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Original Article

Azospirillum brasilense, urease inhibitor, and sulfur contributes on initial growth of wheat

Azospirillum brasilense, inibidor de urease e enxofre contribuem no crescimento inicial do trigo

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Abstract

Wheat (*Triticum aestivum* L.) is an annual crop with potential for cultivation expansion, requiring nutritional management that can favor the nitrogen use efficient and plant growth. We aimed to evaluate the initial growth of wheat in function of different nitrogen fertilization management, without or with *Azospirillum brasilense* and sulfur. The treatments consisted of the following management: CK (without cover fertilization), urea, urea with urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT), urea NBPT (80%) + ammonium sulfate (20%), without cover fertilizer + *A. brasilense*, urea + *A. brasilense*, urea NBPT + *A. brasilense*, and urea NBPT (80%) + ammonium sulfate (20%) + *A. brasilense*. Nutritional management contributed to phenotypic responses to shoot of the plants and culm diameter, especially compared to CK plants. Plants with NBPT + sulfur, without and with *A. brasilense* showed a higher chlorophyll index. CK plants and those only with *A. brasilense* had a lower number of tillers and numerically the shoot dry mass. Nutritional management with *A. brasilense*, urease inhibitor and sulfur contributed to the initial growth of wheat plants.

Keywords: ammonium sulfate, plant growth-promoting bacteria, nutritional management, N-(n-butyl) thiophosphoric triamide, tillers, urea.

Resumo

O trigo (*Triticum aestivum* L.) é uma cultura anual com potencial de expansão de cultivo, necessitando de manejos nutricionais que possam favorecer o uso eficiente do nitrogênio e o crescimento das plantas. Objetivamos avaliar o crescimento inicial do trigo em função de diferentes manejos da adubação nitrogenada, sem ou com *Azospirillum brasilense* e enxofre. Os tratamentos foram constituídos dos seguintes manejos: CK (sem adubação de cobertura), ureia, ureia com inibidor de urease N-(n-butil) tiofosfórico triamida (NBPT), ureia NBPT (80%) + sulfato de amônio (20%), sem adubação de cobertura + *A. brasilense*, ureia + *A. brasilense*, ureia NBPT + *A. brasilense*, ureia NBPT (80%) + sulfato de amônio (20%) + *A. brasilense*. O manejo nutricional contribuiu nas respostas fenotípicas da parte aérea das plantas e do diâmetro de colmo, especialmente em comparação as plantas CK. As plantas com NBPT + enxofre sem e com *A. brasilense*, apresentaram maior índice de clorofila. As plantas CK e aquelas apenas com *A. brasilense* tiveram menor número de perfilhos e numericamente a massa seca da parte aérea. O manejo nutricional com *Azospirillum brasilense*, inibidor de urease e enxofre contribuiu no crescimento inicial das plantas de trigo.

Palavras-chave: sulfato de amônio, bactéria promotora de crescimento, manejo nutricional, N-(n-butil) tiofosfórico triamida, perfilhos, ureia.

1. Introduction

Wheat (*Triticum aestivum* L., Poaceae), is an annual cultivated grass and its grains are rich in carbohydrates, making it one of the most consumed cereals in the world, whether in human consumption or in animal nutrition through bran. In Brazil, wheat consumption is greater than its production, making it necessary to import the cereal. However, according to estimates, this scenario could change in the coming years, due to the increase in production, which in the 2022/23 harvest reached 9,5 million t ha^{-1} (Brasil, 2023).

In order to increase productivity in cultivated areas, several studies focused on mineral nutrition have been carried out with wheat, especially in relation to nitrogen and sulfur fertilization as they are essential and have a synergistic relationship. Nitrogen (N) is the nutrient required in greatest quantity by the plant (Manschadi and Soltani, 2021), as it is an essential component of proteins, chitin, nucleic acids and cell walls, favoring the synthesis of photosynthetic pigments, amino acids,

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favoring photosynthetic metabolism and plant growth (Fornari et al., 2020; Huang et al., 2021).

However, N can be easily lost to the environment, through leaching in the form of nitrate, volatilization as ammonia, and in lower amount by nitrification and denitrification (Zanin et al., 2016; Lasisi et al., 2019). A technical strategy to mitigate losses due to volatilization of urea applied without incorporation into the soil is the use of urease inhibitor such N-(n-butyl) thiophosphoric triamide (NBPT), as it can delay the conversion to ammonia until is incorporated into the soil by rain (Cantarella et al., 2018; Lasisi and Akinremi, 2022).

Another important macronutrient for wheat is sulfur (S), generally using ammonium sulfate, which is directly linked to the formation of gluten, since its proteins are made up of sulfur amino acids methionine, cysteine and cystine, that is, the amount of S available to the plant will influence in the formation of strong or weak gluten, in addition to participating in protective metabolism (Bona et al., 2016). Sulfur also helps in the N uptake by plant, as it is involved in the reduction of nitrate (Frungillo et al., 2014). As they are essential nutrients and responsible for protein synthesis, mineral, and photosynthetic metabolism in plants, and N–S must be added in a balanced way to optimize their nutritional efficiency (Andrade et al., 2019).

With the aim of supplying the amounts of N required by the plant, the use of bioinputs such as plant growth-promoting bacteria (PGPB) is promising, as they act on mineral and physiological metabolism of plants (Mendonça et al., 2020; Dolkhani et al., 2022), in addition to meeting the sustainable development goals (SDGs). *Azospirillum brasilense* is one of the most used PGPB, being efficient in fixation N atmospheric (Hungria, 2011; Barbosa et al., 2021), in addition to favoring the availability of other essential nutrients for plants (Galindo et al., 2021) and stimulating the synthesis of phytohormones (Fukami et al., 2017; Zaheer et al., 2022).

Although wheat is a species of socioeconomic importance, the information on isolated effect or interaction of PGPB, urea, NBPT, and S for wheat were insufficient, we hypothesized that adoption of these nutritional management strategies can optimize the nutrition and physiological efficiency, reflected on plant growth. We aimed to evaluate the initial growth of wheat in function of different nitrogen fertilization management, without or with *A. brasilense* and sulfur.

2. Material and Methods

The experiment was carried from April to June 2023, in pots 7 kg (16 and 32.5 cm of diameter and depth, respectively) under a greenhouse at the Faculty of Agricultural Sciences, Universidade Federal da Grande Dourados-UFGD (22°11'43" S, 54°56'08" W, 450 m), in Dourados, Mato Grosso do Sul, Brazil. The experimental design used was completely randomized, consisting of eight treatments, with four replicates, and the experimental unit consisting of a pot with six plants in each.

The treatments consisted of the following nutritional management: CK (without cover fertilization), urea, urea with urease inhibitor N-(n-butyl) thiophosphoric triamide (NBPT), urea NBPT (80%) + ammonium sulfate (20%), without cover fertilizer + *A. brasilense*, urea + *A. brasilense*, urea NBPT + *A. brasilense*, and urea NBPT (80%) + ammonium sulfate (20%) + *A. brasilense*. The N dose was 500 kg ha^{-1} based in Mitchell and Sheehy (2018).

The variety used was IPR Potyporã, whose seeds were previously treated with Cropstar® and Certeza®. The sowing was carried out at a depth of 2.0 cm, in pots previously filled with Oxisol (USDA classification) corresponding to Distroferric Red Latosol (Brazilian classification). The soil presented 22.50, 16.25 and 61.25% of sand, silt and clay, respectively, and the following chemical attributes: pH $CaCl_2$= 6.30, P= 38.25 mg dm^3, K, Ca, Mg, H + Al, and cation exchange capacity: 0.34, 8.00, 2.72, 1.72, and 12.78 cmol$_c$ dm^3, respectively, S= 5.58 mg dm^3, B, Cu, Fe, Mn, and Zn= 0.26, 9.50, 25.20, 35.40, and 1.10 mg dm^3, and V%= 86.54.

A. brasilense was inoculated via seed at a dose of 250 mL ha^{-1}, with strains Abv5 and Abv6 (2 x 10^8 viable cells mL^{-1}). At 15 days after sowing (DAS), thinning was carried out and six plants were kept per pot. After 22 DAS, when the wheat was at the double ring stage, cover fertilization was carried out according to the treatments described. During the experimental period, irrigation was carried out maintaining 70% water retention capacity according to Souza et al. (2000).

From 28 to 56 DAS, weekly, stem diameter assessments were carried out using a digital caliper. From 42 days, every 7 days, until 56 DAS, a period in which the plants presented a flag leaf with a considerable area, chlorophyll index was determined using a portable chlorophyll meter SPAD 502 Minolta (Soil Plant Analyzer Development), between 8 and 9 am. After 56 DAS, number of tillers per plant was counted, and the shoot was subsequently harvested, packaged in Kraft® paper bags, and dried in a forced air circulation oven at 60 ± 5 °C for 72 hours, and weighed on a scale with thousandths of a precision (0.0001 g).

The data were subjected to analysis of variance (F test, $p \leq 0.05$), and the effect of nutritional management was analyzed individually at each evaluation time, and the means compared by Scott-Knott test ± standard error ($p \leq 0.05$), using Agroestat software.

3. Results and Discussion

We observed that phenotypic response of wheat plants at 56 DAS varied according to the management adopted (Figure 1), in which CK plants had a lower volume of shoot compared to plants in others managements, demonstrating that PGPB, NBPT, and sulfur were efficient to contributing in growth and vigor of plants, confirming our initial hypothesis.

The nutritional management influenced the culm diameter at 28, 35, and 56 DAS, the chlorophyll index at 49 and 56 DAS; in addition to the number of tillers at 56 DAS (Table 1). Conversely, did not influence the shoot dry mass of the plants ($p > 0.05$).

Regarding culm diameter, at 28 DAS the highest values were observed in plants cultivated with urea NBPT + sulfur and those only inoculated with *A. brasilense*. Conversely,

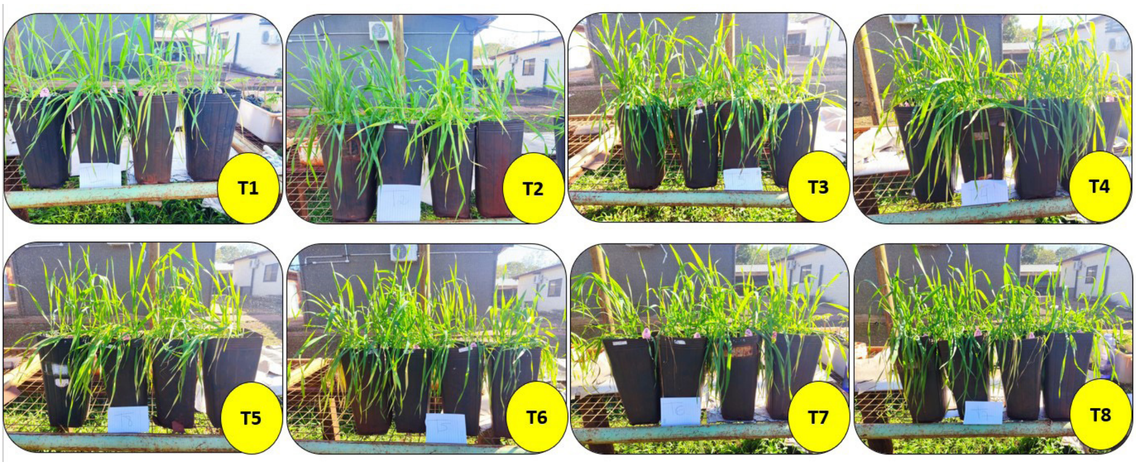

Figure 1. Visual appearance of wheat plants var. IPR Potyporã grown with different nutritional managements at 56 days after sowing. T1) CK (without cover fertilization), T2) urea, T3) urea with NBPT, T4) urea NBPT + ammonium sulfate, T5) without cover fertilization + A. brasilense, T6) urea + A. brasilense, T7) urea NBPT + A. brasilense, T8) urea NBPT + + ammonium sulfate + A. brasilense.

Table 1. Summary of the analysis of variance (p value) of the effect of treatments based on nitrogen fertilization management, inoculation of *Azospirillum brasilense* and sulfur on the initial growth and biomass of wheat variety IPR Potyporã.

Evaluation time	C.V. (%)	F	p
Culm diameter (mm)			
28 DAS	6.22	13.44	< 0.0001
35 DAS	9.04	10.13	< 0.0001
42 DAS	8.11	2.49	0.0453
49 DAS	8.16	2.64	0.0358
56 DAS	6.71	3.49	0.0101
Chlorophyll index (SPAD)			
42 DAS	5.98	1.22	0.3321
49 DAS	1,02	25,12	< 0,0001
56 DAS	5,23	6,96	< 0,0001
Number of tillers			
56 DAS	19.76	4.16	0.0039
Shoot dry mass (g per plant)			
56 DAS	25.48	2.19	0.0715

C.V. = coefficient of variation; F = F value, *p* = p-value.

the lowest values occurred in CK plants and with NBPT + sulfur + *A. brasilense* in same evaluation period (Figure 2a). At 35, 42, 49, and 56 DAS, only CK plants showed low culm diameter values compared to the other nutritional managements (Figures 2b-e), indicating an important role of managements adopted in increasing wheat growth during the initial cultivation cycle.

Wheat plants with only *A. brasilense* showed values that did not differ from the other management conditions adopted, including nitrogen fertilization, suggesting that this group of PGPB is an alternative that contributes positively to the nutritional management of wheat in this condition. *A. brasilense* has potential to biological N fixation, contributing to development of the root system, consequently, greater uptake of water and nutrients (Barbosa et al., 2021), reflecting in vegetative growth.

In addition, in literature there are reports that *A. brasilense* stimulates the synthesis of hormones such as auxin (Zaheer et al., 2022), which explains the higher diameter values in this condition. Although we have not performed a production cost analysis, believed that it is possible to obtain greater profitability owing to low costs with urea. Similarly, Galindo et al. (2020) described that inoculation with *A. brasilense* provided greater profitability in wheat production, regardless of N doses, similar to our results.

At 49 DAS, CK plants and those with urea and *A. brasilense* managed in isolation had lower chlorophyll index values

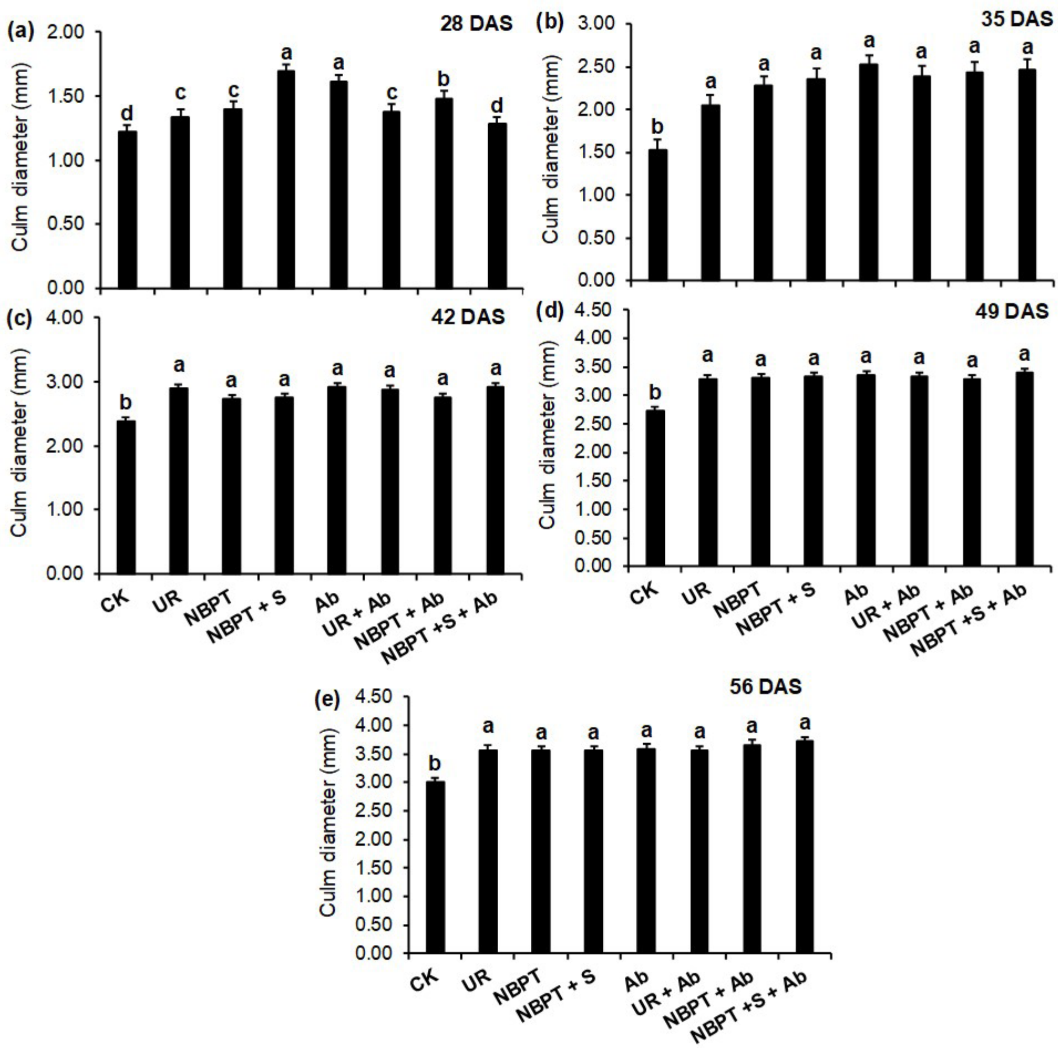

Figure 2. Culm diameter of wheat plants var. IPR Potyporã grown under different fertilization managements, without and with Azospirillum brasilense, during cultivation cycle. Different letters between columns differ statistically by Scott-Knott test ± standard error ($p \leq 0.05$). Ab: Azospirillum brasilense, CK: control, NBPT: N-(n-butyl) thiophosphoric triamide, S: ammonium sulfate, and UR: urea.

(Figure 3b), suggesting that although this group of PGPB contributes to growth, it is necessary to consider this practice as a supplement to physiological management. However, when combined they favor this response, N and S are necessary to obtain higher chlorophyll indices in wheat. Similarly, Prando et al. (2019) founded that the chlorophyll index had a linear increase according to the increase in nitrogen fertilization.

Fertilization with urea alone was not different from the control, and we suggest that this response may be associated with N losses to environment through volatilization as reported by Zanin et al. (2016), which did not occur in the management of urea with urease inhibitor. This is because NBPT acts by slowing down the hydrolysis of urea in the soil (Lasisi and Akinremi, 2022). In study to Cassimiro et al. (2020), NBPT contributed to minimizing ammonia ($N\text{-}NH_3$) losses through volatilization, corroborating our study.

At 56 DAS, wheat plants with NBPT + sulfur without and with *A. brasilense* showed a higher chlorophyll index value (> 40 SPAD), not differing statistically from plants with NBPT and sulfur (Figure 3c), demonstrating that presence of ammonium sulfate as a source of sulfur is important in increasing this characteristic, that is, it contributes to better photosynthetic efficiency and possibly production of photoassimilates, reinforced by the visual volume of shoot. Sulfur in addition to contributed to chlorophyll index and being involved in the reduction of nitrate, favoring the N uptake (Frungillo et al., 2014), also acts in the synthesis of amino acids, contributing to the growth of plants, including wheat (Sharma et al., 2024), similar to observed in our study with wheat.

The higher number of tillers occurred in plants that received nitrogen fertilization, differing statistically from those cultivated without nutritional management (CK) and only inoculated with *A. brasilense* (< 3.00 tillers per plant),

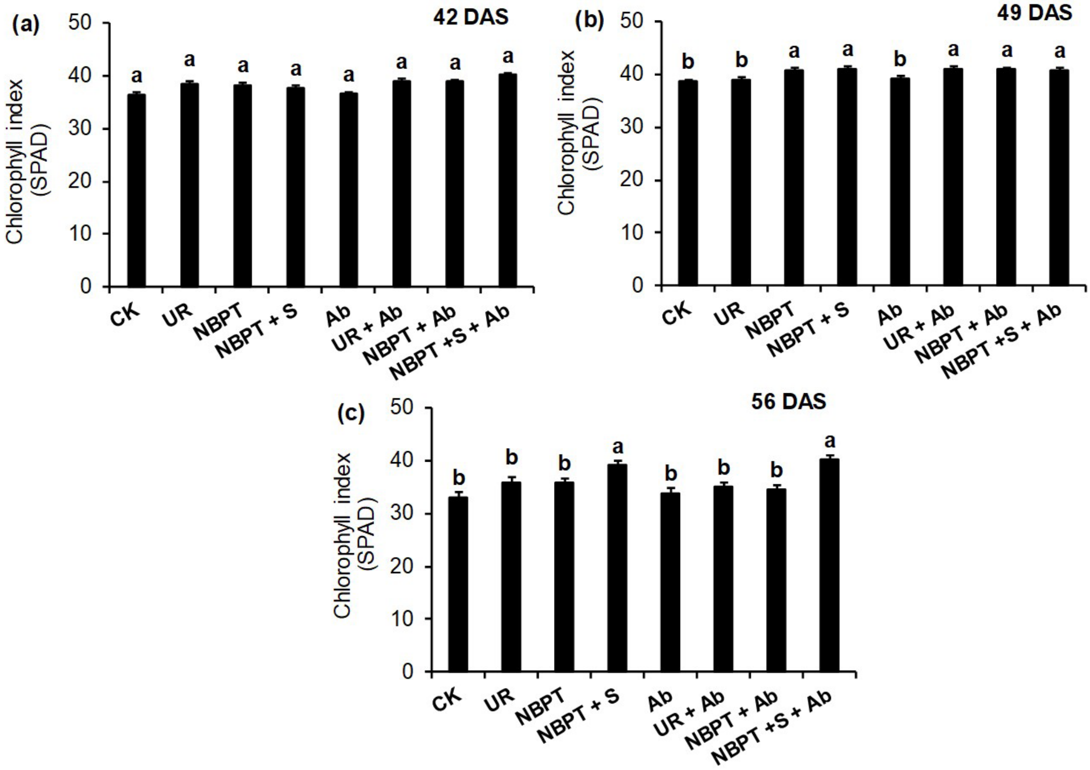

Figure 3. Chlorophyll index of wheat plants var. IPR Potyporã grown under different fertilization managements, without and with Azospirillum brasilense, during cultivation cycle. Different letters between columns differ statistically by Scott-Knott test ± standard error ($p \leq 0.05$). Ab: Azospirillum brasilense, CK: control, NBPT: N-(n-butyl) thiophosphoric triamide, S: ammonium sulfate, and UR: urea.

reinforcing the need for N fertilization for crop, and even if inoculation with rhizobacteria is carried out, it is important to supplement with mineral N for wheat. According to Ecco et al. (2022) the use of *A. brasiliense* isolated without nitrogen fertilization did not recommend, corroborating the information observed in our study with wheat.

We emphasized that although there was no statistical difference, there was a 46% increase in the accumulation of shoot dry mass in plants with urea + *A. brasilense* and NBPT + S + *A. brasilense* compared to CK plants (2.35 g) which had a lower value (Figure 4b). In study by Groff et al. (2020), the authors were application of N with urea as a source and found a linear increase in dry mass of plants according to the increase in the dose of N in cover fertilization. We believe that the management with NBPT, S or *A. brasilense* contributes to better N efficiency, reflecting in better plant vigor in leaf area volume, not quantified in our study, but visibly evidenced (Figure 1) in function of physiological efficiency.

Based in the results, we accept the hypothesis that nutritional management in wheat in the initial growth phase is fundamental, making it a promising practice that can contribute to cost reduction. In future perspectives, we suggest new studies evaluating physiological and nutritional aspects of plants, and also evaluations carried out over longer periods, especially considering the fact that our study presented preliminary results.

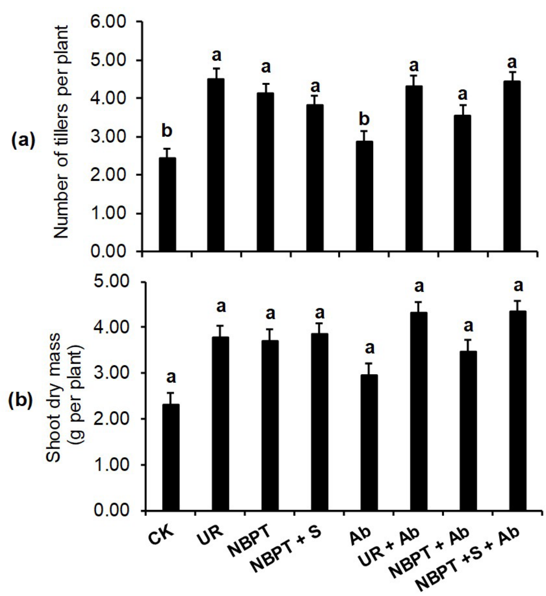

Figure 4. Number of tillers (a) and shoot dry mass (b) of wheat plants var. IPR Potyporã grown under different fertilization managements, without and with Azospirillum brasilense, during cultivation cycle. Different letters between columns differ statistically by Scott-Knott test ± standard error ($p \leq 0.05$). Ab: Azospirillum brasilense, CK: control, NBPT: N-(n-butyl) thiophosphoric triamide, S: ammonium sulfate, and UR: urea.

4. Conclusion

Nutritional management with *A. brasilense*, urease inhibitor, and sulfur contributed to initial growth of wheat plants, improving the efficiency in increasing photoassimilates. Further studies are suggested to evaluate the nutritional status and other physiological indicators of plants using these management methods and longer evaluation periods.

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