

Does *Azospirillum brasilense* and Stimulate® to improve the initial growth of wheat sown at greater depths?

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ABSTRACT: The sowing of the wheat crop is a delicate process given the reduced size of its seed. Errors in adjusting the depth of sowing of this crop may result in damage to the establishment and initial growth of the crop. This study aimed to evaluate the influence of the relationship between inoculation with *Azospirillum brasilense*, Stimulate® treatment and sowing depth on emergence and initial growth of wheat plants. The experimental design was a randomized complete block design with four replications, in a 4 x 4 factorial scheme. The first factor consisted of the following treatments: i) seed inoculation with *A. brasilense*, ii) seed treatment with Stimulate®, iii) inoculation of seeds with *A. brasilense* associated with the treatment of seeds with Stimulate® and iv) without inoculation and seed treatment. The second factor was constituted by four sowing depths, being: 1.0; 3.0; 5.0 and 7.0 cm. The treatment of seeds with Stimulate® favors the higher accumulation of dry mass of stem in wheat plants when sown in greater depths. Seeding at greater depths up to 7.0 cm resulted in the delayed emergence of seedlings, a smaller number of tillers and less dry matter accumulation in wheat plants.

Key words: biological fixation; diazotrophic bacteria; emergence; tiller; *Triticum aestivum* L.

Azospirillum brasilense e Stimulate® favorecem o crescimento inicial do trigo semeado em maiores profundidades?

RESUMO: A semeadura da cultura do trigo é um processo delicado tendo em vista o tamanho reduzido de sua semente. Erros no ajuste da profundidade de semeadura desta cultura podem resultar em prejuízos para o estabelecimento e crescimento inicial da cultura. Este estudo teve por objetivo avaliar a influência da relação entre a inoculação com *Azospirillum brasilense*, o tratamento com Stimulate® e a profundidade de semeadura sobre a emergência e o crescimento inicial de plantas de trigo. O delineamento experimental utilizado foi o de blocos ao acaso com quatro repetições, em esquema fatorial 4 x 4. O primeiro fator foi constituído pelos seguintes tratamentos: i) inoculação de sementes com *A. brasilense*, ii) tratamento de sementes com Stimulate®, iii) inoculação de sementes com *A. brasilense* associado ao tratamento de sementes com Stimulate® e iv) sem inoculação e tratamento de sementes. O segundo fator foi constituído por quatro profundidades de semeadura, sendo: 1,0; 3,0; 5,0 e 7,0 cm. O tratamento de sementes com Stimulate® favorece o maior acúmulo de massa seca de colmo em plantas de trigo quando semeado em maiores profundidades. A semeadura em maiores profundidades, até 7,0 cm, tem como consequência o atraso na emergência plântulas e, a redução do número de filhotes e acúmulo de massa seca em plantas de trigo.

Palavras-chave: fixação biológica; bactéria diazotrófica; emergência; filhote; *Triticum aestivum* L.

Introduction

Like other grasses, wheat is highly responsive to the use of nitrogen fertilizers. Currently the main source of nitrogen is urea, which in turn is a non-renewable source, derived from petroleum, representing a high cost for agricultural production (Bartchechen et al., 2010). Aiming at the supply of this nutrient to the wheat crop, alternatives are sought to chemical fertilizers, such as the bacteria of the genus *Azospirillum*, which are promoters of plant growth and have a beneficial role in the yield of agricultural crops, acting specifically in the biological fixation of nitrogen (Stets et al., 2015).

The inoculation of strains of *A. brasilense* can promote greater development of the shoot of the wheat, greater absorption of nutrients and minerals, besides synthesizing plant hormones essential for the development of plants (Rodrigues et al., 2014; Abbasi et al., 2015; Cohen et al., 2015). This set of characteristics allows a significant reduction in the use of non-renewable sources of nitrogen when associated with the use of *A. brasilense*.

Another technology that has been receiving attention from producers and technicians is the biostimulants. For wheat, there are already positive results from the use of biostimulants (Báez-Peres et al., 2015). These products are composed of natural or synthetic plant hormones, which act directly on the physiology of plants with the potential to increase their development (Calvo et al., 2014). The evolution stage of some cultivated plants makes it necessary to use a high technological level to achieve higher productivity, justifying the use of biostimulants in agriculture (Castro et al., 2009).

Stimulate® is a biostimulant consisting of plant regulators such as auxin, cytokinin and gibberellin, hormones mediators of physiological processes and with action that tends to provide the growth and development of plants, acting in the division, differentiation and cellular stretching, besides become more efficient the rate of absorption of water and nutrients and their use (Garcia et al., 2009).

Besides the use of diazotrophic bacteria and biostimulants, the success in the production of any crop is also related to the fast and uniform establishment of the plant stand, a factor that is directly related to the seeding depth. For wheat (*Triticum aestivum* L.), the time required to complete the emergency process is an important factor in the phenological development, growth, and productivity, as well as to influence the competitiveness with weeds present in the crop area (Wang et al., 2009).

It is recommended to sow the wheat between 2 and 5 cm deep (Embrapa, 2017). If the sowing is deeper than the adequate, it is necessary more time to complete the emergence, and there is greater potential for the attack of plagues present in the ground (Reis et al., 2007). If the sowing is shallower than the adequate, there is less contact of soil with seeds, and it results in damages to germination and emergence of the crop (Klein et al., 2008).

The work was based on the hypothesis that the inoculation of wheat with *A. brasilense* associated with the use of biostimulant like Stimulate® may favor the establishment and initial growth of wheat plants sown at greater depths. This study aimed to evaluate the influence of the relationship between inoculation with *A. brasilense*, treatment with Stimulate® and depth of sowing on emergence and initial development of wheat plants.

Material and Methods

Experimental condition

The experiment was conducted under a protected environment at the Agronomic Experimental Station of the State University of Mato Grosso do Sul, Cassilandia - MS (latitude: 19° 05' 30.50", longitude: 51° 5' 55.64" and altitude: 510 m). According to the climatic classification of Köppen-Geiger, the region presents a tropical climate with dry winter season (Aw).

The soil used is classified as Arenic Entisol (95 g kg⁻¹ clay, 50 g kg⁻¹ silt and 855 g kg⁻¹ sand). Prior to the implementation of the experiment, soil samples were collected from 0 - 20 cm depth to carry out the chemical analyzes and the results are as follows: pH_(CaCl2): 5.4; O.M.: 14.0 g dm⁻³; P_(resina): 2.0 mg dm⁻³; K_(Melich-I): 1.11 cmol_c dm⁻³; Ca_(KCl): 10.0 cmol_c dm⁻³; Mg_(KCl): 7.0; H+Al: 22.0 cmol_c dm⁻³; Al: 0.14 cmol_c dm⁻³; SB: 46.0%; S-SO₄: 2.0 mg dm⁻³; B: 0.08 mg dm⁻³; Cu: 0.60 mg dm⁻³; Fe: 8.00 mg dm⁻³; Mn: 5.70 mg dm⁻³; Zn: 0.30 mg dm⁻³.

Experimental design

The experimental design was a randomized complete block design with four replications, in a 4 x 4 factorial scheme. The first factor consisted of the following treatments: i) inoculation of seeds with *Azospirillum brasilense* (100 ml for 20 kg of seeds), ii) seed treatment with Stimulate® (120 ml for 20 kg of seeds), iii) seed inoculation with *A. brasilense* associated with seed treatment with Stimulate® and iv) control - without inoculation and seed treatment. The second factor was composed of four seeding depths, being: 1.0; 3.0; 5.0 and 7.0 cm.

Experimental conduction

Each experimental unit consisted of a pot with a volume of 5 dm³, totalizing 64 pots. The wheat cultivar used was Quartz, classified as bread type, of medium size (85 cm), medium season (145 days) and medium tillering. Seeds were treated with Carboxine + Tiram (Vitavax + Thiram SC) 30 days before the sowing. The dose used was 0.25 L for 100 kg of seeds. On the day of sowing, in the morning, the seeds were inoculated with *A. brasilense* and / or treated with Stimulate® and allowed to dry in the shade. The sowing was done on the same day, however, in the afternoon.

As a source of *A. brasilense* a liquid commercial inoculant containing the AbV5 and AbV6 strains was used, with 200 million cells per ml. The dose used for the liquid inoculant was 100 ml of the commercial product for 20 kg of seeds. The

commercial product Stimulate® is a biostimulant composed of the following plant regulators: Kinetin (0.09 g L⁻¹), Gibberellic acid (0.05 g L⁻¹) and 4-Indol-3-ylbutyric acid (0.05 g L⁻¹). The dose of Stimulate® used was 120 ml for 20 kg of seeds.

To perform sowing at depths determined as treatment, the dry soil was deposited on the bottom of the pots, and then ten seeds were distributed in each pot. Subsequently, the seeds were covered by a layer of dry soil equivalent to the depth determined as treatment. All pots were filled with the same amount of soil changing only the proportion of soil above and below the seed, according to the sowing depth.

The emergence of seedlings was noted out daily, and when the stabilization of the emergence was confirmed, thinning was performed. It was left only one plant per pot. Watering was performed daily, applying 180 ml of water per pot.

Ten days after the emergency (DAE), 75 mg dm⁻³ of K₂O, 131 mg dm⁻³ of P₂O₅ and 38 mg of dm⁻³ of N were applied. The fertilizer was diluted in water and then applied to the pot.

Experimental evaluations

After sowing, it was daily counting of seedlings emerged in each pot and, from the data obtained, the following variables were estimated:

a) emergence percentage - emerged seedlings were considered to have emerged aerial part, with size greater than 0.5 cm; b) Emergency speed index (ESI) - for the estimation of the emergency speed index, the equation proposed by Maguire (1962) was used:

$$ESI = \frac{N_1}{D_1} + \frac{N_2}{D_2} + \dots + \frac{N_n}{D_n} \quad (1)$$

where:

- N₁ - number of seedlings emerged on the first day;
- N₂ - number of seedlings emerged on the second day;
- N_n - accumulated number of emerged seedlings;
- D₁ - first counting day;
- D₂ - second counting day; and,
- D_n - number of days counted after sowing.

c) Mean emergence time (MET) - estimated according to the equation proposed by Labouriau (1983):

$$MET = \frac{\sum N_i + T_i}{\sum N_i} \quad (2)$$

where:

- N_i - number of seedlings emerged per day; and,
- T_i - time of evaluation (days).

At 51 days after the emergency, the following evaluations were performed:

d) Number of leaves per plant - obtained by counting the number of leaves; e) Number of tillers per plant - obtained by counting the total number of tillers; f) Main stem diameter

(mm) - obtained with pachymeter in the first internode of the main stem; g) Plant height (cm) - obtained by evaluating the height of the main stem with a ruler, it was considered the distance between the soil surface and the highest point of the plant;

After, the plants were cut close to the ground and sectioned into leaves and stems. The root system was washed in tap water with the aid of a fine mesh sieve. The leaves, stems, and roots were placed in a forced circulation oven, with a temperature of 65 °C for 72 h to obtain:

h) Leaf dry mass (g plant⁻¹); i) Stem dry mass (g plant⁻¹); j) Root system dry mass (g plant⁻¹); k) Shoot dry mass (g plant⁻¹) - estimated from the sum of the leaf dry mass and the stem dry mass; l) Total dry mass (g plant⁻¹) - estimated from the sum of the shoot dry mass and the root system dry mass ; m) Leaf area (dm² plant⁻¹) - was determined following the methodology proposed by Benincasa (2003). Ten leaf discs of the known area were removed from each experimental unit, which was considered as the leaf area of the sample (LAS). Then, the dry mass of the sample (DMS) and the leaf dry mass (LDM) were determined after drying in a forced air circulation oven at 65 °C for 72 h. Leaf area (LAR) was estimated with the following equation:

$$LAR = \frac{LAS \times (LDM - DMS)}{DMS} \quad (3)$$

Statistical procedures

Data were submitted to normality tests (Kolmogorov-Smirnov test) and homoscedasticity (Hartley test) and, when necessary, were transformed into \sqrt{x} . The data were submitted to analysis of variance, and the significance of the mean squares obtained was tested by the F test at the 5% probability level. The means were compared by the Tukey test at a 5% probability level. For the means related to the sowing depths, regression equations were adjusted. The significance of the regression coefficients was tested by Student's t-test at the 5% probability level.

Results and Discussion

It was observed the interaction of seed treatment with Stimulate® and inoculation with *Azospirillum brasilense* with sowing depth only for stem dry mass (Table 1). Seed treatment with Stimulate®, inoculation with *A. brasilense* and their association did not influence any of the evaluated variables (Table 1). With the exception for emergence percentage, stem diameter, and plant height, the influence of sowing depth was verified for all other variables evaluated (Table 1).

The inoculation with *A. brasilense* and / or the seed treatment with Stimulate did not influence the variables related to seedling emergence (Table 2). The average of emergency percentage was 93.6%, the average of emergency speed index was 1.60 and the mean emergency time was 6.09 days (Table 2). The number of leaves per plant, number

Table 1. Summary of variance analysis for emergence percentage (EMER), emergency speed index (ESI), mean emergence time (MET), number of leaves per plant (NLV), number of tillers per plant (NTL), stem diameter (SD), plant height (PHT), leaf area (LAR), stem dry mass (STD), leaf dry mass (LDM), shoot dry mass (SHD), root system dry mass (RSM) and total dry mass (TDM) of wheat plants submitted to the treatment of seeds with Stimulate® and / or inoculation with *Azospirillum brasilense* and sown at different depths.

V.F.	Mean square						
	EMER	ESI	MET	NLV	NTL	SD	PHT
Seed treatment (T)	0.46 ^{ns}	0.62 ^{ns}	0.43 ^{ns}	0.72 ^{ns}	1.46 ^{ns}	0.16 ^{ns}	0.02 ^{ns}
Sowing depth (D)	1.27 ^{ns}	19.96 ^{**}	43.00 ^{**}	7.74 ^{**}	9.47 ^{**}	2.15 ^{ns}	2.77 ^{ns}
Interaction (T x D)	0.98 ^{ns}	0.23 ^{ns}	0.64 ^{ns}	1.65 ^{ns}	1.68 ^{ns}	1.19 ^{ns}	1.05 ^{ns}
C.V. (%)	5.26	7.11	4.10	14.28	14.95	8.85	8.75
	LAR	STD	LDM	SHD	RSM	TDM	
Seed treatment (T)	1.04 ^{ns}	0.86 ^{ns}	1.05 ^{ns}	1.04 ^{ns}	0.46 ^{ns}	0.76 ^{ns}	
Sowing depth (D)	6.70 ^{**}	12.04 ^{**}	6.69 ^{**}	9.77 ^{**}	5.48 ^{**}	7.96 ^{**}	
Interaction (T x D)	0.74 ^{ns}	3.03 ^{**}	0.74 ^{ns}	1.31 ^{ns}	0.71 ^{ns}	1.03 ^{ns}	
C.V. (%)	23.55	19.10	23.65	19.67	18.98	18.19	

**significant at 1% probability by the test F. not significant by the test F. C.V - coefficient of variation.

of tillers per plant, stem diameter, plant height, and leaf area were not influenced by inoculation with *A. brasilense* and / or seed treatment with Stimulate® (Table 2). The inoculation with *A. brasilense* and / or the treatment of seeds with Stimulate did not influence the variables related to plant dry mass (Table 2).

Among the reasons that may explain the non-effectiveness of the *A. brasilense* bacteria about the evaluated variables, it can be mentioned that the low organic matter content (14 g dm⁻³) contained in the soil used in the experiment may have been the limiting factor. When these bacteria are associated with humic acids, there is an increase in the initial development of wheat plants (Rodrigues et al., 2014). Also, the seeds used in the experiment were treated with fungicides, which, according to Vogel et al. (2015), may be an inhibitory factor of inoculation.

The absence of response of the wheat to the treatment of seeds with Stimulate® may be associated to the non-efficiency of the stimulants to the low or non-absorption of the same by the seeds, and may be caused by the increase in the sensitivity of the cell membrane of the seed due to the accumulation

of plant hormone or even by the inhibitory effect of one hormone on another when at high concentrations (Moterle et al., 2011). Abati et al. (2014) found similar results for seed germination treated with Stimulate®, in which there was no influence of biostimulant on germination and growth characteristics of wheat plants.

The sowing depth did not influence the percentage of the emergence of wheat seedlings. However, a decrease was observed in the emergence speed index (ESI) and an increase in the mean emergence time (Figure 1A and 1B) as sowing was performed in greater depths. It was estimated that for each centimeter that the sowing depth increases, there is a reduction of 4.8% of emergence speed index and an increase of 6.4% in the mean emergence time of wheat (Figure 1A and 1B). This is due to the higher demand for seed energy reserves for the germination and emergence stages (Han et al., 2017), which also delay the development of the aerial part, responsible for producing photo-assimilates that will be translocated in the plant.

In quinoa seeds, it was observed higher hydrolysis of the starch reserves 24 h after seed hydration, with subsequent

Table 2. Emergence percentage (EMER), emergency speed index (ESI), mean emergence time (MET), number of leaves per plant (NLV), number of tillers per plant (NTL), stem diameter (SD), plant height (PHT), leaf area (LAR), leaf dry mass (LDM), shoot dry mass (SHD), root system dry mass (RSM) and total dry mass (TDM) of wheat plants submitted to the treatment of seeds with Stimulate® and / or inoculation with *Azospirillum brasilense* and sown at different depths.

Treatments	EMER (%)	ESI	MET (days)	NLV (n° plant)	NTL	SD (mm)
	Control	93.75	1.62	6.04	15.31	4.25
Stimulate® (S)	91.88	1.54	6.19	16.94	5.06	2.18
<i>A. brasilense</i> (A)	93.13	1.60	6.02	17.63	4.81	2.20
S+A	95.63	1.64	6.09	16.75	4.44	2.11
Average	93.59	1.60	6.09	16.66	4.64	2.16
	PHT (cm)	LAR (dm ² plant ⁻¹)	LDM	SHD	RSM	TDM
Control	27.88	1.19	0.38	0.57	0.65	1.22
Stimulate® (S)	27.75	1.43	0.45	0.66	0.74	1.40
<i>A. brasilense</i> (A)	27.97	1.49	0.49	0.70	0.76	1.46
S+A	27.88	1.41	0.45	0.66	0.68	1.34
Average	27.87	1.38	0.44	0.65	0.71	1.35

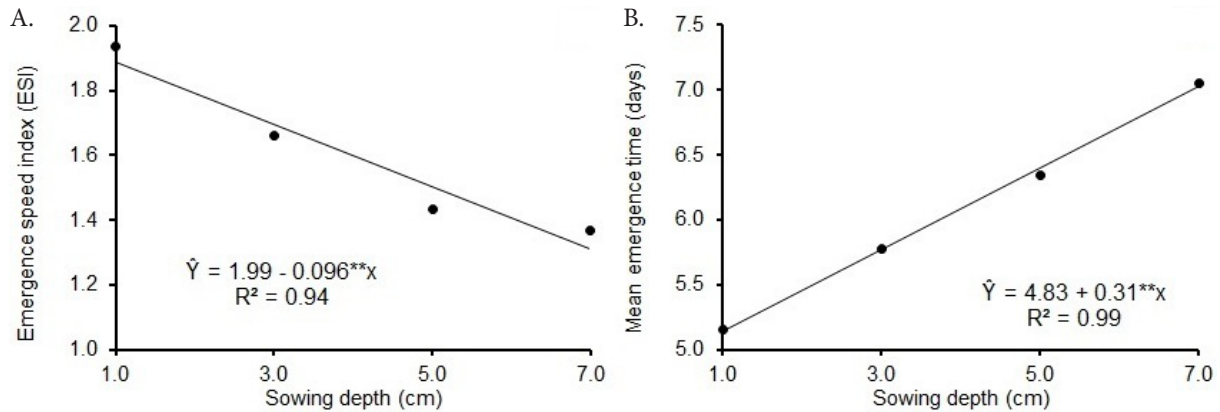


Figure 1. Emergence speed index (A) and mean emergence time (B) of wheat seedlings sown at different depths.

reduction of the rates of this reaction up to 72 h after hydration. This also occurs for other crops such as avocado and wheat itself, culminating in a marked fall in the reserves of this compound and consequent increase in the amount of sugars used in the early development of the seedling (Hager et al., 2014). Thus, the long period observed in the present study for the emergence of wheat plants submitted to sowing at greater depths around 168h can lead to energy deficit for the continuity of organ development.

The number of leaves and tillers per plant were higher when sown at lower depths, decreasing as sowing was performed at greater depths. It was estimated that for each centimeter that the sowing depth increases, there is a reduction of about 5.5% in the number of leaves per plant and 6.1% in the number of tillers per plant (Figure 2A and 2B).

The soil deposited on the seed at the time of sowing acts as a physical barrier, which must be transposed so that the seedling can emerge. During this process, there is a high demand for energy, which comes from the reserves found in the endosperm of the seed and in the embryo itself (Taiz et al., 2017). Thus, little reserve remains available for the plant to develop its photosynthetic and root organs, which will allow the onset of its autotrophic activity (Taiz et al., 2017).

For seedlings of wheat at different depths, it was found that seeds deposited at 8 cm depth resulted in lower plants, compared to those sown at 4 cm depth (Alam et al., 2014). Similar results were also observed for the maize crop, for

which 6 and 12 cm seedlings were used, which originated plants with higher and lower height, respectively (Hussem et al., 2013).

In relation to the unfolding of the treatment and inoculation of wheat seeds within the depth of sowing factor, it was not verified influence of the inoculation with *A. brasilense* and / or the treatment of seeds with Stimulate[®] on the stem dry mass at the depths of sowing of 1.0, 3.0 and 5.0 cm (Table 3). At the seeding depth of 7.0 cm, a higher stem dry mass was verified with the Stimulate[®] seed treatment about the combination of the seed treatment with Stimulate[®] and the inoculation with *A. brasilense* (Table 3).

The ratio of the plant hormones presents in the commercial product Stimulate[®] favored the accumulation of stem dry mass under conditions of greater energy expenditure to

Table 3. Dry stem mass of wheat plants submitted to seed treatment with Stimulate[®] and / or inoculation with *Azospirillum brasilense*.

Treatments	Sowing depth (cm)			
	1	3	5	7
Control	0.203	0.308	0.102	0.120 ab
Stimulate [®] (S)	0.197	0.215	0.204	0.230 a
<i>A. brasilense</i> (A)	0.302	0.229	0.164	0.148 ab
S + A	0.305	0.270	0.203	0.064 b
Average	0.204			

Different lowercase letters in the columns indicate significant difference by the Tukey test at the 5% probability level.

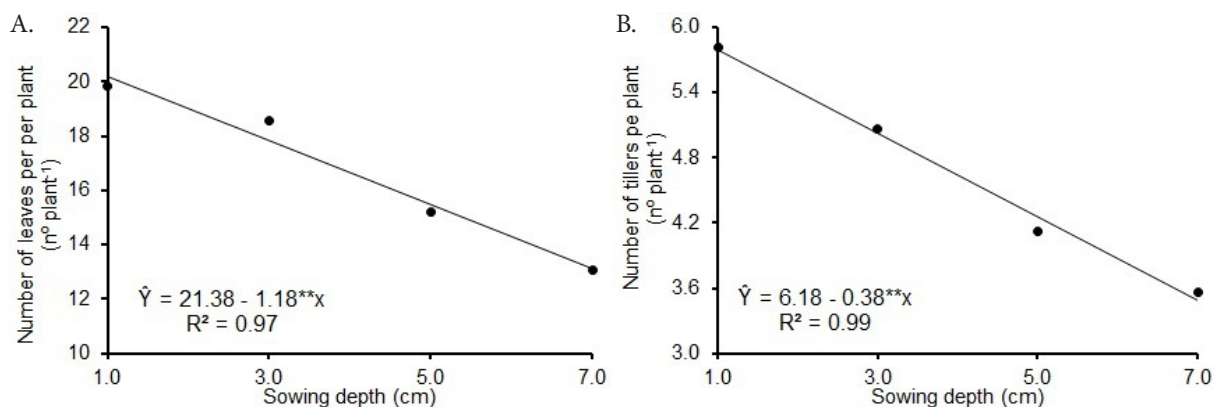


Figure 2. Number of leaves (A) and number of tillers (B) per wheat plant sown at different depths.

complete the seedling emergence, represented by sowing at 7.0 cm depth (Table 3). The low auxin: cytokinin ratio, as in the commercial Stimulate® product (0.56) favors the formation of shoot

In conditions of lower energy expenditure, that is, with sowing at lower depths, the hormones provided by Stimulate® were not enough to promote a greater accumulation of dry mass of the stem about the other treatments.

The inferiority of the association of seed treatment with Stimulate® with inoculation of seeds with *A. brasilense* in relation to the treatment of seeds with Stimulate® isolated is because to the fact that the bacteria of the genus *Azospirillum*, have the capacity to synthesize phytohormone related to the plant development (Rodrigues et al., 2014; Abbasi et al., 2015; Cohen et al., 2015), however, when inoculation was carried out in isolation, the amount of phytohormone was not sufficient to influence the accumulation of dry mass in the stem. However, when associated with the phytohormones present in Stimulate®, there was an excess of phytohormone that was harmful to the accumulation of dry mass in the stem.

The deleterious effects of exposure to excessively high levels of plant hormone, in the form of commercial biostimulant products, were also observed for the cotton crop (Vendruscolo et al., 2018). In this study, it was demonstrated that, at doses above ideal for the development of the crop, there was a harmful interference with the height of the plants, stem diameter and mass accumulation of the plants during their initial development, independently of the cultivar used.

It was verified that the sowing when performed at greater depths, it results in lower leaf area and dry mass of leaves and aerial part of wheat plants (Figure 3A, 3B and 3D). At each centimeter that the seeding depth is increased, there

is reduction around 7.2, 7.3 and 7.7% for leaf area, dry leaf mass and shoot dry matter, respectively. In deeper sowing, more time was required, and there was a higher expenditure of reserve of the seed for the seedling to complete the emergence. This delay in the emergence associated with the less amount of available reserve that the seedling possessed after the emergence resulted in the lower initial growth of the plant, which can be observed by the values of leaf area, leaf dry mass and shoot dry mass.

These results can be verified by metabolomic analysis in which the mobilization of the present reserves both in the embryo and the endosperm of the wheat seeds, was observed during the first hours after the beginning of the germination process. It culminates in the breakdown of carbohydrates, proteins, lipids, among other sources, into sugars used in the establishment of the seedling (Han et al., 2017). In this way, the high initial availability of energy facilitates the rapid development of aerial and root structures of the seedlings until the beginning of photosynthetic processes and the absorption of nutrients. In cases where these two processes are established late, as observed under conditions of deep seeding, the demand for energy may not be supplied.

Regression equation adjustment for the stem dry mass as a function of sowing depth was not obtained when using seed treatment with Stimulate® (Figure 3C). For inoculation with *A. brasilense*, the inoculation with *A. brasilense* associated with seed treatment with Stimulate® and the control treatment, it was verified a reduction of the stem dry mass as the sowing depth increased. (Figure 3C).

Reduction of the dry mass of the root system and total dry mass of wheat plants was observed as sowing was performed at higher depths (Figure 4A and 4B). The reduction was

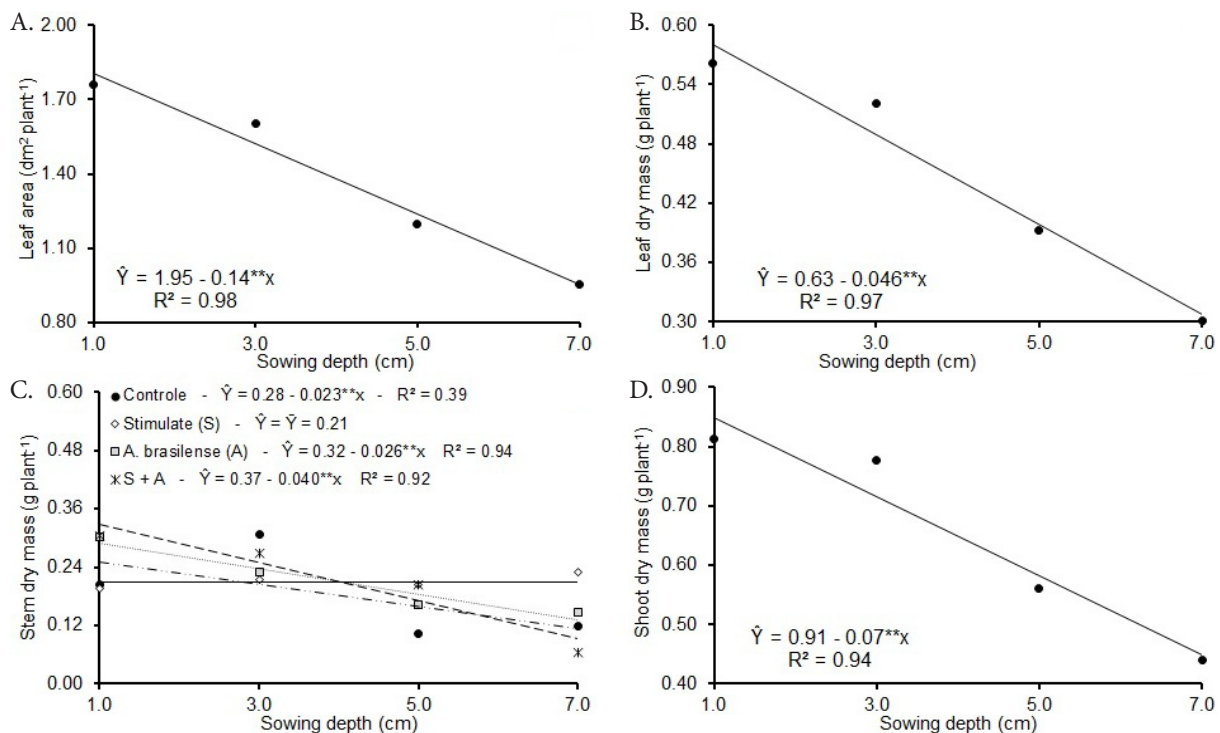


Figure 3. Leaf area (A), leaf dry mass (B), stem dry mass (C) and shoot dry mass (D) of wheat plants sown at different depths.

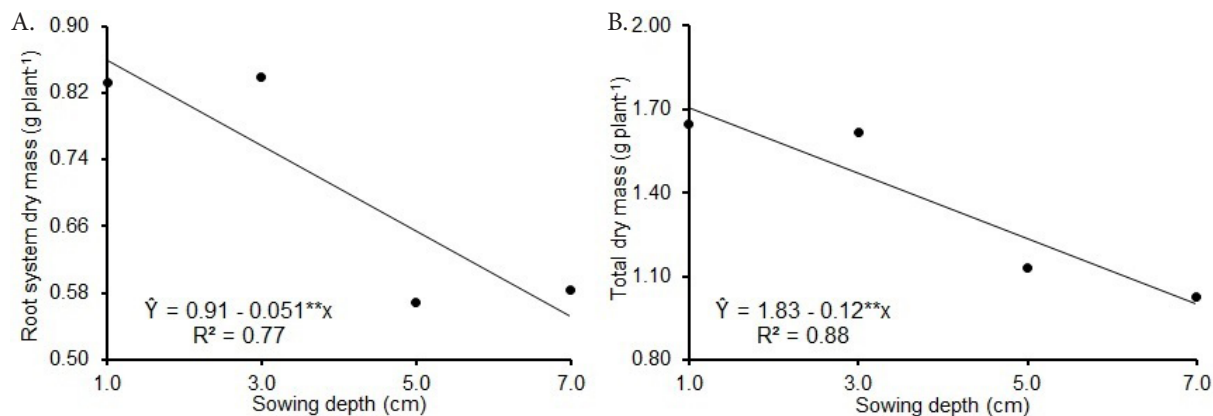


Figure 4. Dry mass of the root system (A) and total dry mass (B) of wheat plants sown at different depths.

around 5.6 and 6.6% for the dry mass of the root system and total for each centimeter that increases the seeding depth, respectively. These effects are linked to the lower amount of reserve observed in small seed species such as wheat. For these species, there is a need for less deep seeding, to optimize the development of plants (Pacheco et al., 2010). This fact was visualized for grass species, for which a better development and accumulation of dry mass was observed in the aerial organs when smaller seeding depths were used (Pacheco et al., 2010).

The inoculation with *Azospirillum brasilense* and treatment of seeds with Stimulate® isolated or combined did not improve the initial growth of the wheat crop, independently of the seeding depth adopted. Therefore, to reduce the cost of production, inoculation with *Azospirillum brasilense* and seed treatment with the Stimulate® in the wheat crop is not recommended.

The definition of wheat sowing depth should be carried out with caution, because sowing at greater depths does not alter the emergence percentage of seedlings, but results in delayed emergence, less accumulation of dry mass of the plant and reduced number of tillers per plant. The last one being directly related to wheat yield (Liu et al., 2018).

Conclusions

The inoculation with *Azospirillum brasilense* and the treatment of seeds with Stimulate® isolated or combined did not improve the initial growth of the wheat crop, regardless of the seeding depth employed.

Seeding at greater depths up to 7.0 cm results in the delayed emergence of seedlings and the reduction of number of tillers and dry matter accumulation in wheat plants.

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