of Brazil. Fifty soybean field trials were conducted in the Brazilian States of Paraná, Goiás and Mato Grosso. In each site, seven seed treatments were established in combination with twelve chemical products [*i)* seeds without inoculation; *ii)* fertilization with 200 kg ha^{-1} N without rhizobia inoculation; *iii)* seeds inoculated with rhizobia at planting; *iv)* to *vii)* seeds inoculated with rhizobia and biological protectants applied at planting and at approximately 38, 52and 66 days before planting. Plant nodulation, vegetative growth and grain yield components were evaluated and the data analyzed based on ANOVA and regression procedures. Independent of the time of pretreatment of inoculant as a seed treatment, the combined use of the inoculant, with a specially formulated protectant, favored greater nodulation and grain productivity than the control without inoculant. These results validate that, under regular Brazilian soybean production conditions, pretreatment of seed with inoculant from 26 to 78 days before planting, using a formulation containing *Bradyrhizobium elkanii* strains, combined with chemicals and a specially formulated microbial protectant, is as effective as the inoculation applied at planting.

INTRODUCTION

A beneficial feature of soybean [*Glycine max* (L.) Merrill] is its ability to associate with rhizobia bacteria, that can be highly efficient delivering nitrogen, by the symbiosis of biological nitrogen fixation (Herridge *et al*. 2008; Thilakarathna and Raizada, 2017). Inoculants containing elite strains of *Bradyrhizobium spp.* for soybeans, have been widely used in South America, and strongly contribute to soybean nitrogen nutrition supporting high crop productivity (Hungria *et al*., 2006; Legget *et al*. 2017).

In Brazil, under current production practices, the regular application of inoculants containing *Bradyrhizobium sp*. promotes high productivity of crops. Recent studies show that, inoculation practices, can be performed, not only at planting, but also several days before planting, and in combination with fungicide, insecticide and nematicide seed treatments. Based on results from several studies by Anghinoni *et al*. (2017); Araujo *et al.* (2017); Machineski *et al*. (2018); Ribeiro Neto *et al.* (2018) and Silva *et al*. (2018), among others, the use of modern inoculation application results in similar nodulation and grain yield responses, compared to current inoculant formulations applied at planting. Other strategies to improve soybean yields, without adding chemical nitrogen fertilizers, are to co-inoculate *Bradyrhizobium spp*. with *Azospirillum brasilense* (Hungria *et al.* 2015a; Cerezini *et al*. 2016) or with other plant growth promoters (Buso, 2017), however these techniques are still under development.

It is known that rhizobia survival on seeds decreases as the duration of the pretreatment of seeds with rhizobial inoculant increase, because of oxidative and other physiological processes (Deaker *et al*. 2004). This behavior varies depending on environmental conditions, like temperature and humidity, as well as with the presence of compounds applied in combination with the inoculant, that protect the microbial cells (Bikrol *et al*. 2005; Hartley *et al*. 2012). Diverse fungicides can reduce the survival of *Bradyrhizobium sp*. cells when are applied on the seeds, and its severity increases when increasing the time of pretreatment applied on the seed (Campo *et al.* 2009). Several studies show that the use of selected additives facilitates the survival of inoculated rhizobia on seed when the seeds are also treated in combination with several chemical products. For example, Araujo *et al*. (2017) showed that pretreatment of soybean with inoculant, in combination with selected fungicides and insecticides, was feasible when the seeds were also treated with microbial protectant. The protective properties of biopolymers have been attributed to their ability to maintain water activity levels optimal for rhizobia survival, and their ability to limit heat transfer. Detailed studies are still needed, however, that consider complex and multiple interactions between seed and rhizobial cell biology, and the physicochemical properties of seed additives (O'Callaghan, 2016).

The crop nodulation and grain yield response to the inoculation practice is, under certain limits, directly related with the quantity and the quality of the rhizobia applied on the seeds. Any factor that reduces the population of inoculum rhizobia on the seeds and, consequently, nodulation, may decrease the contribution of biological nitrogen fixation (Hungria *et al*. 2015). Thus, the modern industrial inoculation processes, including the use of protective additives as well as specific seed handling and storage conditions, could favor the on-seed survivability of rhizobia until planting, maximizing the benefits of inoculants pretreated on seed. However, field information to support the use of these biological treatments on the seed is still scarce and based on limited environmental conditions.

Our objective was to describe how increasing the duration of the pretreatment of inoculant on the seeds with a formulation containing *Bradyrhizobium elkanii*, affects nodulation and grain production of soybean crops, cultivated under a wide range of Brazilian environmental conditions.

MATERIALS AND METHODS

During the 2016/17 growing season, fifty soybean field trials were performed along three Brazilian States (Paraná, Goiás and Mato Grosso) covering a wide range of agricultural production environments (Table 1). In all locations, the crops were grown following best recommended practices for high productivity, based on the use of PK fertilizers (Table 2), and weed, pest and disease preventive protection. The planting of soybean varieties (Table 1) were selected based on growing location. The selected sites were all under long-term agriculture

Table 1. Location and main crop management practices of 50 soybean field trials in the States of Paraná, Goiás and Mato Grosso, Brazil.

Sites	City, State	Latitude	Longitude	Previous crop	Soybean variety	Planting date
1, 4, 7, 10, and 13	Palmeira, PR	25°25'30"S	50°03'01"O	*Triticum aestivum*	M5947IPRO	Nov/14/2016
2, 5, 8, 11, and 14	Palmeira, PR	25°25'12"S	50°03'18"O	*Triticum aestivum*	M5947IPRO	Nov/17/2016
3, 6, 9, 12 and 15	Palmeira, PR	25°25'42"S	50°03'09"O	*Triticum aestivum*	M5947IPRO	Nov/22/2016
16, 17 and 18	Maringa, PR	23°22'57"S	52°03'45"O	*Avena sativa*	M5947IPRO	Dec/01/2016
19 and 20	Maringa, PR	23°22'57"S	52°03'45"O	*Avena sativa*	M5947IPRO	Dec/02/2016
21, 22 and 23	Paiçandu, PR	23°29'58"S	52°04'31"O	*Zea mays*	M5947IPRO	Dec/07/2016
24 and 25	Paiçandu, PR	23°29'58"S	52°04'31"O	*Zea mays*	M5947IPRO	Dec/08/2016
26, 29 and 32	Rio Verde, GO	18°00'26"S	50°31'02"O	*Zea mays*	M7739IPRO	Nov/15/2016
27, 30 and 33	Rio Verde, GO	17°49'55"S	50°50'48"O	*Zea mays*	M7739IPRO	Nov/17/2016
28, 31 and 34	Rio Verde, GO	17°29'55"S	50°37'34"O	*Zea mays*	M7739IPRO	Nov/20/2016
35 and 38	Rio Verde, GO	18°00'26"S	50°31'02"O	*Zea mays*	M7739IPRO	Nov/22/2016
36 and 39	Rio Verde, GO	17°49'55"S	50°50'48"O	*Zea mays*	M7739IPRO	Nov/25/2016
37 and 40	Rio Verde, GO	17°29'55"S	50°37'34"O	*Zea mays*	M7739IPRO	Nov/26/2016
41 and 42	Campo Verde, MT	15°42'40"S	55°21'29"O	*Brachiaria brizanta*	M7739IPRO	Nov/30/2016
43, 44, 45 and 46	Campo Verde, MT	15°42'39"S	55°21'30"O	*Brachiaria brizanta*	M7739IPRO	Dec/01/2016
47, 48, 49 and 50	Campo Verde, MT	15°42'41"S	55°21'28"O	*Brachiaria brizanta*	M7739IPRO	Dec/02/2016

crop rotation, including soybean crops, and the soils mostly classified as Cambisols and Ferralsols (designated Latossolo-type soil) (Table 2). During the studied season, the mean rainfall between November (planting) to March (harvest) varied between 888 and 1242 mm and no limitations for normal soybean growth were noted (Table 3).

In each location, the seven treatments studied were: *i)* seeds without inoculant (UTC); *ii)* seeds without rhizobia inoculant but fertilized with 200 kg ha^{1} N (46: 0: 0) and applied 50 % at planting and 50 % at the R1 growing stage (Fehr and Caviness, 1977) (N200); *iii)* seeds inoculated with *Bradyrhizobium japonicum* and *Bradyrhizobium diazoefficiens* at planting (IP) and *iv)* to *vii)* seeds inoculated with *Bradyrhizobium elkanii* and biological protectants, applied at planting (IST) and approximately at 38 (SAT), 52 (MAT) and 66 (LAT) days before planting (Table 3). In all the treatments, commercially available fungicides and insecticides were applied to the seeds (Table 4).

In the treatment *iii)* (IP) a commercial peat formulation containing *B. japonicum* (SEMIA 5079) and *B. diazoefficiens* (SEMIA 5080) with a concentration of 5.5 x 10^{9} colonies formation units g^{1}) was used at an application rate of 1.6 g kg^{-1} of seed. The seed treatments *iv)* to *vii)* were applied using industrial machines and at an application rate of 2.0 g kg^{-1} of seed with a peat inoculant containing *B. elkanii* (SEMIAS 587 and 5019 at a concentration of 2.1 x 10^{9} colonies formation units g^{-1}) in combination with the chemical seed treatments (Table 4), as well as specific polymers and other additives for microbial protection. The compounds used for microbial protection against osmotic damage with adhesive polymers are based on liquid formulations applied at 3.3 mL kg^{-1} of seed.

At the v2 to v3 growing stage (Fehr and Caviness, 1977), 5 adjacent plants were collected from each of the plots for counting the number of nodules located on the main root and lateral roots, and for measuring nodule dry matter. The main root and lateral root nodules were counted together from one plant and used as the total number of nodules, and when dried, was used as the dry matter of total nodules. The shoot height was measured from the soil surface to the upper node; this portion of the plant was dried and weighed and defined as shoot dry matter.

Table 2. Soil type and main properties (0 to 20 cm top layer) and applied fertilization treatments in 50 soybean field trials in the States of Paraná, Goiás and Mato Grosso, Brazil. Soil type based on Brazilian Soil Classification System (EMBRAPA, 2018), SOM = soil organic matter, Pe = soil extractable phosphorus.

Sites	Soil type	SOM	pH	Pe	Sand	Silt	Clay	Fertilizer	
		%		mg kg^{-1}	%			N-P-K	kg ha^{-1}
1, 4, 7, 10, and 13	Cambisolo	2.50	5.40	37.0	63.4	15.2	21.4	04-14-02	300
2, 5, 8, 11, and 14	Cambisolo	2.90	4.80	20.0	57.1	14.2	28.7	04-14-02	300
3, 6, 9, 12 and 15	Cambisolo	3.30	4.40	20.0	44.0	28.2	27.8	04-14-02	300
16, 17 and 18	Latossolo Vermelho distroférrico	1.26	5.10	12.8	70.0	5.0	25.0	04-28-08	300
19 and 20	Latossolo Vermelho distroférrico	1.26	5.10	12.8	70.0	5.0	25.0	04-28-08	300
21, 22 and 23	Latossolo Vermelho distroférrico	3.17	6.10	12.4	20.0	13.0	67.0	04-28-08	300
24 and 25	Latossolo Vermelho distroférrico	3.17	6.10	12.4	20.0	13.0	67.0	04-28-08	300
26, 29 and 32	Latossolo Vermelho argiloso	3.71	4.90	33.9	27.0	13.0	60.0	00-20-18	350
27, 30 and 33	Latossolo Amarelo argiloso	4.28	4.47	3.9	41.0	13.0	46.0	11-52-00	200
28, 31 and 34	Latossolo Roxo	5.41	5.08	16.6	27.0	12.0	61.0	00-20-10	400
35 and 38	Latossolo Vermelho argiloso	3.71	4.90	33.9	27.0	13.0	60.0	00-20-18	350
36 and 39	Latossolo Amarelo argiloso	4.28	4.47	3.9	41.0	13.0	46.0	11-52-00	200
37 and 40	Latossolo Roxo	5.41	5.08	16.6	27.0	12.0	61.0	00-20-10	400
41 and 42	Latossolo Vermelho Amarelo distrófico	2.13	6.00	15.6	69.0	6.6	24.4	05-25-25	200
43, 44, 45 and 46	Latossolo Vermelho Amarelo distrófico	2.13	6.00	15.6	69.0	6.6	24.4	05-25-25	200
47, 48, 49 and 50	Latossolo Vermelho Amarelo distrófico	2.13	6.00	15.6	69.0	6.6	24.4	05-25-25	200

Table 3. Duration of the pretreated anticipated seed inoculation treatments and monthly rainfall in 50 soybean field trials in the States of Paraná, Goiás and Mato Grosso, Brazil. SAT = short anticipated treatment, MAT = medium anticipated treatment, LAT = long anticipated treatment.

Sites	Planting date	Anticipated treatments (days before planting)			Rainfall (mm month^{-1})			
		SAT	MAT	LAT	November	December	January	February	March
1, 4, 7, 10, and 13	Nov/14/2016	26	40	54	129	182	263	212	139
2, 5, 8, 11, and 14	Nov/17/2016	29	43	57	129	182	263	212	139
3, 6, 9, 12 and 15	Nov/22/2016	34	48	62	129	182	263	212	139
16, 17 and 18	Dec/01/2016	43	57	71	37	215	334	141	161
19 and 20	Dec/02/2016	44	58	72	37	215	334	141	161
21, 22 and 23	Dec/07/2016	49	63	77	37	215	334	141	161
24 and 25	Dec/08/2016	50	64	78	37	215	334	141	161
26, 29 and 32	Nov/15/2016	28	42	56	169	200	335	241	297
27, 30 and 33	Nov/17/2016	30	44	58	169	200	335	241	297
28, 31 and 34	Nov/20/2016	33	47	61	169	200	335	241	297
35 and 38	Nov/22/2016	35	49	63	169	200	335	241	297
36 and 39	Nov/25/2016	38	52	66	169	200	335	241	297
37 and 40	Nov/26/2016	39	53	67	169	200	335	241	297
41 and 42	Nov/30/2016	43	57	71	22	260	269	254	243
43, 44, 45 and 46	Dec/01/2016	44	58	72	22	260	269	254	243
47, 48, 49 and 50	Dec/02/2016	45	59	73	22	260	269	254	243
Mean		**38**	**52**	**66**					

The main root length was measured from the soil surface to the root tip; this portion of the plant was dried and weighed and defined as root dry matter. The perimeter around the main root was removed in a soil block composed of a volume of 20 cm depth and 15 cm diameter and used for nodulation and root measurements. Each block was carefully washed with tap water over a sieve to capture loose roots and nodules. Nodules located in the main root were counted as those directly adhered to the main root or on a lateral root within 1.25cm to the main root.

During the early reproductive (R1) growth stages (Fehr and Caviness, 1977) the total nitrogen content was measured from 30 to 35 plants per plot using the upper developed leaves. After physiological maturity of the crops, the measurements performed in each of the plots were grain nitrogen, grain number, grain weight, grain yield and nitrogen yield. The number of grains per unit area was estimated from the ratio between the grain yield and the single grain weight; the grain productivity was calculated at 13.5% moisture content; and the nitrogen yield was calculated by multiplying grain nitrogen by grain yield.

The studies were configured using a randomized complete block design in 24 m^2 plots (8 rows with 0.5 m row spacing and 6 m long) with separation of 1 m between plots. The analysis of variance (ANOVA) of all the data was performed using the mean across 50 locations with the differences between means determined using the Scott Knott test at a significance level of 0.10 (Canteri *et al*. 2001); correlation and regression analysis were calculated between selected variables. The efficacy of the inoculation treatments, also named as the percent of positive cases, was calculated as the percentage of experiments with positive absolute increments in grain production compared with the treatment without rhizobia inoculation.

RESULTS

Early nodulation and vegetative growth

In all field trial sites, nodules were observed on both the main root and the laterals roots of soybean plants in all treatment groups; differences in nodule number were observed between the main and lateral roots (Table 5). The number of nodules per plant varied between 3 and 102, with little relationship between nodules located on the main root and the lateral roots. When the nodulation was measured across the fifty field trial sites, nodulation was lowest when 200 kg ha^{-1} was applied or in the absence of inoculation (Table 5). However, regardless of the time of pretreatment with inoculant, the use of a seed treatment inoculant combined with chemical seed treatment produced plants with more number of nodules found in the main root than that of the control without inoculation (Table 5). The nodules observed on the main roots varied across treatment groups from 31 to 36 nodules plant^{-1}, however, seeds treated with inoculant at the short, medium and long pretreatment times, developed the most nodules (34 to 36 nodules plant^{-1}). The mean dry matter of the nodules varied across treatment group from 100 and 132 mg plant^{-1} with pretreated seeds generating greater nodule dry matter (Table 5). The available information did not reveal differences in the mass of nodules across treatment groups when pretreating seeds with inoculant (Table 5).

At the v2-v3 growth stages, the mean height of plants across treatment groups was 22.0 cm, and the mean length of roots was 13.5 cm (Table 6). Although differences in plant nodulation were observed, in general, no differences in the early growth of the plants was observed among the seed treatment groups (Table 6).

Grain production and yield components

Across the 50 trial locations, the soybean grain yields ranged from 1516 to 6555 kg ha^{-1}, however, using the mean grain yield across the 50 trial

Table 4. Chemical seed treatments in 50 soybean field trials in the States of Paraná, Goiás and Mato Grosso, Brazil.

Sites	Active ingredients (concentration in g l^{-1})	Doses (mL kg^{-1})
1, 2, 3, 16, 21, 26, 27, 28, 41, and 42	Fipronil (250), Pyraclostrobin (25), Thiophanate metyl (225)	2.0
4, 5, 6, 17, 22, 29, 30, 31, 43 and 44	Imidacloprid (150), Thiodicarb (450)	5.0
	Carbendazim (150), Thiram (350)	2.0
7, 8, 9, 18, 23, 32, 33, 34, 45 and 46	Chlorantraniliprole (625)	1.0
	Carbendazim (150), Thiram (350)	2.0
10, 11, 12, 19, 24, 35, 36, 37, 47, and 48	Abamectin (500)	1.0
	Thiametoxan (350)	2.0
	Metalaxyl-M (10), Fludioxonil (25)	1.0
13, 14, 15, 20, 25, 38, 39, 40, 49 and 50	Cyantraniliprole (600)	1.0
	Thiametoxan (350), Metalaxyl-M (10)	2.0
	Fludioxonil (25)	1.0

Table 5. Effects of seed inoculum pretreatment on the nodulation of soybean crops. Mean of 50 field trials in the States of Paraná, Goiás and Mato Grosso, Brazil. NMR = nodules in the main root, NLR = nodules in lateral roots, TN = total number of nodules, MRNDM = dry matter of nodules from the main root, LRNDM = dry matter of nodules from lateral roots, TNDM = dry matter of total nodules. UTC = untreated control, N200 = untreated control with Nitrogen fertilization, IP = inoculated control at planting, IST = industrial seed treatment at planting, SAT = short anticipated treatment, MAT = medium anticipated treatment, LAT = long anticipated treatment. CV = coefficient of variation. In each column, different letters show significant differences between treatments (Scott-Knott, p<0.10).

	NMR	NLR	TN	MRNDM	LRNDM	TNDM
Treatments	nodules plant^{-1}			mg plant^{-1}		
UTC	10.1 c	20.9 b	31.0 b	40.6 b	65.7 b	106.2 b
N200	8.4 d	18.4 c	26.8 c	38.9 b	61.7 b	100.6 b
IP	11.0 b	21.8 b	32.8 b	52.4 a	77.0 a	129.4 a
IST	11.8 b	22.8 a	34.6 a	52.7 a	71.6 a	124.3 a
SAT	12.7 a	23.4 a	36.1 a	49.1 a	82.7 a	131.9 a
MAT	12.4 a	21.7 b	34.1 a	50.0 a	74.3 a	124.3 a
LAT	11.7 b	21.5 b	33.3 b	46.7 a	73.9 a	120.6 a
CV (%)	29.2 20.0	19.4 40.8	34.4 26.3			

locations revealed differences between the control treatment group and pretreated seed treatment groups. On average, the productivity of crops pretreated with inoculant was 14 % greater the control treatment without inoculant, with 73 % of positive cases, independent of the pretreated time of application (Table 7). The number of harvested grains ranged from 1270 to 6679 m^2 across the 50 trial locations and was greater in the crops pretreated with inoculant, mainly when the inoculant was specially formulated for microbial protection (Table 7). No significant differences in the weight of the single grains was observed between the studied treatments (Table 7). The concentration of nitrogen in the grains showed small variability among the studied sites and treatment groups, and it was approximately 1 % greater in the crops with pretreated inoculant on seed (Table 7). The total nitrogen production of the crops ranged from approximately 91 to almost 390 kg ha^{-1} across the 50 locations, and, in average, total nitrogen was 15 % greater in the crops pretreated with inoculant than in the treatment groups without the application of inoculant (Table 7). The use of the inoculant specially formulated for microbial protection, both at planting or seeds pretreated prior to planting, improves nitrogen production compared to the control without inoculant seed treatment (Table 7).

Differences in nodulation parameters (i.e. number or dry matter) demonstrated a correlative trend with grain productivity of soybean across the 50 trial locations (Fig. 1) among the treatment groups. In general, approximately 12 nodules per plant, or, a nodule dry matter of approximately 80

Table 6. Effects of seed inoculum pretreatment on soybean vegetative growth and evaluated during v2-v3 growth stages. Mean of 50 field trials in the States of Paraná, Goiás and Mato Grosso, Brazil. UTC = untreated control, N200 = untreated control with Nitrogen fertilization, IP = inoculated control at planting, IST = industrial seed treatment at planting, SAT = short anticipated treatment, MAT = medium anticipated treatment, LAT = long anticipated treatment. CV = coefficient of variation. In each column, different letters show significant differences between treatments (Scott-Knott, p<0.10).

Treatments	Shoot measurements		Root measurements	
	Height cm	Dry matter kg ha^{-1}	Length cm	Dry matter kg ha^{-1}
UTC	22.3 a	1064 a	13.8 a	165.9 a
N200	22.4 a	1047 a	14.0 a	163.8 a
IP	22.2 a	990 b	13.9 a	161.9 a
IST	22.5 a	1050 a	14.1 a	160.4 a
SAT	22.0 a	1084 a	13.7 a	160.5 a
MAT	22.0 a	1061 a	13.6 a	163.7 a
LAT	22.2 a	1090 a	13.8 a	170.3 a
CV (%)	6.8	16.1	10.9	15.5

Table 7. Effects of soybean seed inoculum pretreatment on grain yield components and nitrogen grain content. Mean of 50 field trials in the States of Paraná, Goiás and Mato Grosso, Brazil. UTC = untreated control, N200 = untreated control with Nitrogen fertilization, IP = inoculated control at planting, IST = industrial seed treatment at planting, SAT = short anticipated treatment, MAT = medium anticipated treatment, LAT = long anticipated treatment. CV = coefficient of variation. In each column, different letters show significant differences between treatments (Scott-Knott, p<0.10).

Treatments	Grain N (g kg^{-1})	Grains (number m^{-2})	Grains weight (mg $grain^{-1}$)	Grain yield (kg ha^{-1})	N yield (kg ha^{-1})
UTC	6.08 b	2595 b	139.7 a	3173.9 c	193.0 c
N200	6.14 a	2705 b	139.5 a	3238.5 c	198.8 c
IP	6.14 a	2679 b	137.8 a	3451.4 b	211.9 b
IST	6.16 a	2867 a	138.7 a	3761.4 a	231.7 a
SAT	6.16 a	2752 a	137.7 a	3732.3 a	229.9 b
MAT	6.12 b	2756 a	138.3 a	3609.6 b	220.9 b
LAT	6.18 a	2776 a	139.0 a	3549.5 b	219.4 b
CV (%)	1,6	11.7	4.4	15.8	16.1a

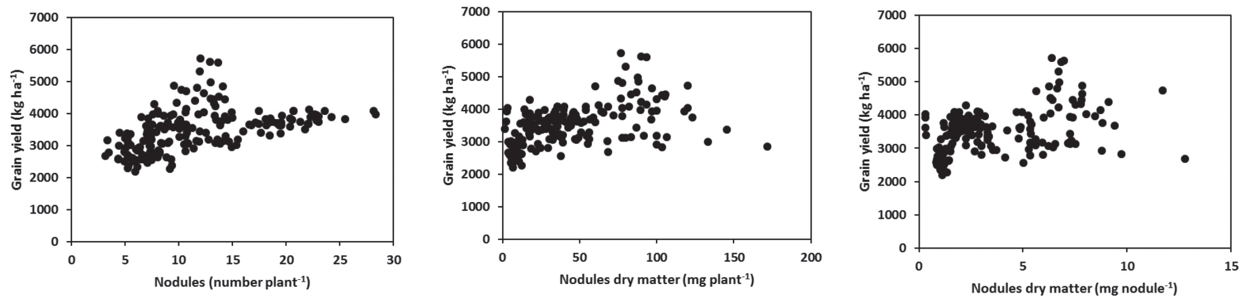

Fig. 1. Relationship between nodulation and soybean grain production in 50 dryland production sites from Brazil.

mg per plant, supported achieving maximum grain yield, under the studied conditions (Fig. 1).

When using a pretreatment of seeds with an inoculant specially formulated for microbial protection, the pretreatment of 26 to 78 days modified the nodulation as well as the grain productivity (Fig. 2). No significant effects of the duration of pretreatment with inoculant was observed on nodule dry matter (Fig. 2). While the number of nodules in the main root decreased with increasing duration of inoculant pretreatment ($p<0.05$), the rate of reduction in grain yield was modest over the same duration of inoculant pretreatment ($p<0.15$) (Fig. 2).

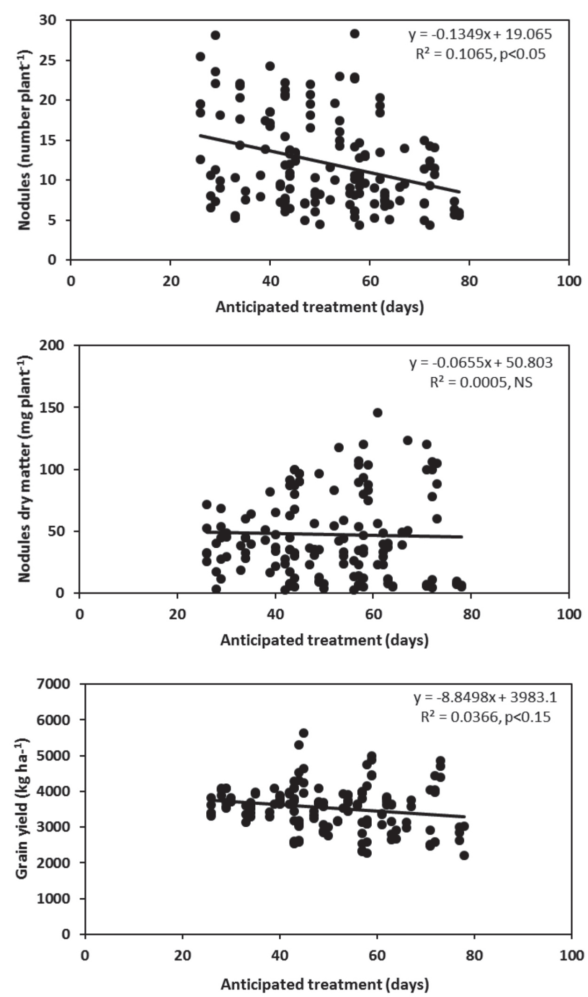

Fig. 2. Soybean nodulation and productivity in relation with the duration of anticipated seed inoculation in 50 dryland production sites from Brazil.

DISCUSSION

The interest and need of pretreatment of soybean with rhizobia inoculant for soybean production in Brazil, and other regions, is increasing and its feasibility depends, not only on the composition of the applied inoculants, but also the conditions for the application of the products and for the storage of the treated seeds until planting (Deaker *et al*., 2012). In the current study, the process of pretreating seeds with an inoculant, combined the use of a special inoculant formulation for microbial protection with compatible seed treatments (Table 4), while carefully considering the sequence and timing for the application of the products, as well as, storing the treated seeds in standard storage facilities close to each of the experimental sites (Table 1).

The field evaluation of the crops showed differences in nodulation, crop production and nitrogen accumulation in response to the applied treatments, mainly due to the use of the inoculant; with minor differences, due to increasing the duration of the pretreatment of seeds with inoculant (Tables 5, 6 and 7). The studied sites were all planted in soils with previous soybean crop rotation and rhizobial inoculation; nodule formation occurred in the uninoculated control treatments due to symbiosis with native soil rhizobial bacteria (Table 5). Bulegon *et al*. (2016), in Paraná (Brazil), also observed that non-inoculated (control) soybean plants showed nodules in response to native soil rhizobia strains, but less nodulation that when using inoculation treatments with *Bradyrhizobium* *japonicum*. In this study, the number of the nodules formed on the main roots, the grain number and production increased in response to the inoculation seed treatments, compared with the untreated control (Tables 5, 6 and 7). It is recognized that seeds treated with rhizobial inoculum favors nodule formation, and occupies the main roots of soybean with the selected strains (Mc Dermott and Graham, 1989). Thus, in this study, we demonstrate more nodules located in the main root when treating seeds with this special formulation for microbial protection indicating the successful contribution of rhizobia from the inoculation process in combination with other chemical seed treatments, within a range from 26 to more than 78 days of duration when pretreated with inoculum before planting.

The application of the studied inoculant formulation, specially formulated for microbial protection, provided more production and nitrogen accumulation in the grains than when the standard inoculation treatment was applied at planting (Table 7), suggesting the beneficial effects of use of these types of seed treatment formulations on rhizobial performance under field conditions. In general, the responses to the inoculation agreed with the mean productivity gain expected under Brazilian environmental conditions, in response to yearly inoculation of soybean crops, and on lands with a history of previous cultivation (EMBRAPA Soja, 2013). Part of the variability in crop productivity may be related to the number of nodules and nodule biomass per plant, suggesting that, under the studied environmental conditions, a minimum of 11 to 12 nodules on the main root per plant or the accumulation of at least 80 mg of nodules in the main root could support attaining higher grain yields. Fernández-Canigia and Díaz-Zorita (2004), evaluated 370 soybean dryland management conditions in Argentina, and described similar trends in the relationship between productivity and nodulation. Their study concluded that mean grain yields in the range of 3,000 kg ha^{-1} were attained with at least 6.5 nodules on the main root per plant, equivalent to 21 % of the total nodulation located on the main root of the inoculated plants. The quantitative differences between their study and the current research is attributed to the more stressful environmental conditions in the Brazilian environments in this study, and the resulting greater grain yields.

In agreement with the expected results, the number of nodules formed on the main roots of the inoculated plants, decreased when increasing the duration of the inoculum pretreatment. However, the effect of the duration of the storage of treated seeds on the magnitude of grain yields was not significant (Fig.2). Souza *et al*. (2008) indicted that, under field observations, the dry weight of the nodules is related with the response of soybean plants to the symbiosis with *Bradyrhizobium sp*. In the current study, no significant change in nodule dry matter were observed when increasing the duration inoculum pretreatment supporting the idea that nodulation parameters may not affect grain yield Furthermore, other field studies also showed that the small reduction in nodulation parameters, due to stressful conditions at planting, has minor effects on crop production (Costa *et al*. 2013).

All the treatment groups with inoculum pretreatment on the seed, including the standard control treatment, showed positive responses in grain productivity, compared to uninoculated soybean control, This positive response in grain productivity confirms that, under the studied conditions, representative of current dryland Brazilian soybean management practices, that pretreatment of seeds with a specially formulated inoculum containing selected strains of *Bradyrhizobium elkanii* is possible when treated an average of 60 days before planting. These results support the desire, as described from a previous study by Hungria *et al*. (2015b), that demand for seeds pretreated with inoculum perform with new and developing technologies that, do not affect survival of inoculated bacteria, but also contribute to yield increase.

CONCLUSION

Under regular Brazilian soybean production conditions, the pretreatment of seeds with inoculant 26 to 78 days before planting, using a *Bradyrhizobium elkanii* strains specially formulated for microbial protection, and combined with chemical seed treatments is as effective as inoculation performed at planting.

Soybean seed inoculation increases nodulation on the main root, and has correlation to the increment in grain productivity observed. While nodulation and grain productivity diminished over increasing duration of seed pretreatment, the soybean response remained positive when compared to the control without inoculation.

These results support the feasibility of effectively implementing pretreatment of soybean with rhizobial seed treatment inoculum, under the tropical and subtropical regions of Brazil, providing integral benefits for the current crop production systems.

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