










Interactive Performance of Wheat Nitrogen Fertilization and Inoculation with Growth-Promoting Bacteria

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Scopus Author ID 23096241300

Received: 6.07.2022; Accepted: 7.09.2022; Published: 10.09.2022

Abstract: The exploitation of plant growth-promoting rhizobacteria (PGPR) emerges as an important strategy for fixing atmospheric nitrogen (N) and making it available to plants. Correspondingly, the purpose of this study was to evaluate the inoculation with *Azospirillum brasilense* via foliar with distinct N doses (40, 20, and 0 kg/ha) for different wheat cultivars (Sossego, Toruk, and Quartzo). The experiment was conducted during the 2020 harvest at the State University of Rio Grande do Sul, Cachoeira do Sul, Brazil. A completely randomized design (CRD) in a 3x6 factorial scheme with four replications was applied. Yield components and grain yield were established. Furthermore, the physicochemical characterization of wheat silique biomass was executed based on subcritical water hydrolysis (SWH). Gluten and mass analysis was established. Appropriately, the grain yield was up to 3197.05 kg/ha for the Sossego cultivar with the N dose of 40 kg/ha. Up to 0.84 g reducing sugars/100 g wheat silique was obtained based on the SWH. Finally, this study promoted N management as a key factor in increasing grain yield. This scenario reports the importance of the association of PGPRs as a fundamental promoter of N for plants to express their maximum potential.

Keywords: biological nitrogen fixation; byproducts management; growth-promoting bacteria; subcritical water hydrolysis.

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most cultivated plants globally and is fundamentally the food base of approximately 35% of the population worldwide [1]. Global wheat production is estimated to reach around 750 million tons annually, with a large production concentrated in North America, Europe, China, and India [2]. The species has been considered for several applications, mainly as a source in disease prevention, directly with anticancer, antidiabetic, antioxidant, and antimicrobial action [3]. Consequently, wheat was one of the first species domesticated on a large scale in the agricultural context. The grain is also used in manufacturing non-food products, such as adhesive mixtures, printing, soluble or edible packaging, the pharmaceutical industry (production of antibiotics and other medicines), animal feed, biogas, and bioethanol synthesis [4–6]. Nonetheless, wheat production is

relatively modest compared to summer crops, such as soybean and maize, considering the growing food demand and crop production, promoting a requirement to investigate management techniques to obtain increased productivity and, consequently, ensure profits to the producer.

Appropriately, the main determining factor associated with the potential wheat yield is the N availability in an adequate amount to satisfy the plant requirements, in which it plays a fundamental role, as it is the nutrient found in higher concentration in vegetative tissues and grains [7]. There is a significant N requirement for proper wheat growth, and the absolute yield and quality of the final product are directly dependent on substantial N incorporation [8]. Nevertheless, the maximum N use efficiency (NUE) is concentrated in the range of 30-40% of the total N fertilizers applied to cereals [9]. The lower values result from the significant portion of N lost by the phenomena of volatilization, denitrification, and lixiviation [10]. Moreover, with the growing demand for N fertilizers and the constant concern about the pollution of natural resources, the necessity arises to investigate alternatives to reduce losses and explore possibilities for N supplementation. Accordingly, biological nitrogen fixation (BNF) is considered a valuable option to increase wheat yield with substantially reduced damage to natural resources [11].

BNF is one of the main tools for N availability to plants. It is a process conducted by some microorganisms that inhabit the soil, providing N in the form of NH_3 or amino acids to plants from atmospheric N_2 , supplying the N necessity for plants [12]. Based on the microbial population, more specifically, plant growth-promoting rhizobacteria (PGPR) with N-fixing capacity are indicated as a valuable N source that results in a direct reduction in fertilizer use and promotes soil fertility stability [13]. The BNF phenomenon by diazotrophic bacteria has been the main strategy to save N in the ecosystem, with up to 50% of the total N in agricultural systems [14]. These organisms have been extremely beneficial and advantageous in improving water and nutrient absorption and resistance to various biotic and abiotic factors [15].

Furthermore, the rhizobacteria establish considerably higher plant growth through the action of a higher number of microbial colonies in the root zone, causing a higher root absorption of nutrients and water to encourage plant growth [1]. Although numerous soil bacteria have been widely reported as growth promoters and development of leguminous species, the mode(s) of action by these bacteria to exhibit beneficial activities in grass species is scarce. Contextually, in grasses, the use of bacteria of the genus *Azospirillum* spp. has provided improvements for N application in fertilization, reducing costs with fertilizers [16].

The *Azospirillum* spp. includes a group of free-living PGPB that are established in a diversity of global zones, which can cooperate with distinct mechanisms of crop N nutrition [17]. As in legumes with *Bradyrhizobium*, effective root nodules are formed. Nevertheless, in associations of grasses with N-fixing bacteria, this phenomenon does not occur. A process of colonization of the plant and root surface zones by adhesion emerges, and the bacteria perform the atmospheric N fixation, providing this nutrient available to the plant [18]. In wheat, *A. brasilense* promotes a substantial increase in grain nutrients and significant advances in grain yield and higher root development [19].

The microbial seed inoculation procedure represents a substantial portion of the direct availability of PGPB to plants. Nonetheless, seed application presents incompatibility with certain types of herbicides, fungicides, and insecticides that can express negative effects [20]. Recent research specified that foliar inoculation of Ab-V5 and Ab-V6 strains promoted plant growth and phytohormone synthesis [21,22]. Moreover, the bacterium promotes prominent

water absorption, promoting higher resilience to extreme abiotic conditions and considerable gains in yield [23]. Furthermore, the direct foliar application of *A. brasilense* in wheat promotes higher absorption of nutrients by the leaves and, associated with N, establishes productivity gains at doses of up to 61 kg/ ha [24]. The nutritional increment of Fe, Cu, Mn, and Zn establishes a substantial grain yield increase and higher root growth, promoting a higher plant yield [25]. In this context, with the large economic costs related to N fertilization associated with the necessity for more sustainable agricultural production, this scenario demonstrates the importance of developing research evaluating the feasibility of inoculating N-fixing bacteria in grasses in an attempt to verify the benefits of this technology for wheat cultivation.

Accordingly, the current status of the application of rhizobacteria in grasses is scarce and indicates substantial gaps. Therefore, significant perspectives are identified in exploring this community of organisms that act directly on plant growth and promote plants to express their maximum productive potential. Correspondingly, this study reports the role of PGPR in foliar application in wheat, based on investigating these microorganisms in the plant's productive potential. Additionally, valuable information on some specific topics projected important purposes developed in the study, such as (i) stipulating the proportion of N in coverage that results in the best performance of different wheat cultivars; (ii) evaluating the response of different wheat cultivars to inoculation with *A. brasilense*; and (iii) establish the composition and quantification of chemical compounds based on the application of sustainable technologies that promote the aggregation of value to wheat products and byproducts.

2. Materials and Methods

2.1. Geographical characterization.

The experiment was conducted in the 2020 crop year with wheat (*Triticum aestivum* L.) in an experimental area of the State University of Rio Grande do Sul, Três Vendas, Cachoeira do Sul, in the Central Region of the State of Rio Grande do Sul, Brazil (29°53' S and 53° 00' W; altitude of 125 m above sea level). The region's climate is classified by [26] as humid subtropical, predominant in the South region. The soil was classified as Typical Dystrophic Red Argisol [27].

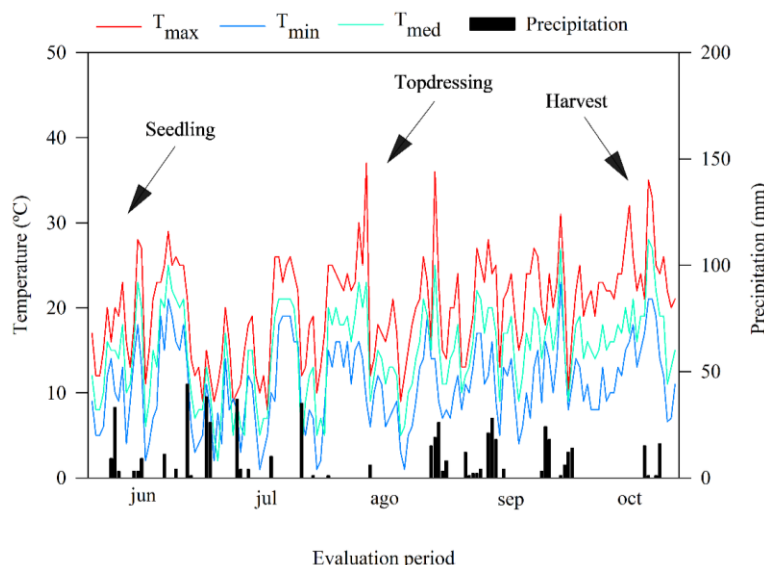


Figure 1. Maximum (Tmax, °C), average (Tmed, °C), and minimum (Tmin, °C) temperature and precipitation (mm) obtained from the automatic station in Cachoeira do Sul, the Rio Grande do Sul, Brazil, during wheat cultivation from June to October 2020.

The chemical characteristics of the soil were determined before the installation of the experiment: clay (%): 27.0; organic material (%): 2.9; SMP (cmol_c dm⁻³): 6.1; CTC (cmol_c dm⁻³): 7.0; Al (cmol_c dm⁻³): 0; H+Al (cmol_c dm⁻³): 3.9; pH (H₂O): 5.8; P (mg dm⁻³): 10.3; K (mg dm⁻³): 172.0; soil saturation (%): 64.1. Soil analysis was conducted by the Soil Analysis Laboratory, Soil Department, Federal University of Santa Maria, Santa Maria, Rio Grande do Sul, Brazil. Moreover, the area has been constantly cultivated with annual crops in experimental studies. Figure 1 reports the behavior of meteorological variables, such as maximum, minimum, and average temperature (T_{\max} , T_{\min} , and T_{med} , respectively) and precipitation during the study.

2.2. Experimental design.

The experimental arrangement applied was completely randomized, in a 3x6 factorial scheme, with four replications. The treatments consisted of three wheat cultivars (TBIO Sossego, TBIO Toruk, and TBIO Quartzo) submitted to inoculation or not via foliar inoculation with *A. brasilense* (Ab-V5), combined with the application of N in coverage (100, 50, and 0% of the recommended application).

The experiment was implemented in succession to soybean (*Glycine max* (L) Merrill), and weeds were managed with the herbicide based on glyphosate, using 720 g a.i./ ha before sowing. It was installed under a no-tillage system on June 4, 2020, using a fine-grain seeder with 17 rows at a sowing density of 110 kg/ ha seeds. The plots consisted of 17 lines of wheat cultivation, spaced at 17 cm and 5 meters in length, totaling 14.5 m² per plot.

For fertilization, the recommendations of the Soil Chemistry and Fertility Commission - RS/SC (2016) were considered, setting as a standard for all treatments a sowing dose of 20 kg/ ha N, 95 kg/ ha/ P₂O₅, and 75 kg/ ha K₂O, sprayed in urea (45% N), triple superphosphate (41% P₂O₅), and potassium chloride (58% K₂O), respectively. Nitrogen topdressing occurred according to the established treatments, in which 40 kg/ ha N was applied for treatments with 100% of the recommended dose and 20 kg/ ha N for 50% of the recommended dose. N fertilization was managed at V4 and V6 plant stages, using urea as an N source. Cultural practices, such as weed, pests, and disease management, were followed according to the recommendations of [28].

2.3. Inoculation procedure.

For inoculation, *A. brasilense*, strain Ab-V5, was utilized with a concentration of approximately 200,000,000 (2×10⁸) colony forming unit – CFU/ mL, with the foliar application, at a dose of 0.3 L/ ha. The inoculation procedure was conducted associated with the first N topdressing.

2.4. Analytical procedure.

2.4.1. Yield components evaluation.

The harvest was conducted in October, when the culture presented a state of senescence and drought, in which the assessments of plant height were performed (maturation), from the distance from the ground level to the tip of the cobs, excluding edges (cm). The hectoliter weight (PH) was determined at the time of wheat maturation, performed in triplicate, and the results were expressed in kg/ hL.

The number of grains per cob was evaluated in 10 cobs per experimental unit, and cob size was evaluated for 10 cobs per plot, using a graduated measuring tape. To determine grain yield, 10 lines with 2 meters in length (3.4 m²) were harvested from the central area of the plots, threshed, and weighed, which was converted to kg/ ha and corrected to 13% moisture. The thousand-grain mass was performed by triplicates of 1,000 grains weight obtained from the experimental plot area. The agronomic efficiency was calculated by Equation 1, described by [29].

$$AE = (GYF - GY_wF) \div Total\ N\ applied \quad (1)$$

Where:

AE: agronomic efficiency, kg grains/ kg N applied;

GYF: Grain yield with fertilizer application, kg;

GY_wF: Grain yield without fertilizer application, kg.

2.4.2. Total reducing sugars,

The analysis of total reducing sugars and the determination of Y_{RS} were conducted by the dinitrosalicylic acid (DNS) calorimetric method, as described by [30]. To determine the absorbance, a spectrophotometer (UV-2700, Shimadzu, Japan) at a wavelength of 575 nm was employed. The reducing sugars yield (YRS) was established for each hydrolyzate sample based on equation 2.

$$Y_{RS} = (m_{RS} \div m_{SA}) \times 100 \quad (2)$$

Where:

Y_{RS}: reducing sugars yield, g/ 100 g wheat siliqua;

m_{RS}: mass of total reducing sugars in the hydrolyzed solution, g;

m_{SA}: initial mass of wheat siliqua introduced in the reactor vessel at the beginning of the process, g.

2.4.3. Grain and flour analysis.

As well as SWH, for grain and flour analyses, the combination of treatments applied was: 50% N, 300 mL *A. brasilense* (Ab), and Sossego; 50% N and Toruk; 50% N, 900 mL Ab, and Audaz; 100% N, 900 mL Ab, and Audaz; 100% N, 300 mL Ab, and Audaz; and 100% N and Toruk. The moisture analysis of the grain and wheat flour was performed according to the methodology of AACC 44-15-A (1995). The falling number was estimated according to the method of AACC 56-81-B (1995). The alveography was performed according to the method of AACC 54-30-A (1995) to determine the mass deformation energy (W) and the relationship between toughness and extensibility (P/L). The water absorption capacity was performed according to the AACC method 54-21 (1995). The color (L*, a*, and b*) was determined according to the method of AACC 14-22 (1995).

Wet gluten and dry gluten analyzes were conducted as described by [31], with some modifications. Approximately 10g of each sample was weighed, and 4.5 mL of a 2% sodium chloride solution was added. The saline solution was incorporated into the flour until a dough was obtained, left to rest for 10 min, then washed in running water over a sieve until the water was clear. The sample was then dried and weighed. The value obtained on the scale was

multiplied by 10 to obtain the value of wet gluten and subsequently divided by 2.9 to obtain the value of dry gluten.

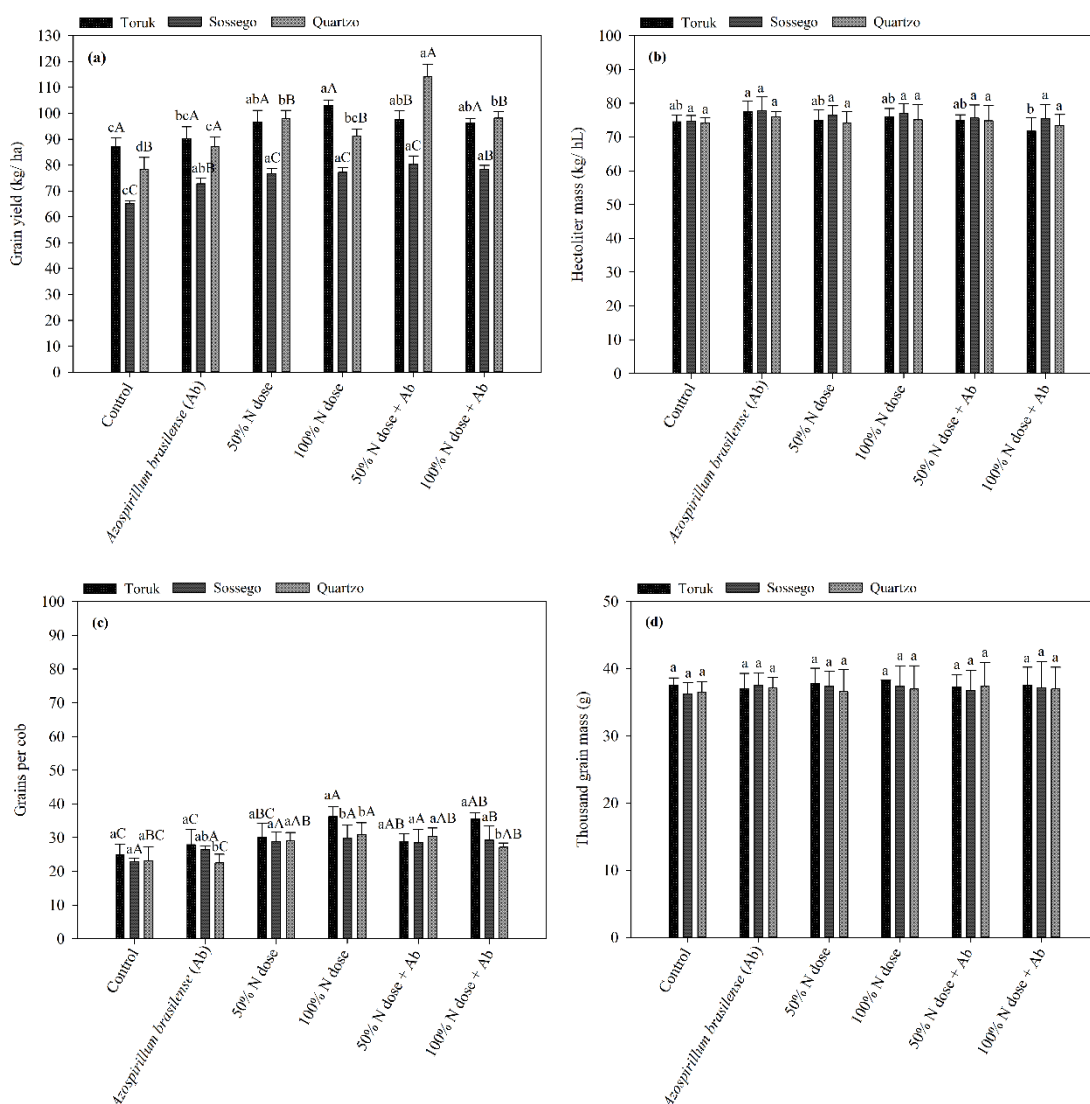
2.5. Statistical analysis.

All described parameters were submitted for analysis of variance. When significant differences between treatments were seen, the samples were analyzed by Tukey's test at 5% probability of error, using the software RStudio®.

3. Results and Discussion

3.1. Yield components.

Figure 2 reported the wheat yield components based on the different treatment combinations and cultivars. Figure 2 (a) expressed plant height (cm) (CV, 4.30%) data of different wheat cultivars and their respective treatment combinations. Considering the treatments, there was a significant difference for all the combinations. The highest results were observed for treatments with 50% N dose (20 kg/ha) + *A. brasilense* (Ab) (114.25±4.60 cm) for Quartzo, 100% N dose (40 kg/ha) (103.03±2.16 cm) for Sossego, and 50% N dose (20 kg/ha) + Ab (80.33±3.21 cm) for Toruk.



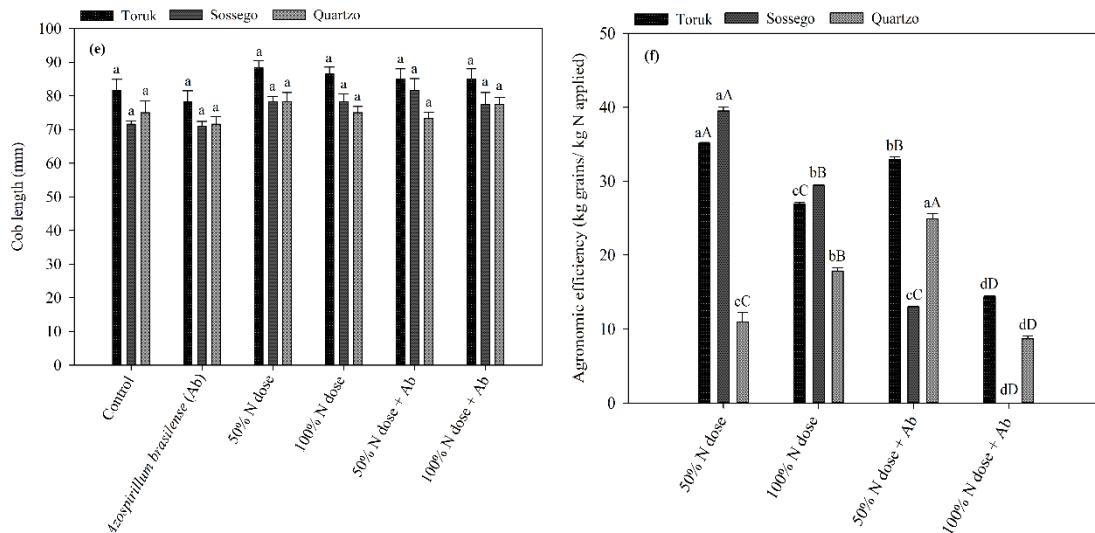


Figure 2. Wheat yield components plant height (cm) (a), hectoliter mass (kg/ hL) (b), grains per cob (c), thousand grain mass (g) (d), cob length (mm) (e), and agronomic efficiency (kg grains/ kg N applied) (f) for Toruk, Sossego, and Quartzo cultivars in control, *Azospirillum brasilense* (Ab), 50% N, 100% N, 50% N + Ab, and 100% N + Ab treatment combinations; means followed by the same lowercase letter corresponding to the comparison between the different treatment combinations and the uppercase letter corresponding to the comparison between cultivars do not differ significantly by Tukey's test at the level of 5% error probability.

Considering the cultivar variable, all treatments indicated a significant difference for the different cultivars in this study. Plant height was influenced by the N doses associated with inoculation with *A. brasilense*. The Sossego cultivar was more responsive to the increase in wheat height as the N fertilization dose increased.

Plant height was influenced by the N applied in topdressing associated with *A. brasilense*, and the Sossego cultivar was more responsive to the increase in wheat stature as the N fertilization dose increased. According to [32], this increase in plant height is due to the N supply, which promotes plant elongation and increases the number of leaves and tillers. Sossego and Toruk cultivars had a higher height when N fertilizer was applied, with 40 kg/ ha and 20 kg/ ha. The cultivar Quartzo had a higher development associated with inoculation and 20 kg/ ha N.

According to [33], this increase in plant height is related to N's functions directly in cell division, plant metabolism, and photosynthetic processes. In a study conducted by [34], the inoculation of *A. brasilense* associated with N fertilization in topdressing provided a higher height of wheat plants. Nonetheless, for the corn crop there was no significant difference for the inoculation via foliar of *A. brasilense* and N doses. [35] analyzed that for each 1.0 kg of N added to the soil, it provided an increase of 0.05 cm in plant height.

Figure 2 (b) showed the hectoliter mass (kg/ hL) (CV, 2.54%), where the cultivars Toruk and Quartzo did not differ statistically for the treatment combinations applied. The cultivar Sossego showed a significant difference between the treatment with 100% N dose (40 kg/ha) + Ab (71.76±3.91 kg/ hL) and the other combinations. The highest results for all cultivars were verified for the treatment with *A. brasilense* without the association with the N dose. Up to 77.86±4.10 kg/ hL, 77.53±3.19 kg/ hL, and 76.00±1.60 kg/ hL were indicated for Toruk, Sossego, and Quartzo, respectively. According to the grains per cob component (Figure 2 (c)) (CV, 8.78%), it was observed that the treatments without inoculation and only inoculation presented lower values for Sossego and Toruk when compared to N associated with inoculation. The two treatments did not differ where complete nitrogen fertilization was applied. The

cultivar Sossego expressed the highest results for all cultivars with the N dose of 40 kg/ ha (36.2 ± 2.98 grains per cob). Up to 30.88 ± 3.52 grains per cob and 29.88 ± 3.96 grains per cob were indicated for Quartzo and Toruk, respectively, in the same treatment. Moreover, Figure 2 (d) and Figure 2 (e) expressed the components thousand-grain mass (g) (CV, 1.35%) and cob length (mm) (CV, 6.22%), where no significant difference was observed between the treatments. For thousand grain mass, the highest results were indicated for Sossego in the treatments 100% N (38.2 ± 3.75 g) and 50% N (37.76 ± 2.66 g), respectively. The same scenario was observed for the cob length variable, in which Sossego showed higher results in treatments without the association with *A. brasilense* (88.30 ± 2.14 mm, 50% N, and 86.60 ± 1.92 mm, 100% N, respectively). Finally, Figure 2 (f) indicated the agronomic efficiency (kg grains/ kg N applied) (CV, 2.42%), where the Sossego cultivar showed the highest value in the treatment with a 50% N dose (20 kg/ ha) (39.49 ± 0.53 kg). There was a significant difference for this treatment in all cultivars, with AE being null for 100% N + Ab (0.0 kg). The same scenario was verified for Toruk (35.13 ± 0.09 kg), and the association with *A. brasilense* promoted the best result for Quartzo (24.91 ± 0.71 kg), which differed from the other treatments.

Figure 3 indicates the grain yield (kg/ ha) (CV, 13.44%) of different wheat cultivars and their respective treatment combinations. It was verified that there was a significant difference between the cultivars Sossego and Toruk, and the treatments that examined only N fertilization proved to be superior. The highest results were observed for treatments with 100% N dose (40 kg/ha) for Sossego (3197.05 ± 35.12 kg/ ha), Toruk (2396.07 ± 20.69 kg/ ha), and Quartzo (2013.37 ± 27.85 kg/ ha). These results differed statistically from treatments involving *A. brasilense*. Considering the cultivars, all treatments involving only N doses indicated a significant difference. The treatment combinations with *A. brasilense* did not express a significant difference.

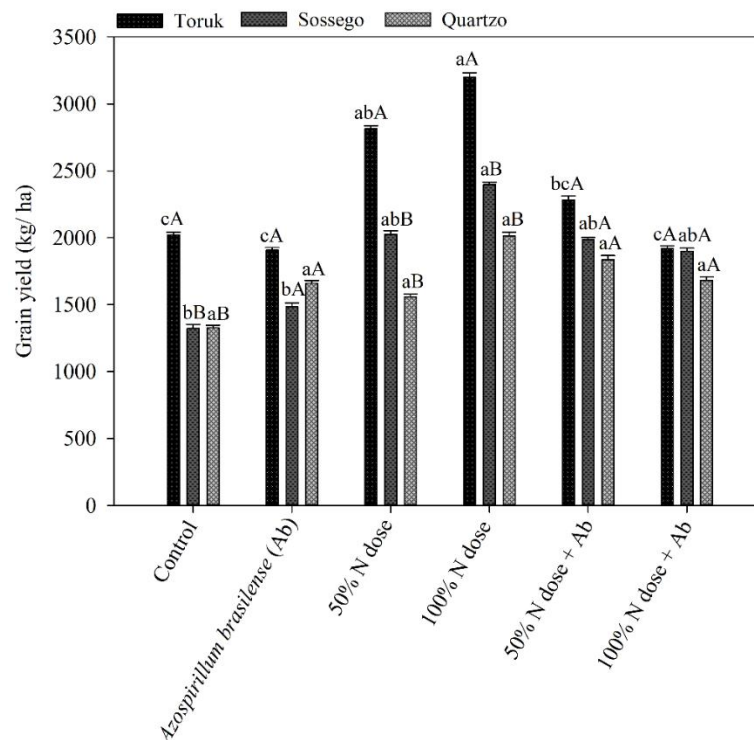


Figure 3. Wheat grain yield (kg/ ha) for Toruk, Sossego, and Quartzo cultivars in control, *Azospirillum brasilense* (Ab), 50% N, 100% N, 50% N + Ab, and 100% N + Ab treatment combinations; means followed by the same lowercase letter corresponding to the comparison between the different treatment combinations and the uppercase letter corresponding to the comparison between cultivars do not differ significantly by Tukey's test at the level of 5% error probability.

Moreover, the *A. brasilense* inoculation reduced wheat yield in the absence of N fertilization in topdressing and increased yield when N was applied. Nevertheless, in a study involving corn crops, inoculation with *A. brasilense* responded positively to doses of 142.8 kg/ha, 285.6 kg/ha, and 428.4 kg/ha of N fertilization in topdressing [32]. Appropriately, [19] found an increase in the accumulation of nutrients, such as B and Cu in different wheat cultivars, when associated with *A. brasilense* a. These results reinforce the fact that the inoculation of *A. brasilense* must always be related to the application of N fertilizer, which is beneficial for the agronomic characteristics of the wheat crop and contributes to grain yield.

For all treatments, the cultivar Quartzo showed no significant difference in grain yield, showing the variation in the interaction between the genotype and the diazotrophic bacteria strains. Nonetheless, when comparing treatments with and without inoculation submitted to less N dose, it was observed that there was an increase of 278 kg/ha in grain yield. Sala *et al.* (2005) and Sala *et al.* (2007), evaluating wheat cultivars inoculation with *A. brasilense*, found that some cultivars may present an increase in grain production from 27% to 45% in relation to the control treatment. One of the variables that contribute to the complexity of responses related to inoculation is the interaction of the plant genotype and the inoculated strain.

According to the evaluated cultivars, the treatments in which inoculation was associated with a complete N dose obtained a lower grain yield than the treatment in which half of the fertilization associated with inoculation was used. According to [36], wheat seeds inoculated with *Azospirillum* spp., combined with different doses of N (40, 60, and 80 kg/ha), obtained higher productivity in the treated plants with inoculation and N fertilization, particularly at 60 kg/ha. Additionally, the wheat yield treated with 80 kg/ha of N did not differ statistically when compared to that of 60 kg/ha of N. Moreover, inoculation with *A. brasilense* saved about 20 kg/ha of total N, showing that the use of these bacteria is economically viable.

Finally, many factors for the development and action of inoculation with *A. brasilense*, mainly via foliar, still require further results in terms of research related to these growth-promoting bacteria. One of the variables that contribute to the complexity of responses to inoculation is the interaction of the plant genotype and the inoculated strain, as evidenced by the results. According to [37], the factors that interfere with cultures' responses to the inoculation of *A. brasilense* are still not fully comprehended. Nevertheless, factors such as plant genotype, *Azospirillum* genus, inoculant placement, cell viability, and cultivar characteristics can influence the association of these bacteria with crops. Accordingly, there are many paradigms about the inoculation of *A. brasilense* for the wheat crop, and the foliar application becomes an inoculation alternative, which aims to improve its establishment since they can translocate and penetrate via the stoma and colonize the roots via xylem. Nonetheless, reports of the absorption mechanism in the leaf and how the N conversion occurs are scarce for this inoculation method, which depends on some factors, such as plant genetics, the inoculant strain, and the conditions at the moment of application [32].

3.2. Subcritical water hydrolysis.

The Y_{RS} kinetics of the hydrolyzed samples of wheat siliqua after 15 minutes of the SWH reaction is indicated in Figure 4. According to Y_{RS} , this study showed no significant difference between treatments and treatment combinations. Comparing the treatments, the cultivar Audaz, under application conditions of 100% N dose (40 kg/ha) and 900 mL *A. brasilense* showed the best results (0.84 ± 0.14 g/100 g wheat siliqua). Subsequently, the highest Y_{RS} were verified for Toruk (20 kg/ha N, 0.82 ± 0.09 g/100 g wheat siliqua) and Audaz with

50% N dose (20 kg/ha) and 300 mL *A. brasilense* (0.79 ± 0.13 g/ 100 g wheat siliqua). In general, the behavior of Y_{RS} accumulated as a result of reaction time was similar for all treatments, with higher growth up to 10 minutes of reaction and lower growth after this period.

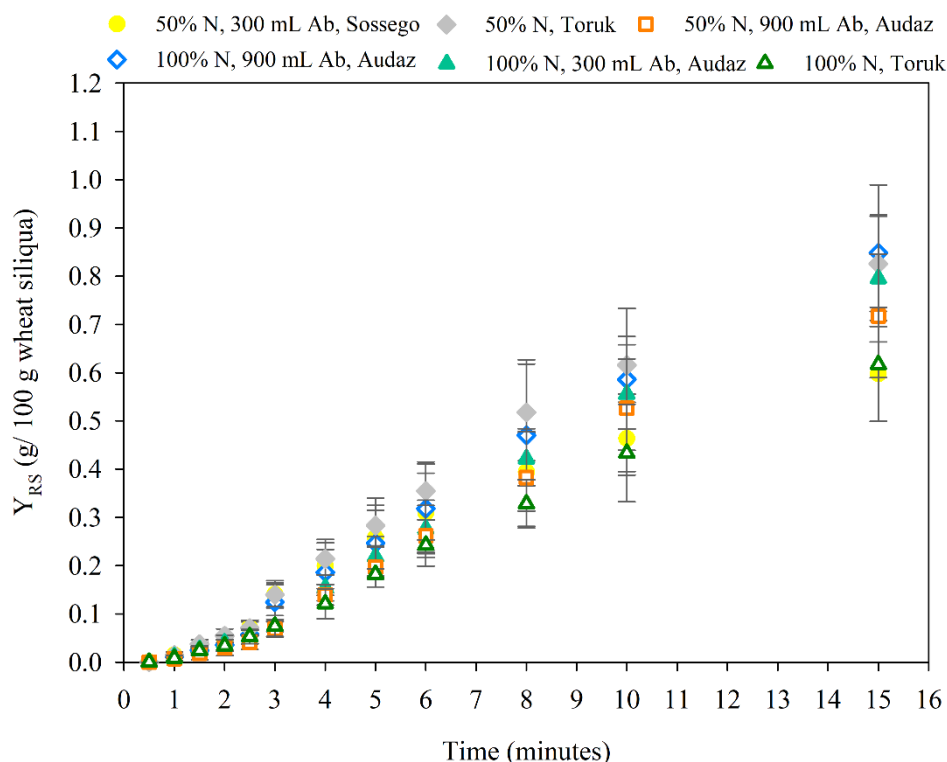
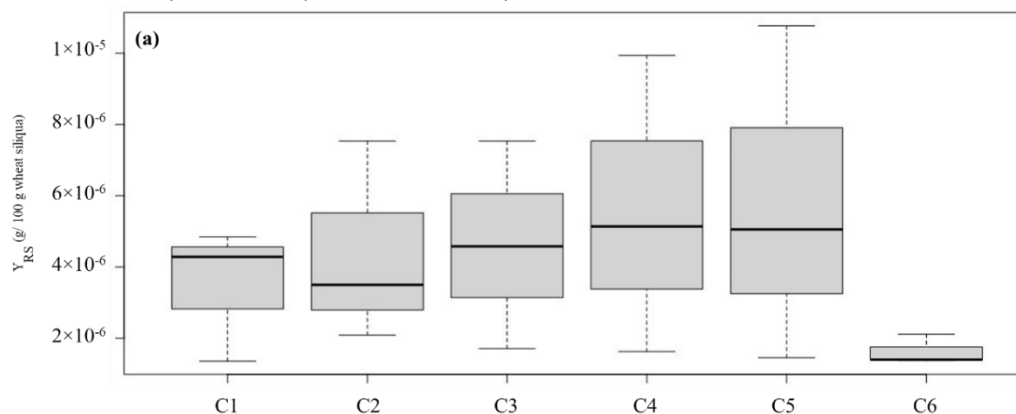
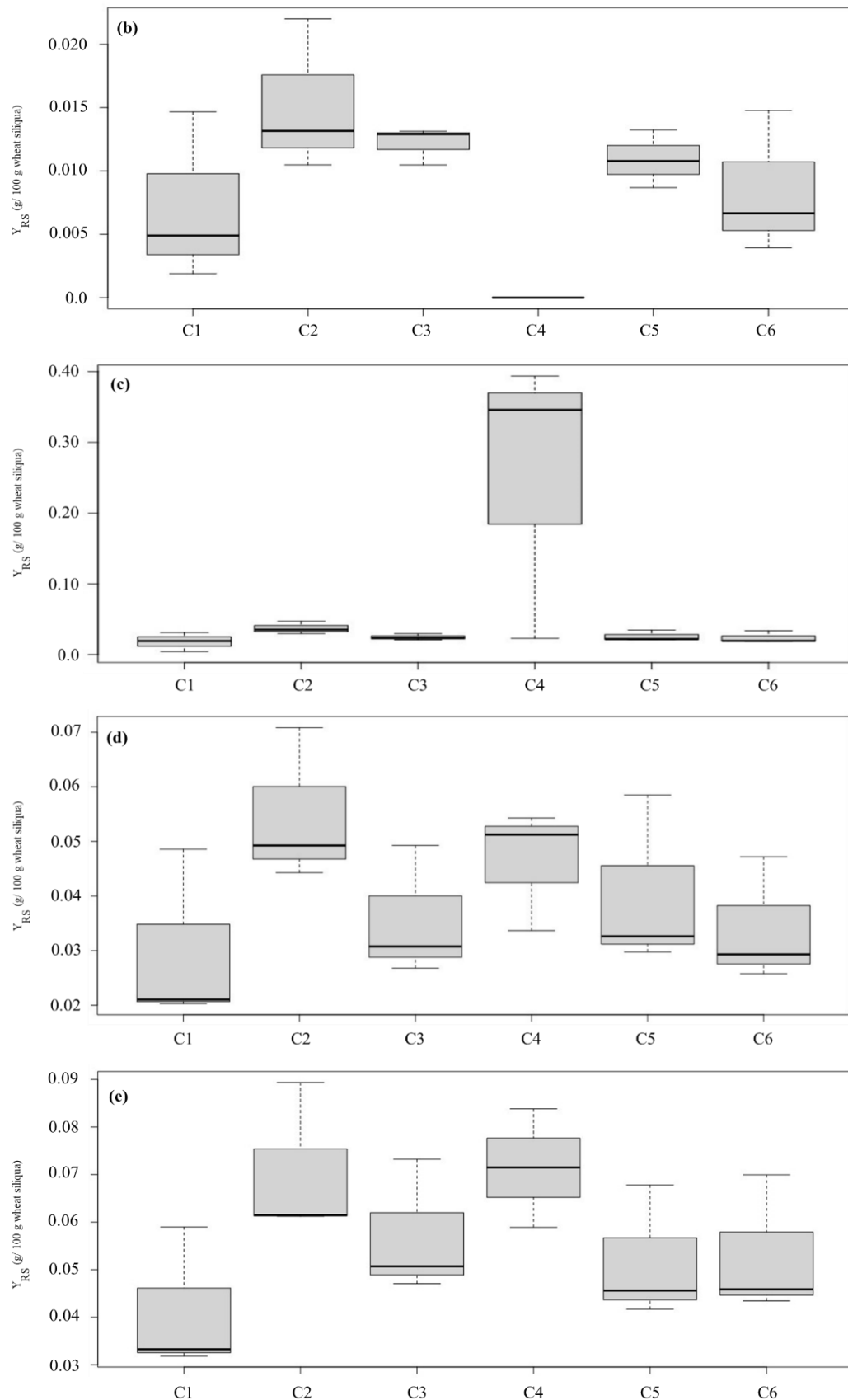
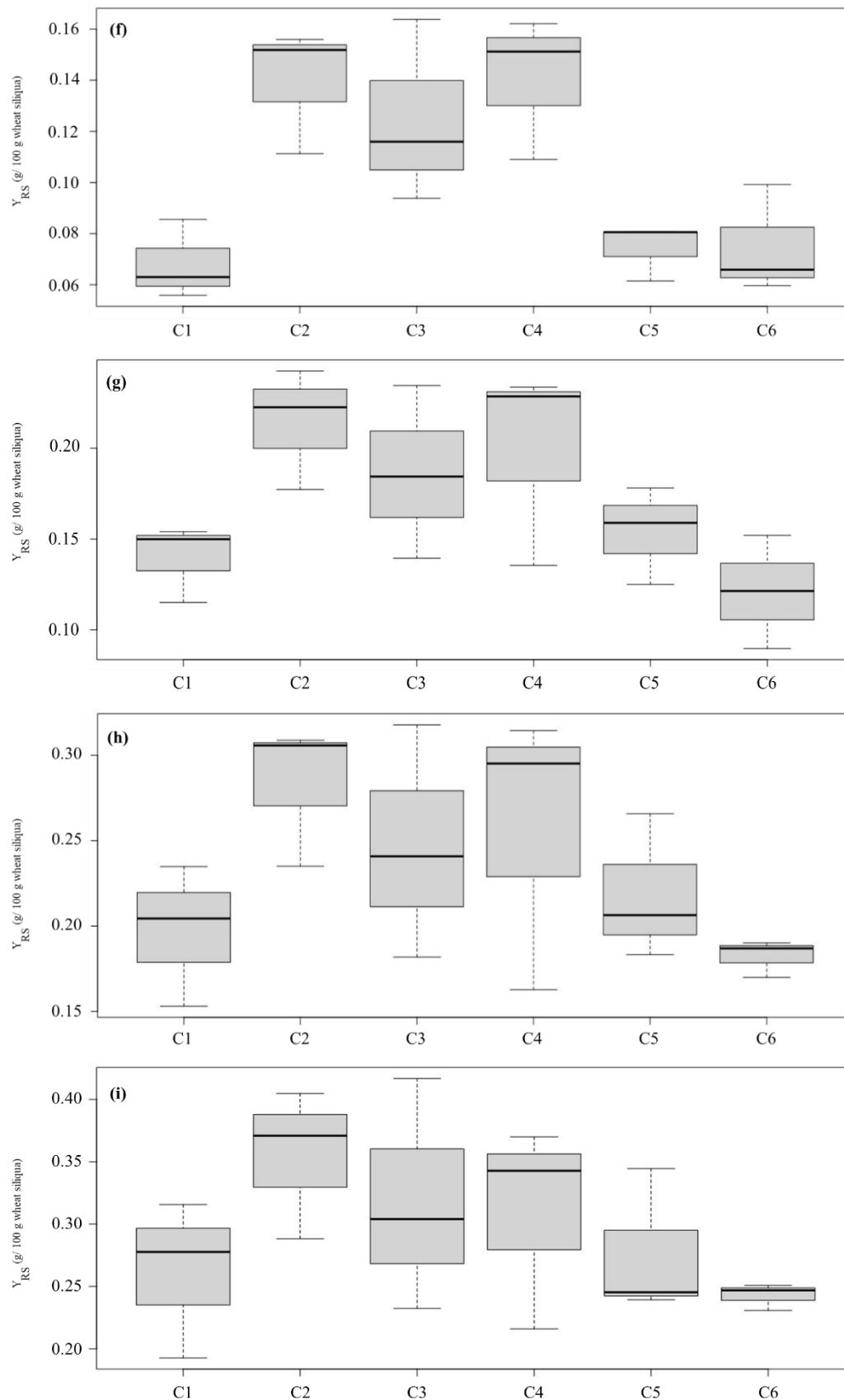


Figure 4. Wheat siliqua reducing sugars yield (Y_{RS}) (g/ 100 g wheat siliqua) kinetics for Toruk, Sossego, and Audaz cultivars in 50% N, 300 mL *Azospirillum brasilense* (Ab), and Sossego; 50% N and Toruk; 50% N, 900 mL Ab, and Audaz; 100% N, 900 mL Ab, and Audaz; 100% N, 300 mL Ab, and Audaz; and 100% N and Toruk treatment combinations; means followed by the same lowercase letter corresponding to the comparison between the different treatment combinations and the uppercase letter corresponding to the comparison between cultivars do not differ significantly by Tukey's test at the level of 5% error probability.

Additionally, Figure 5 reports the evolution of Y_{RS} in the different reaction times 0.5 (a), 1 (b), 1.5 (c), 2 (d), 2.5 (e), 3 (f), 4 (g), 5 (h), 6 (i), 8 (j), 10 (k), and 15 (l) minutes. Treatment combinations were arranged, where C1 (Sossego, 50% N, 300 mL *A. brasilense*), C2 (Audaz, 100% N, 900 mL Ab), C3 (Toruk, 50% N), C4 (Audaz, 100% N, 300 mL Ab), C5 (Sossego, 50% N, 900 mL Ab), and C6 (Toruk, 100% N).







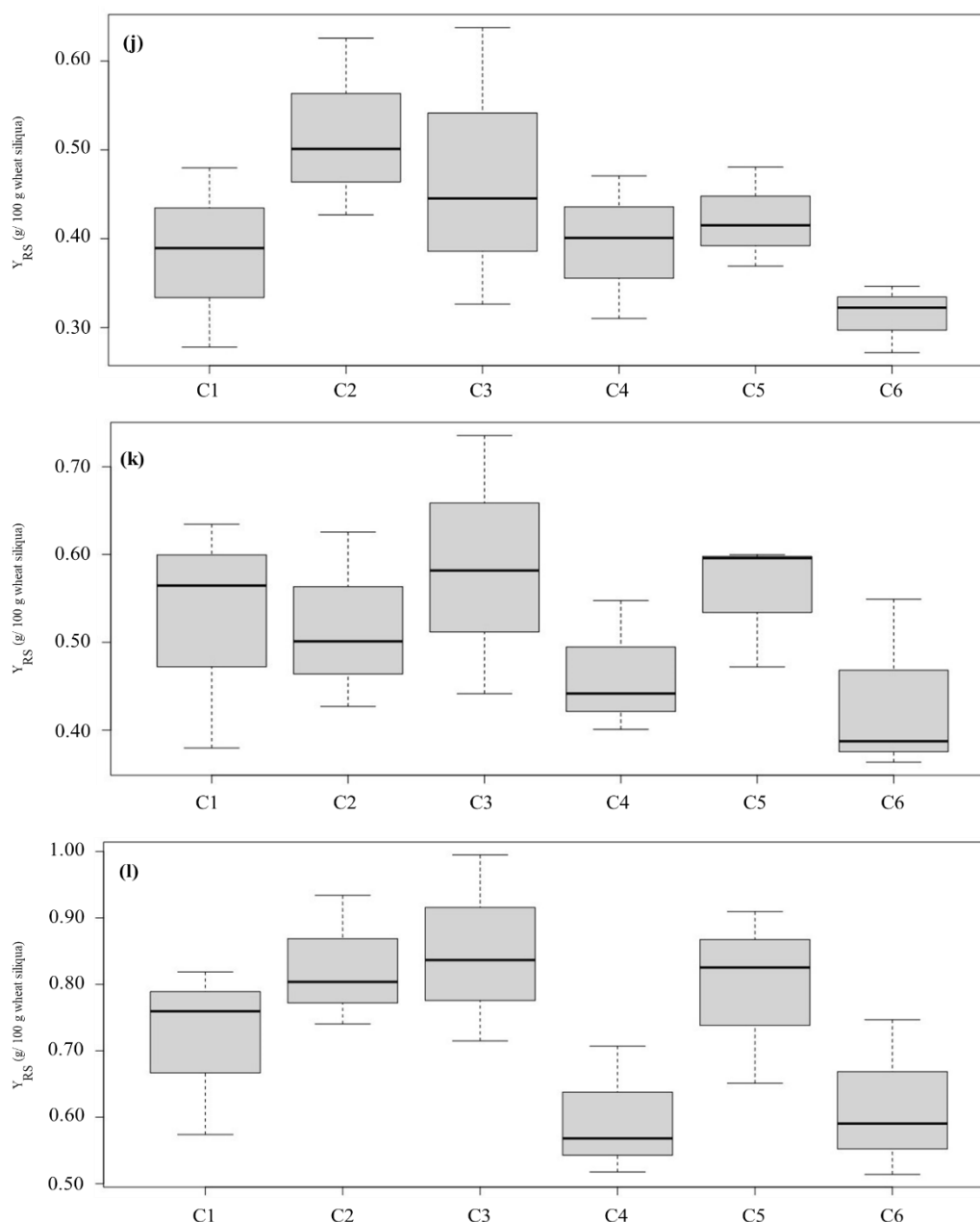


Figure 5. Wheat siliqua reducing sugars yield (YRS) (g/ 100 g wheat siliqua) kinetics for Toruk, Sossego, and Audaz cultivars in 50% N, 300 mL *Azospirillum brasilense* (Ab), and Sossego; 50% N and Toruk; 50% N, 900 mL Ab, and Audaz; 100% N, 900 mL Ab, and Audaz; 100% N, 300 mL Ab, and Audaz; and 100% N and Toruk treatment combinations in 0.5 (a), 1 (b), 1.5 (c), 2 (d), 2.5 (e), 3 (f), 4 (g), 5 (h), 6 (i), 8 (j), 10 (k), and 15 (l) minutes.

Considering the initial Y_{RS} , at 0.5 minutes (Figure 5 (a)), the highest values were obtained for C4 ($5.75 \times 10^{-6} \pm 4.69 \times 10^{-6}$ g/ 100 g wheat siliqua) and C5 ($3.49 \times 10^{-6} \pm 1.87 \times 10^{-6}$ g/ 100 g wheat siliqua). The rate suffered a higher reduction in reaction times 2 (d) and 2.5 (e) minutes, and Y_{RS} increased after 3 minutes (Figure 5 (f)). Ultimately, the highest results corroborate Figure 4, showing that C2 and C3 indicated the highest accumulated Y_{RS} ($0.84 \times 10^{-6} \pm 0.14 \times 10^{-6}$ g/ 100 g wheat siliqua e $0.82 \times 10^{-6} \pm 0.09 \times 10^{-6}$ g/ 100 g wheat siliqua, respectively).

Table 1. Grain and flour moisture, falling number, W, P/L, and water absorption for Sossego, Audaz, and Toruk cultivars. ^{a-e}mean values with different lowercase superscript letters within each column denote significant differences ($p < 0.05$), and values are mean \pm SD.

Treatment	Grain moisture (g/100g)	Flour moisture (g/100g)	Falling number (s)	W (10^{-4} J)	P/L	Water absorption (mL)
1 – 50% N, AZ 300 mL, Sossego	13.10 \pm 0.03 ^c	14.30 \pm 0.07 ^d	450	168	1.01	31.00
2 – 50% N, AZ 900 mL, Sossego	13.40 \pm 0.02 ^b	14.90 \pm 0.02 ^{ab}	425	202	1.20	41.90
3 – 100% N, AZ 300 mL, Audaz	13.70 \pm 0.04 ^a	14.70 \pm 0.05 ^c	373	199	0.81	16.30
4 – 100% N, AZ 900 mL, Audaz	12.90 \pm 0.02 ^{de}	14.90 \pm 0.08 ^{ab}	366	231	0.96	48.00
5 – 50% N, Toruk	12.80 \pm 0.08 ^e	14.80 \pm 0.02 ^{bc}	345	148	0.79	38.40
6 – 100% N, Toruk	13.00 \pm 0.06 ^{cd}	15.00 \pm 0.03 ^a	356	140	0.87	41.20

Table 2. Color and gluten analysis of Sossego, Audaz, and Toruk cultivars. ^{a-e}mean values with different lowercase superscript letters within each column denote significant differences ($p < 0.05$), and values are mean \pm SD.

Treatment	L*	a*	b*	Wet gluten	Dry gluten
1 – 50% N, AZ 300 mL, Sossego	90.96 \pm 0.04 ^d	0.22 \pm 0.01 ^b	11.17 \pm 0.03 ^b	34.86 \pm 0.80 ^a	12.02 \pm 0.68 ^a
2 – 50% N, AZ 900 mL, Sossego	90.79 \pm 0.01 ^d	0.36 \pm 0.01 ^a	11.15 \pm 0.01 ^b	34.52 \pm 0.55 ^a	11.90 \pm 0.52 ^a
3 – 100% N, AZ 300 mL, Audaz	88.13 \pm 0.06 ^e	0.07 \pm 0.01 ^c	9.56 \pm 0.01 ^d	28.51 \pm 0.47 ^c	9.83 \pm 0.51 ^b
4 – 100% N, AZ 900 mL, Audaz	91.4 ^c \pm 0.05 ^c	0.06 \pm 0.01 ^c	11.95 \pm 0.02 ^a	31.17 \pm 0.32 ^b	10.74 \pm 0.24 ^{ab}
5 – 50% N, Toruk	92.16 \pm 0.09 ^a	0.07 \pm 0.02 ^c	10.82 \pm 0.02 ^c	34.17 \pm 1.02 ^a	11.78 \pm 1.09 ^a
6 – 100% N, Toruk	91.70 \pm 0.11 ^b	0.06 \pm 0.01 ^c	10.80 \pm 0.02 ^c	35.27 \pm 0.16 ^a	12.16 \pm 0.13 ^a

3.5. Grain and wheat flour:

Table 1 presents the results of moisture of the grain and the wheat flour, falling number, the energy of deformation of the mass (W), the relation between the tenacity and extensibility (P/L), and the water absorption of the six wheat cultivars evaluated. The moisture of the grain varied between 12.80 and 13.70 g/100g, and the flour's moisture varied between 14.30 and 15.00 g/100g. For the cultivars, Sossego and Audaz, an increase in the value of W was observed when the value of AZ increased from 300 to 900 mL. It is also possible to observe a higher water absorption for cultivars with AZ 900 mL and 100% N compared to the other cultivars, as well as a slight increase in the moisture content in the flours and a higher P/L ratio. Table 2 presents the results for color and wet and dry gluten.

According to the physicochemical evaluation, the falling number is related to the enzymatic activity of α -amylase. When the falling number is high, the enzyme activity is low in the flour [38]. The falling number varied between 345 and 450s, with the highest values observed for the cultivar Sossego and the highest values for the P/L ratio. Table 2 indicates the results for color and wet and dry gluten. As for luminosity, the samples varied between 88.13 and 92.16, values similar to those found in the literature [39]. The b^* values were higher for the cultivars Audaz AZ 900 mL (11.95) and Sossego (11.15 - 11.17) compared to the other samples, which indicates the presence of yellow pigments [40]. According to the classification of wet gluten content by Tian *et al.* 2018, the flours in this study had a high gluten content (> 30%), with the exception of the cultivar Audaz AZ 300 mL, which exhibited a medium gluten content (26% ~ 30%).

4. Conclusions

The foliar inoculation of *A. brasilense* in wheat provided an increase in yield components when associated with N fertilization. The application of 50% (20 kg/ha) and 10% (40 kg/ha) of the recommended amount of N resulted in higher grain yields compared with the other treatments. In general, the Sossego cultivar presented a higher grain yield (up to 3197.05 kg/ha). Up to 0.84 g reducing sugars/ 100 g wheat silique was indicated by the SWH procedure. Hence, this study concluded that N management was more responsive than association with *A. brasilense* for the expression of the crop maximum potential. Nonetheless, the association of wheat with *A. brasilense* promoted promising results when added to applied N. Additionally, new studies should be encouraged to demonstrate the effects of *A. brasilense* on the productivity of different wheat cultivars as a tool for programming management strategies and benefits for the farmers.

Funding

This work was supported by Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council of Technological and Scientific Development (CNPq), and the Research Support Foundation of the State of Rio Grande do Sul (FAPERGS). G. L. Zabot thanks CNPq (304882/2018-6) for the productivity grant. M. V. Tres thanks CNPq (308936/2017-5; 428180/2018-3; 306241/2020-0) for the productivity grant.

Acknowledgments

The authors would like to thank the anonymous reviewers for their valuable comments and suggestions to improve the paper's quality.

Conflicts of Interest

The authors declare no conflict of interest.

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