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**INOCULATION WITH *Azospirillum brasilense* ASSOCIATED WITH SILICON
APPLIED TO CORRECT SOIL ACIDITY AND NITROGEN RATES IN CORN AND
WHEAT CROPS**

Ilha Solteira
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**PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA (SISTEMAS
DE PRODUÇÃO)**

FERNANDO SHINTATE GALINDO

**INOCULATION WITH *Azospirillum brasilense* ASSOCIATED WITH
SILICON APPLIED TO CORRECT SOIL ACIDITY AND NITROGEN
RATES IN CORN AND WHEAT CROPS**

Tese apresentada à Faculdade de
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Orientador

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TRECHO DA MÚSICA: MINHA PRECE (Milionário e José Rico)

“(...) Enquanto existir
Um amigo sequer
Sozinho não caminharei
Enquanto existir
Uma mão amiga
A me amparar eu não cairei
Enquanto no céu
Uma estrela brilhar
Nas trevas não ficarei
Enquanto existir motivo
Que é a razão pra gente lutar
Em frente seguirei
E derrota jamais irei gritar (...)”

ABSTRACT

Alternative management practices are needed to minimize the need for chemical fertilizer use in non-leguminous cropping systems. The use of biological agents which can fix atmospheric nitrogen and promote plant growth has shown potential as an option to improve soil nutrient availability to grass crops. In recent years, studies investigating the effects of silicon (Si) have increased substantially, especially in grain crops. This increased interest in Si is likely due to the beneficial effects of Si application on plant resistance to abiotic and biotic stresses, reflecting on greater plant development. This research was developed to investigate if inoculation of corn and wheat with *Azospirillum brasilense* associated with Si can enhance nitrogen use efficiency (NUE) and improve plant nutrition and yield, leading to a greater economic profit. The study was set up in a Rhodic Haplustox under no-till system, located in Selvíria, state of Mato Grosso do Sul, Brazil. The field trial took place during the 2015/16 (November-March), 2016 (April-September), 2016/17 (November-March) and 2017 April-September) seasons, with corn and wheat crops in succession (spring/summer and winter seasons for corn and wheat, respectively). Treatments were tested in a randomized completely block design with four replicates, arranged in a full factorial design $5 \times 2 \times 2$ and included: i) five N application rates, as urea source, applied in side-dressing (0, 50, 100, 150 and 200 kg ha⁻¹); ii) two liming sources (Ca and Mg silicate as Si source and dolomitic limestone); and iii) and two seed inoculation (with and without *A. brasilense*). Increasing N rates positively influenced corn and wheat nutrition with increased nutrient and Si uptake in shoot, straw and grain, plant development and grain filling, leading to a greater grain yield. The average nutrient uptake in corn straw and grain in descending order was K > N > Ca > Si > Mg > S > P > Fe > Mn > Zn > Cu > B for straw; and N > K > P > S > Mg > Ca > Si > Fe > Zn > Mn > B > Cu for grains. For wheat, the average nutrient uptake was K > N > Si > Ca > S > Mg > P > Fe > Mn > Zn > B > Cu in straw; and N > K > P > S > Mg > Si > Ca > Fe > Mn > Cu > Zn > B in grains. The application of Ca and Mg silicate as a Si source had small effects on corn and wheat development and grain yield. Although silicate application as Si source did not greatly favor NUE, Si also did not negatively affect the inoculation and N fertilization, and correlates positively with nutritional, productive components, soil chemical attributes and corn and wheat grain yield. After four cropping seasons (2015/16, 2016, 2016/17 and 2017, with corn and

wheat in succession), silicate application was more efficient as a liming source to reduce soil acidity, with decreased soil H+Al and Al content, and increased pH and base saturation (V). Inoculation with *A. brasilense* increased NUE in corn crop by 19.1% and wheat by 38.6% (average of both years, in both crops) leading to a greater leaf chlorophyll index (LCI), nutrient uptake, corn development, grain filling, grain yield and operating profit. Also, inoculation could increase corn and wheat leaf N concentration when associated with silicate application. Inoculation provided greater corn grain yield by 5.6% (average of both years). Under high N application rates (200 kg N ha⁻¹), inoculation provided an increase of 11.4% on corn grain yield, in 2015/16. In the absence of N fertilization, inoculation provided an increase of 20.2% in grain yield, in 2016/17. In wheat crop, with application of 200 kg N ha⁻¹, inoculation provided an increase of 22.2% in shoot N uptake, in 2016. Inoculation provided greater wheat grain yield by 6.8% (average of both years). The application of 200 and 140 kg N ha⁻¹ in side-dressing with dolomitic limestone, associated with *A. brasilense* inoculation provided greater corn grain and wheat yield, respectively. However, the highest economic return was obtained with 100 kg N ha⁻¹, with limestone and inoculation, ensuring profitability from production of irrigated corn and wheat under tropical condition (Brazilian Savannah - Cerrado).

Keywords: Biological nitrogen fixation on grasses. Economic viability. Nitrogen fertilization with urea. Plant growth promoting bacteria. Silicon use on grasses. *Zea mays* L. *Triticum aestivum* L.

RESUMO

Práticas alternativas de manejo são necessárias para minimizar a necessidade por fertilizantes químicos em sistemas de cultivos com culturas não leguminosas. O uso de agentes biológicos que fixam nitrogênio atmosférico e promovem crescimento de plantas tem demonstrado potencial para aumento na disponibilidade de nutrientes do solo às culturas de gramíneas. Nos últimos anos, estudos investigando os efeitos do silício (Si) têm crescido substancialmente, especialmente em culturas agrícolas de grãos. O crescente interesse no estudo com Si ocorre principalmente em função dos efeitos benéficos de sua aplicação, principalmente em condições de estresse biótico e abiótico, refletindo em maior desenvolvimento das plantas. O objetivo deste estudo foi investigar se a inoculação com *Azospirillum brasilense* no milho e no trigo associado à aplicação de Si pode aumentar a eficiência de uso do nitrogênio (EUN) e refletir positivamente na nutrição de plantas e produtividade de grãos, propiciando aumento no retorno econômico. O estudo foi desenvolvido em um Latossolo Vermelho distrófico típico em sistema plantio direto, localizado no município de Selvíria, Mato Grosso do Sul. O experimento foi conduzido durante as safras 2015/16 (Novembro-Março), 2016 (Abril-Setembro), 2016/17 (Novembro-Março) e 2017 (Abril-Setembro) com as culturas do milho e trigo em sucessão (milho e trigo cultivados nas estações de primavera/verão e inverno, respectivamente). O delineamento experimental utilizado foi em blocos casualizados, com quatro repetições, disposto em esquema fatorial $5 \times 2 \times 2$, incluindo: i) cinco doses de N em cobertura na forma de ureia (0, 50, 100, 150 e 200 kg ha⁻¹); ii) duas fontes de corretivo de acidez do solo (silicato de Ca e Mg como fonte de Si e calcário dolomítico); iii) com e sem inoculação das sementes com *A. brasilense*. O incremento nas doses de N influenciou positivamente na nutrição do milho e do trigo, com aumento no acúmulo de nutrientes e Si em parte aérea, palhada e grãos, no desenvolvimento das plantas e no enchimento de grãos, propiciando aumento na produtividade de grãos. O acúmulo de nutrientes médio na palhada do milho e nos grãos, em ordem decrescente foi K > N > Ca > Si > Mg > S > P > Fe > Mn > Zn > Cu > B na palhada; e N > K > P > S > Mg > Ca > Si > Fe > Zn > Mn > B > Cu nos grãos. Para o trigo, o acúmulo de nutrientes foi K > N > Si > Ca > S > Mg > P > Fe > Mn > Zn > B > Cu na palhada; e N > K > P > S > Mg > Si > Ca > Fe > Mn > Cu > Zn > B nos grãos. A utilização do silicato de Ca e Mg como fonte de Si pouco influenciou no

desenvolvimento e na produtividade do milho e do trigo. Embora o silicato como fonte de Si não tenha influenciado sobremaneira a EUN, o Si não afetou negativamente a inoculação e a adubação nitrogenada, além de correlacionar-se positivamente com as avaliações nutricionais, componentes produtivos, atributos químicos do solo e produtividade de grãos de milho e de trigo. Após quatro safras (2015/16, 2016, 2016/17 e 2017, com milho e trigo em sucessão), a aplicação do silicato de Ca e Mg foi mais eficiente como corretivo de acidez de solo do que o calcário dolomítico, com redução do H⁺Al e Al e aumento do pH da saturação por bases (V) do solo. A inoculação com *A. brasilense* aumentou a EUN no milho em 19,1% e no trigo em 38,6% (média das suas safras, para ambas as culturas), propiciando aumento no índice de clorofila foliar (ICF), no acúmulo de nutrientes, no desenvolvimento de plantas, no enchimento de grãos, na produtividade de grãos, com reflexo positivo no lucro operacional do milho e do trigo. Além disso, a inoculação pode aumentar a concentração de N foliar no milho e trigo quando associado à aplicação do silicato. A inoculação propiciou aumento na produtividade de grãos de milho em 5,6% (média das duas safras). Quando associada à dose de N elevada (200 kg N ha⁻¹) a inoculação aumentou a produtividade de grãos de milho em 11,4% em 2015/16. Na ausência de adubação com N em cobertura, a inoculação propiciou aumento em 20,2% na produtividade de grãos de milho, em 2016/17. Para a cultura do trigo, com aplicação de 200 kg N ha⁻¹, a inoculação propiciou aumento de 22,2% no acúmulo de N em parte aérea em 2016. A inoculação propiciou aumento na produtividade de grãos de trigo em 6,8% (média das suas safras). A aplicação de 200 e 140 kg N ha⁻¹ em cobertura, com aplicação de calcário dolomítico e inoculação com *A. brasilense* propiciou as maiores produtividades de grãos de milho e trigo, respectivamente. Entretanto, o maior retorno econômico foi obtido com a aplicação de 100 kg N ha⁻¹ associado à utilização de calcário dolomítico e inoculação com *A. brasilense*, garantindo lucratividade na produção de milho e trigo irrigados em região tropical (Cerrado de baixa altitude).

Palavras-chave: Fixação biológica de nitrogênio em gramíneas. Viabilidade econômica. Adubação nitrogenada com ureia. Bactérias promotoras de crescimento em plantas. Uso de silício em gramíneas. *Zea mays* L. *Triticum aestivum* L.

LIST OF FIGURES

Figure 1	- Study area at the Selvíria, state of Mato Grosso do Sul, Brazil. Map created by using QGIS software and Google Earth program. QGIS Development Team (2019). QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org . Image obtained in Google Earth program. Google company (2020)	47
Figure 2	- Rainfall, maximum, average and minimum temperatures obtained from the weather station located in the Education and Research Farm of FE / UNESP during the corn cultivation in the period November 2015 to April 2016 (A) November 2016 to April 2017 (B)	49
Figure 3	- Interaction between N rates and liming sources in LCI in 2015/16 (A) and 2016/17 (B), leaf NH_4^+ concentration in 2015/16 (C) and 2016/17 (D), interaction between N rates and inoculation with <i>A. brasilense</i> in leaf N concentration in 2016/17 (E) and interaction between liming sources and inoculation with <i>A. brasilense</i> in leaf N concentration in 2016/17 (F) in corn crop	58
Figure 4	- Interaction between N rates and inoculation with <i>A. brasilense</i> in shoot N uptake in 2015/16 (A), interaction between N rates and liming sources in root N uptake in 2015/16 (B), and 2016/17 (C), interaction between N rates and inoculation in root N uptake in 2015/16 (D) and 2016/17 (E) in corn crop	61
Figure 5	- Interaction between N rates and liming sources in root biomass in 2015/16 (A), interaction between N rates and inoculation with <i>A. brasilense</i> in number of grains per ear in 2015/16 (B), interaction between N rates and liming sources on corn grain yield in 2015/16 (C) and 2016/17 (D), interaction between N rates and inoculation in grain yield in 2015/16 (E) and 2016/17 (F) in corn crop	68
Figure 6	- Interaction between N rates and inoculation in grain N uptake in	

	2015/16 (A), grain S uptake in 2015/16 (B) and grain Zn uptake in 2015/16 (C) in corn crop	81
Figure 7	- Rainfall, maximum, average and minimum temperatures obtained from the weather station located in the Education and Research Farm of FE / UNESP during the wheat cultivation in the period May 2016 to October 2016 (A) May 2017 to October 2017 (B)	99
Figure 8	- Interaction between N rates and liming sources in LCI in 2017 (A), leaf NO_3^- concentration in 2016 (B), interaction between liming sources and inoculation in leaf NO_3^- concentration in 2016 (C), interaction between N rates and inoculation in leaf N concentration in 2016 (D), and 2017 (E) and interaction between liming sources and inoculation in leaf N concentration in 2016 (F) in wheat crop	108
Figure 9	- Interaction between N rates and inoculation in leaf Si concentration in 2017 (A) and shoot N uptake in 2016 (B) in wheat crop	110
Figure 10	- Interaction between N rates and inoculation in grain yield in 2016 (A) 2017 (B), interaction between N rates and liming sources in grain yield in 2017 (C) in wheat crop	118

LIST OF TABLES

Table 1	- Soil chemical attributes and granulometry in 0-0.20m and 0.20-0.40m layers before the application of liming sources	48
Table 2	- Nutrient accumulation and C/N ratio in corn straw (2015 corn - predecessor crop)	51
Table 3	- Leaf chlorophyll index (LCI), leaf NO_3^- , NH_4^+ , N and Si concentration as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	57
Table 4	- Leaf N and Si concentration, shoot NO_3^- , N and Si uptake and root NO_3^- , NH_4^+ and Si uptake as a function of N rates in corn crop	59
Table 5	- Shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	62
Table 6	- Shoot and root biomass, plant height, stem diameter, ear length, ear diameter, number of rows per ear, grains per row and grains per ear, mass of 100 grains, straw biomass and harvest index as a function of N rates in corn crop	67
Table 7	- Shoot and root biomass, plant height, stem diameter, ear length, ear diameter and number of rows per ear as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	69
Table 8	- Number of grains per row, grains per ear, mass of 100 grains, straw biomass, harvest index and grain yield as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	70
Table 9	- Nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use (NUE) as a function of N rates in corn crop	74
Table 10	- Nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use (NUE) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	75
Table 11	- Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a	

	function of N rates in corn crop	78
Table 12	- Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	79
Table 13	- Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates in corn crop	82
Table 14	- Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	83
Table 15	- Soil P-resin, organic matter (OM), Ca, sum of bases (SB), cation exchange capacity (CEC), Zn and Si content as a function of N rates in corn crop	86
Table 16	- Soil P-resin, organic matter (OM), pH, K, Ca, Mg, sum of bases (SB), S-SO ₄ , cation exchange capacity (CEC), base saturation (V) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	87
Table 17	- Soil H+Al, Al, B, Cu, Fe, Mn, Zn and Si content as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	89
Table 18	- Total operating cost (TOC) structure model of corn crop for the control treatment (0 kg N ha ⁻¹ in side-dressing), with limestone application and without inoculation with <i>A. brasilense</i> per hectare	92
Table 19	- Total operating cost (TOC), grain yield and gross revenue (GR) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	94
Table 20	- Operating profit (OP) and profitability index (PI) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	95
Table 21	- Accumulated operating profit (AOP), equilibrium price (EP) and equilibrium yield (EY) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop	96
Table 22	- Nutrient accumulation and C/N ratio in corn straw (2015/16 corn	

	- predecessor crop)	101
Table 23	- Leaf chlorophyll index (LCI), leaf NO_3^- , NH_4^+ , N and Si concentration as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	106
Table 24	- Leaf chlorophyll index (LCI), leaf NH_4^+ concentration, shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rates in wheat crop	107
Table 25	- Shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	108
Table 26	- Shoot and root biomass, plant height, number of spikes per meter, spike length, number of defective grains, spikelets per spike and grains per spikelet as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	115
Table 27	- Shoot and root biomass, number of spikes per meter, spike length, spikelets per spike, grains per spike and straw biomass as a function of N rates in wheat crop	115
Table 28	- Number of grains per spike, hectoliter mass, mass of 1000 grains, straw biomass, harvest index and grain yield as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	116
Table 29	- Nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use (NUE) as a function of N rates in wheat crop	121
Table 30	- Nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use (NUE) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	121
Table 31	- Straw N, P, K, Ca, Mg, S, B, Mn, Zn and Si uptake as a function of N rates in wheat crop	125
Table 32	- Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	126
Table 33	- Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Zn and Si uptake as a function of N rates in wheat crop	128

Table 34	- Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	129
Table 35	- Soil P-resin, K and organic matter (OM) as a function of N rates in wheat crop	133
Table 36	- Soil P-resin, organic matter (OM), pH, K, Ca, Mg, sum of bases (SB), S-SO ₄ , cation exchange capacity (CEC), base saturation (V) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	134
Table 37	- Soil H+Al, Al, B, Cu, Fe, Mn, Zn and Si content as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	135
Table 38	- Total operating cost (TOC) structure model for wheat crop in the control treatment (0 kg ha ⁻¹ N in side-dressing), with limestone application and without inoculation with <i>A. brasilense</i> per hectare	137
Table 39	- Total operating cost (TOC), grain yield and gross revenue (GR) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	139
Table 40	- Operating profit (OP) and profitability index (PI) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	140
Table 41	- Accumulated operating profit (AOP), equilibrium price (EP) and equilibrium yield (EY) as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in wheat crop	141

SUMMARY

1	INTRODUCTION	22
2	LITERATURE REVIEW	26
2.1	CORN AND WHEAT CROP PRODUCTION IN BRAZIL AND IN THE WORLD	26
2.2	NITROGEN MANAGEMENT IN CEREAL CROPS	29
2.1.1	Nitrogen fertilization in corn crop under tropical conditions	33
2.1.2	Nitrogen fertilization in wheat crop under tropical conditions	34
2.3	PLANT GROWTH PROMOTING BACTERIA: <i>Azospirillum brasilense</i>	36
2.3.1	Inoculation with <i>Azospirillum brasilense</i> in corn and wheat associated with N application under tropical conditions	39
2.4	SILICON IN AGRICULTURE	41
Study i	CORN RESPONSE TO INOCULATION WITH <i>Azospirillum brasilense</i> ASSOCIATED WITH SILICON AND NITROGEN RATES	46
3	MATERIAL AND METHODS	46
3.1	FIELD SITE DESCRIPTION	46
3.2	EXPERIMENTAL DESIGN AND TREATMENTS	50
3.3	FIELD MANAGEMENT.....	51
3.4	MEASUREMENTS	52
3.4.1	In season evaluations	52
3.4.2	Productive components, NUE, and grain yield	53
3.4.3	Nutrient uptake in corn straw and grain (uptake and removal)	53
3.4.4	Soil chemical attributes after corn cultivation (0-0.20m depth)	54
3.4.5	Economic analysis	54
3.5	STATISTICAL ANALYSIS	55
4	RESULTS AND DISCUSSION	56
4.1	IN SEASON EVALUATIONS	56
4.2	PRODUCTIVE COMPONENTS, NUE AND GRAIN YIELD	65
4.3	NUTRIENT UPTAKE IN CORN STRAW AND GRAIN (UPTAKE AND	

	REMOVAL)	76
4.4	SOIL CHEMICAL ATTRIBUTES AFTER CORN CULTIVATION (0-0.20M DEPTH)	85
4.5	ECONOMIC ANALYSIS	91
Study ii	WHEAT RESPONSE TO INOCULATION WITH <i>Azospirillum brasilense</i> ASSOCIATED WITH SILICON AND NITROGEN RATES	98
3	MATERIAL AND METHODS	98
3.1	FIELD SITE DESCRIPTION	98
3.2	EXPERIMENTAL DESIGN AND TREATMENTS	100
3.3	FIELD MANAGEMENT.....	101
3.4	MEASUREMENTS	102
3.4.1	In season evaluations	102
3.4.2	Productive components, NUE, and grain yield	102
3.4.3	Nutrient uptake in wheat straw and grain (uptake and removal)	103
3.4.4	Soil chemical attributes after wheat cultivation (0-0.20m depth)	103
3.4.5	Economic analysis	103
3.5	STATISTICAL ANALYSIS	104
4	RESULTS AND DISCUSSION	105
4.1	IN SEASON EVALUATIONS	105
4.2	PRODUCTIVE COMPONENTS, NUE AND GRAIN YIELD	113
4.3	NUTRIENT UPTAKE IN CORN STRAW AND GRAIN (UPTAKE AND REMOVAL)	124
4.4	SOIL CHEMICAL ATTRIBUTES AFTER CORN CULTIVATION (0-0.20M DEPTH)	133
4.5	ECONOMIC ANALYSIS	137
6	CONCLUSIONS	143
	REFERENCES	145
	APPENDIX A – Corn F-test values	188

APPENDIX B – Wheat F-test values	192
APPENDIX C – Corn heatmaps (Pearson correlation)	200
APPENDIX D – Wheat heatmaps (Pearson correlation)	206
APPENDIX E – Photos	212

1 INTRODUCTION

Brazil is the world's third largest producer of corn (*Zea mays* L.) and a large importer of wheat (*Triticum aestivum* L.) (CONAB, 2020). Corn is among of the most relevant and cultivated grain crop used for human and animal consumption (FOSTER *et al.*, 2016). However, the Brazilian average yield (average of 5.63 Mg ha⁻¹) is still low compared with high yielding areas of the USA (average of 10.84 Mg ha⁻¹) and Europe (average of 8.39 Mg ha⁻¹) (EUROSTAT, 2020; USDA, 2020; CONAB, 2020). Similarly, wheat is an annual crop that has significant economic importance among the winter season cereals, due to its large grain yield capacity (FANG *et al.*, 2017; FAROOQ *et al.*, 2017). However, in Brazil, the average yield (average of 2.53 Mg ha⁻¹) is still low compared with the high yielding areas of Europe (e.g., Ireland, Netherlands and United Kingdom average 10.10, 9.17 and 8.58 Mg ha⁻¹, respectively), Africa (e.g., Zambia, Namibia and Egypt average of 7.16, 6.67 and 6.51 Mg ha⁻¹, respectively) and USA (average of 3.63 Mg ha⁻¹) (FAO, 2017; USDA, 2020; CONAB, 2020). In the last half decade, more than 50% of Brazil's wheat consumption was imported from other countries (CONAB, 2020).

The increased demand for food production has resulted in an overuse of agricultural resources, among which nitrogen (N) can be considered one of the most yield limiting factors for maximum yield (LASSALETTA *et al.*, 2016; SCHILS *et al.*, 2018). It has been estimated that 15% of the total operating profit of cereals being produced on the Brazilian Savannah (Cerrado) is due to side-dressing N application (GALINDO *et al.*, 2017a, 2018a, 2019a). The Brazilian Savannah area comprises about 204.7 million hectares, equivalent to 23.3% of the country land (IBGE, 2019), being the main area for expanding crops in Brazil. Therefore, knowledge-based N management is critical for global food security and environmental sustainability (XIA *et al.*, 2017). The production and over application of N fertilizer contribute to a series of environmental problems, such as greenhouse gas emissions, global warming, air pollution, contamination of water bodies and eutrophication (LASSALETTA *et al.*, 2016; XIA *et al.*, 2017). Therefore, integrated N management strategies could contribute significantly to

improving N use efficiency (NUE) under Savannah conditions, particularly for corn and wheat, which has high N requirements (LADHA *et al.*, 2016; QUATRIN *et al.*, 2019).

Bacteria with multiple plant-growth promoting traits (PGPB, plant-growth promoting bacteria) can improve NUE and increase the growth and grain yields of cereal crops under tropical conditions (GALINDO *et al.*, 2016, 2017b, 2019b; MARTINS *et al.*, 2018). Numerous PGPB genera show positive associations with different plant species of agricultural importance (for example, *Azospirillum*, *Rhizobium*, *Bradyrhizobium*, *Bacillus* and *Pseudomonas*) (ZEFFA *et al.*, 2018). *Azospirillum* sp. is currently one of the most broadly studied and commercially employed PGPB (CASSÁN; DIAZ-ZORITA, 2016; ZEFFA *et al.*, 2018). This microorganism is able to colonize more than one hundred plant species and significantly improves their growth, development and productivity under field conditions (BASHAN; DE-BASHAN, 2010; CASSÁN; DIAZ-ZORITA, 2016; FUKAMI *et al.*, 2018a).

Previous studies with *Azospirillum* emphasize its capacity to fix N₂, as well as its benefits in terms of promoting plant growth via the synthesis of phytohormones, such as auxins, gibberellins, cytokinins, ethylene and salicylic acid, and nutrient availability increases (VEJAN *et al.*, 2016; FUKAMI *et al.*, 2018a; ZAHEER *et al.*, 2019). Additionally, *Azospirillum* sp. has been associated with reduced biotic and abiotic stresses, for example, increasing plant resistance to pathogens, salinity and drought and reducing the oxidative damage caused by an increase in reactive oxygen species (VEJAN *et al.*, 2016; FUKAMI *et al.*, 2018a; FORNI *et al.*, 2017; MAJEED *et al.*, 2018). An analysis of field trials conducted worldwide for over 20 years, where various non-legume crops were inoculated with *Azospirillum* spp. under different weather and soil conditions, concluded that crop yield can increase up to 30% with inoculation (FUKAMI *et al.*, 2016). Zeffa *et al.* (2018) performed a meta-analysis on the influence of side-dressing N application associated with *Azospirillum* sp. inoculation in corn and reported an average yield increase of 652 kg ha⁻¹. Munareto *et al.* (2019) verified the positive influence of side-dressing N application associated with *Azospirillum brasilense* seed inoculation in wheat and reported an average yield increase of 36.8% and 13.5% when the inoculation was associated with N application rates of 70 and 140 kg ha⁻¹, respectively.

In recent years, the use of silicon (Si) fertilizers has been reported to improve plant growth and development with greater N uptake and NUE in cereal crops (NEU *et al.*, 2017; XU *et al.*, 2018; CONCEIÇÃO *et al.*, 2019; RAMÍREZ-OLVERA *et al.*, 2019). Silicon application has been associated with increased plant resistance to abiotic and biotic stresses, such as attacks from insects and pathogens, salinity, drought, flooding, and high and low temperatures (COSKUN *et al.*, 2016; CRUSCIOL *et al.*, 2018; RAMÍREZ-OLVERA *et al.*, 2019; VEGA *et al.*, 2019). Si enhances primary metabolism by decreasing the transpiration rate (CAMARGO *et al.*, 2017) and improving photosynthesis (ARAUJO *et al.*, 2019; VEGA *et al.*, 2019) and nutrient uptake (HAJIBOLAND *et al.*, 2018) as well as secondary metabolism by stimulating the production of phenolic compounds with either antioxidant (for example, flavonoids) or structural (for example, lignin and cellulose) functions (XU *et al.*, 2015; VEGA *et al.*, 2019). Silicon has also been reported to stimulate germination and increase root length (TORABI *et al.*, 2012; RAMÍREZ-OLVERA *et al.*, 2019), the number of leaves and plant architecture (XU *et al.*, 2015; DORNELES *et al.*, 2018). Calcium and magnesium silicate as a liming source can correct soil acidity; increase soil pH, extractable levels of Si, Ca, Mg and P; and decrease the harmful effects of toxic heavy metals such as Al, Fe and Cd (ADREES *et al.*, 2015; CASTRO *et al.*, 2016; CRUSCIOL *et al.*, 2016; ANTONANGELO *et al.*, 2017).

Silicon is the second most abundant element in the earth's crust after oxygen (TUBANA *et al.*, 2016; NEU *et al.*, 2017). However, Si availability in tropical soils is limited mainly by the high levels of weathering and desilication of primary silicate minerals (HAYNES *et al.*, 2017; WALSH *et al.*, 2018; LINDEN *et al.*, 2019). Therefore, studies on Si applications aiming to increase Si replacement, NUE and N uptake under tropical conditions should be performed. Recently, research on the interaction of Si application and PGPB inoculation has increased (ETESAMI, 2018; CHANDRAKALA *et al.*, 2019; REZAKHANI *et al.*, 2019a, b). Etesami (2018) suggests that the associated use of Si and PGPB can be a powerful and sustainable strategy to enhance plant growth under sub-optimal conditions. Rezakhani *et al.* (2019a, b) concluded that *Pseudomonas* sp. associated with Si application can improve wheat and sorghum (*Sorghum bicolor* (L.) Moench) development. Galindo *et al.* (2019c) studied inoculation

methods associated with Ca and Mg silicate and reported an average wheat grain yield increase of 6.7% when seed inoculation and silicate application were performed. Therefore, new studies with Si application associated with inoculation need to be performed to improve NUE, corn and wheat development and grain yield. Additionally, new studies are needed to define how much N fertilizer needs to be applied when Si is applied in association with *A. brasilense* for optimum grain yield. The hypothesis of this study was that the application of Si associated with *A. brasilense* inoculation would improve NUE and reduce the amount of N required for maximum corn and wheat production under tropical Savannah conditions. This research was developed to investigate if inoculation of corn and wheat with *Azospirillum brasilense* associated with Si can enhance nitrogen use efficiency (NUE) and improve plant nutrition and yield, leading to a greater economic profit.

2 LITERATURE REVIEW

2.1 CORN AND WHEAT CROP PRODUCTION IN BRAZIL AND IN THE WORLD

Global area harvested with cereals is expected to grow by 17.6 million ha between 2017 and 2027. Harvested area in developed countries is expected to slightly decline (- 0.4 million ha) as increased wheat area harvested is offset by lower corn and other grain area (FAO, 2018). Conversely, area harvested in developing countries is projected to expand 18 million ha. Slow global area expansion is largely due to low cereal prices relative to other crops and higher yields that support the growth in production and demand (FAO, 2018). Area growth is also limited by more restrictive land availability compared to the previous decade due to constraints on converting forest or pasture into arable land and on-going urbanization. Global corn and wheat areas are projected to increase by 3.2% and 1.4% while other grains are expected to increase by 2.4% by 2027 (FAO, 2018). Although the overall area to cereals will grow, the growth in yields is expected to contribute more to higher production, especially in developing countries with improving technology and cultivation practices. Global yields for wheat, corn and rice (*Oryza sativa* L.) are projected to increase 9%, 10%, and 12%, respectively, between the 2017 and 2027 (FAO, 2018).

Corn, also called maize or field corn, is one of the most important cereals in the world, with annual global production exceeding that of wheat and rice (1099.61 million Mg compared to 734.74 and 495.87 million Mg of wheat and rice, respectively) (CHAVAS; MITCHELL, 2018). In 2019, corn production accounted for approximately 40% of total grain production in the world (USDA, 2020). While corn is a staple food in parts of the world, it has many uses, including animal feed, biofuel and sweetener (FOSTER *et al.*, 2016; CHAVAS; MITCHELL, 2018). Corn was first domesticated in southern Mexico about 9000 years ago (MATSUOKA *et al.*, 2002; PIPERNO, 2011). Its closest wild relative is teosinte, a wild grass of Mexico, Guatemala and Honduras (MATSUOKA *et al.*, 2002; PIPERNO, 2011). A major puzzle is the great genetic differences between teosinte and corn, indicating how key mutations and human selection contributed to genetic evolution (HUFFORD *et al.*, 2013). As a C4 plant, corn has some photosynthetic advantages in capturing solar energy in warm weather

compared to C3 crops such as wheat, rice and soybean (*Glycine max* (L.) Merr.). Due to its high productivity under various climate conditions, corn is the largest grain crop in the world (CHAVAS; MITCHELL, 2018).

Favorable agro-climatic conditions in the “Corn Belt” have made the USA the largest corn producer. In 2019, corn production in the US accounted for above 30% of world corn production (347.8 million Mg) (USDA, 2020). China (260.8 million Mg) and Brazil (101 million Mg) are the world's second and third largest producer of corn, respectively (USDA, 2020). In Brazil, corn is traditionally grown in two crop seasons: September-April (First season) and February-September (Second season) (CONAB, 2020). The first corn crop season has little representativeness in the total Brazilian supply, since the cereal has the difficult task of competing with soybean crop, which has, at least, until now, the preference of Brazilian producers (CONAB, 2020). The average cropping area in the first and second season are 4147.9 and 12878.0 ha, respectively. In addition, the average yield in the first and second season are 6.42 and 5.51 Mg ha⁻¹, respectively (CONAB, 2020).

Recently a new corn crop season has grown quickly (third season). This crop season occurs mainly in the region so called “Sealba” (Sergipe, Alagoas, northeastern Bahia), Roraima and Amapá (CONAB, 2020). Planting is concentrated between May to June, similar to Northern Hemisphere cropping systems. It is estimated, as soybean crops are consolidated in these regions and, considering that corn appears as the ideal crop for the necessary planting rotation, the growing appearance of this supply, contributing to the regularization of internal supply and also generation of exportable surpluses (CONAB, 2020). The average corn area and grain yield in Brazil, considering the three cropping seasons is 17536.9 ha and 5.63 Mg ha⁻¹, respectively. However, the Brazilian average yield is still low compared with high yielding areas of the USA (average of 10.84 Mg ha⁻¹) and Europe (average of 8.39 Mg ha⁻¹) (EUROSTAT, 2020; USDA, 2020; CONAB, 2020).

Wheat is an annual crop that has significant economic importance among the cool season cereals, due to its large grain yield capacity (FANG *et al.*, 2017; FAROOQ *et al.*, 2017). Wheat is the second most widely cultivated cereal crop belonging to *Poaceae* Family and contribute with nearly 30% of the world grain production. China

(134.34 million Mg), India (98.51 million Mg) and Russia (85.86 million Mg) are the world's first, second and third largest producer of wheat, respectively (AKTER; ISLAM, 2017; FAO, 2020). Brazil is a large importer of wheat and is not even among the world's top ten wheat-producing countries (China, India, Russia, USA, France, Australia, Canada, Pakistan, Ukraine and Germany) (CONAB, 2020; FAO, 2020). In the last half decade, more than 50% of Brazil's wheat consumption was imported from other countries such as Argentina, Canada, Paraguai and USA (CONAB, 2020).

In Brazil, wheat is traditionally grown in winter season: April-November. The average production, cropping area and grain yield are 5154.7 million Mg, 2040.5 ha and 2.53 Mg ha⁻¹, respectively (CONAB, 2020). Similarly to corn, wheat grain yield is still low compared with the high yielding areas of Europe (e.g., Ireland, Netherlands and United Kingdom average of 10.10, 9.17 and 8.58 Mg ha⁻¹, respectively), Africa (e.g., Zambia, Namibia and Egypt average of 7.16, 6.67 and 6.51 Mg ha⁻¹, respectively) and USA (average of 3.63 Mg ha⁻¹) (FAO, 2017; USDA, 2020). Although the southern region is responsible for about 90% of the national production, the cereal has been introduced gradually in Savannah region (Brazilian Cerrado), mainly under irrigation (CONAB, 2020). The Savannah region has great potential for the expansion of the wheat crop, as it offers excellent climate and soil conditions, a strategic market position and industrialization capacity. Also, wheat is very receptive to heat stress (GUPTA *et al.*, 2013a, b). Low latitude zones, where around 100 million hectares of wheat are cultivated, are predominantly heat prone areas worldwide (BRAUN *et al.*, 2010).

Recent reports estimated that the global food production will have to increase by about 70% to accomplish 2050 demands (SHARMA *et al.*, 2015). The increased demand for food production has resulted in an over use of agricultural resources, among which nitrogen (N) can be considered one of the most yield limiting factors for maximum yield (LADHA *et al.*, 2016; GALINDO *et al.*, 2017b, 2019b). The use of high yielding varieties developed by breeding and planting of most suitable and efficient varieties for each location plays an important role in the sustainable intensification of agriculture (STAHL *et al.*, 2017). However, the low corn and wheat yield observed in Brazil is usually attributed to low-fertility soils that require high fertilizer inputs for optimum productivity (MARTINS *et al.*, 2018). Therefore, best practices that optimize N

management should also be developed (LADHA *et al.*, 2016; GALINDO *et al.*, 2017b, 2019b).

2.2 NITROGEN MANAGEMENT IN CEREAL CROPS

Nitrogen is the nutrient that is most demanded by cereal crops and directly affects crop development and yield (GOOD *et al.*, 2018; GALINDO *et al.*, 2019b; LOLLATO *et al.*, 2019). This nutrient play important roles in plant nutrition and development (LOLLATO *et al.*, 2019) such as synthesis and production of phytohormones, coenzymes, nucleic acids, secondary metabolites, chlorophyll and proteins (MARSCHNER, 2012). Nitrogen is the largest by composition in the atmosphere and yet remains the most limiting nutrient in most plants. The plants do not assimilate N₂, therefore its abundance does not indicate availability to plants (OMARA *et al.*, 2019). Natural mechanisms for atmospheric-soil N input include non-symbiotic and symbiotic fixation, and addition in rainfall (PEOPLES *et al.*, 1995; SULLIVAN *et al.*, 2014). However, the natural mechanism of soil N input does not significantly support world food production, as the world population growth rate has outpaced this process (CREWS; PEOPLES, 2004). Therefore, use of N from synthetic sources is inevitable in the face of increasing world population (OMARA *et al.*, 2019).

There is a lot of studies reporting the N fertilization benefits in different cereal crops such as corn (COYOS *et al.*, 2018; ICHAMI *et al.*, 2019), wheat (YU *et al.*, 2018; OMARA *et al.*, 2020), rice (JIA *et al.*, 2019; TATSUMI *et al.*, 2019), sorghum (*Sorghum bicolor* (L.) Moench) (MAW *et al.*, 2016; SAINJU *et al.*, 2018), barley (*Hordeum vulgare* L.) (YOUSIF; EVANS, 2018; KESSIE; TESFAYE, 2019) and oats (*Avena sativa* L.) (MA *et al.*, 2017; ALLWOOD *et al.*, 2018). The optimum amount of N required for best cereal production depends on many factors including soil type, indigenous nutrient concentration in the soil, weather conditions and others (AUSTIN *et al.*, 2019; SANCHEZ *et al.*, 2019). In general, the world average for N rate applied to corn and wheat is about 80 and 52 kg ha⁻¹ yr⁻¹ (LADHA *et al.*, 2016). Under tropical conditions, maximum productivity of corn and wheat has been achieved by applying N rates

ranging between 60 and 240 kg N ha⁻¹ (MENDES *et al.*, 2013; MUNARETO *et al.*, 2019; NUNES *et al.*, 2015; GALINDO *et al.*, 2017b, 2019a).

Despite technological improvements in plant nutrition, soil management and machinery, for example, use of no-tillage system, cover crops, and crop rotation to increase sustainability in Brazilian agricultural system, nutrient management strategies should be improved, in particular N management (GALINDO *et al.*, 2017b, 2019a; GIL *et al.*, 2018). It has been estimated that the cereal crops, mainly corn, wheat and rice, consume approximately 50% of all N-based fertilizer produced (LADHA *et al.*, 2016). In addition, as much as 15% of the total operating profit of the cereals produced in the Brazilian Savannah is due to side-dressing N application (GALINDO *et al.*, 2017a, 2018a, 2019a). It is commonly found that only 30 to 50% of N-fertilizer is taken up by grain crops (DUNCAN *et al.*, 2018; LOLLATO *et al.*, 2019).

Management practices that improve NUE are needed to assure that cereal production is maximized. Cereal world NUE was estimated and reported by Raun; Johnson (1999) at 33%. This implies that 67% of all the N applied is unaccounted for and can be lost within the soil system through leaching and/or gaseous forms, potentially contributing to a decrease in air and water quality (OMARA *et al.*, 2019). The initial documentation for low cereal world NUE sparked curiosity among researchers in many disciplines. Since the initial estimate, several research efforts have advanced from agronomic, environmental, and breeding perspectives in improving the low NUE (RAUN *et al.*, 2002; PRESTERL *et al.*, 2003; FAGERIA; BALIGAR, 2005; HIREL *et al.*, 2007; GARNETT *et al.*, 2009; OMARA *et al.*, 2019). It is well known that the production and the application of N fertilizer contribute to greenhouse gas (GHG) emission and the contamination of water bodies (XU *et al.*, 2012; ZHAO *et al.*, 2012).

According to Pires *et al.* (2015), out of all GHGs released annually into the atmosphere by human activities, the agriculture, forestry, and other land use sector is responsible for approximately a quarter of carbon dioxide (CO₂) emissions through deforestation, depletion of soil organic C, use of machinery and manufactured fertilizers; half of methane (CH₄) emissions via livestock and rice cultivation; and three quarters of nitrous oxide (N₂O) emissions through fertilizer application and animal waste management. Therefore, best management practices that minimize the potential

negative environmental impacts resulting from excessive N application should also be developed (LADHA *et al.*, 2016; LASSALETA *et al.*, 2016; GALINDO *et al.*, 2017b, 2019a).

Nitrogen is a very dynamic nutrient in the soil and is involved in many processes such as, ammonification, nitrification, denitrification and mineralization (ROSOLEM *et al.*, 2017; SILVA *et al.*, 2017). When those processes are coupled with poor fertilizer management practices, such as application of inadequate rates, broadcast application without incorporation or excessively high rates applied to poorly drained soils, low NUE is likely to occur (TASCA *et al.*, 2011; MIRA *et al.*, 2017). The biggest challenge to improving NUE is the ability to associate genetic, agronomic, and environment variables (OMARA *et al.*, 2019). Agronomic practices are responsible for low NUE due to excessive application of N fertilizers (RAUN; JOHNSON, 1999; ZHU *et al.*, 2005). However, improved N economy from reduced N fertilizer inputs must operate within acceptable crop yield levels (HIREL *et al.*, 2007) mainly because food production needs to be intensified on a fixed world land resource while embracing the challenges that comes with as much (TILMAN *et al.*, 2011; OMARA *et al.*, 2019).

Inadequate N management has contributed to increased N losses by ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching, denitrification and N_2O emissions, which have both economic and environmental consequences (LINQUIST *et al.*, 2013; ABALOS *et al.*, 2014; MARTINS *et al.*, 2015, 2017). Ammonia volatilization from the applied fertilizer is an important issue when urea is applied on the soil surface in no-till systems (ROSOLEM *et al.*, 2017). Under tropical conditions, urea is usually applied as side-dressing with plant residues on the soil surface blocking or delaying the contact between soil and fertilizer, which enhances urease activity far above that of bare soil, favoring NH_3 volatilization (MIRA *et al.*, 2017). The rate at which volatilization losses occur depends on fertilizer management practices, and ecological conditions like wind, temperature, rainfall, and soil properties (SOMMER *et al.*, 2004; OMARA *et al.*, 2019). Up to 60% of N-urea fertilizer applied to agricultural soils may volatilize as NH_3 (DUTTA *et al.*, 2016; PAN *et al.*, 2016).

In contrast, NO_3^- leaching was estimated in Brazil from trace to 87 kg ha^{-1} per year, and losses from fertilizers usually exceed $10\text{--}15 \text{ kg ha}^{-1}$ per year (ROSOLEM *et*

al., 2017). Nitrate leaching occurs mostly in sandy permeable soils and also a result of soil having net negative charge, which does not retain the negatively charged nitrate ions (STENBERG *et al.*, 1999; DI; CAMERON, 2002; OMARA *et al.*, 2019). The amount of nitrate that is leached from soil depends on the concentration of nitrate present in soil solution, which in turn depends on the amount of N applied, the nitrification rate, and the denitrification rate (CAMERON *et al.*, 2013; OMARA *et al.*, 2019).

Concerning about denitrification, this is a biological process involving the conversion of nitrate N to gaseous forms under anoxic soil environments. It is believed to be exclusively a bacterial trait and an important component of the N cycle, which reverses the N fixation process (ZUMFT, 1997; OMARA *et al.*, 2019). Denitrification is the loss of N in gaseous form as nitrous oxide, nitrogen monoxide, and nitrogen dioxide. It is probably considered the most important N loss pathway under oxygen limited agricultural systems that require heavy application of N fertilizer (OMARA *et al.*, 2019). Omara *et al.* (2019) reported that losses of the applied N fertilizer due to denitrification are extremely high in flooded or paddy rice. These losses can sometimes be as high as 50% of the applied fertilizer N (HOULTON *et al.*, 2006).

Another N loss pathway is related to plant losses. Plants are known to lose significant quantities of N after uptake and accumulation (PACKER; KRAMER, 2018; OMARA *et al.*, 2019). Loss of N from leaf as ammonia occurs during plant photorespiration (OMARA *et al.*, 2019). The accumulated gaseous N losses have been documented at 40 kg N ha⁻¹. This is a result of the imbalance between N accumulation and assimilation in plant systems (XU *et al.*, 2012). This loss is believed to increase with increased N rate applied and vary significantly with different crop species (OMARA *et al.*, 2019). Kanampiu *et al.* (1997) documented between 7.7 and 59.4% of post anthesis plant N loss in winter wheat. Although these losses were variety specific, they observed that there was a general increase with increased N rate applied. The two most important components of the N economy are comprised of the uptake and partitioning of N between the straw and grain (OMARA *et al.*, 2019). Efficient utilization of N in the production of grain also requires efficiency in the processes of uptake, translocation, assimilation, and redistribution. At favorable environmental conditions that reduce

ammonia volatilization, leaching and denitrification, plant N loss can become the most important factor influencing low NUE (PACKER; KRAMER, 2018; OMARA *et al.*, 2019).

2.2.1 Nitrogen fertilization in corn crop under tropical conditions

Nitrogen fertilization has been quite reported to increase corn development and grain yield under diverse soil conditions, climate and cropping systems (LUCAS *et al.*, 2019; SPACKMAN *et al.*, 2019; FERNANDEZ *et al.*, 2020). Current recommendations for N fertilization in side-dressing are based on response curves, area history and expected yield (FANCELLI, 2010). Under tropical conditions, N management is recommended to be performed at least in two split applications. The first application is recommended to be applied at corn planting (20 to 30 kg N ha⁻¹) next to and below the seeds. The second application is suggested to be applied in side-dressing between the fully expanded four-leaf stages (V4) and eight fully expanded leaves (V8) (FANCELLI, 2010), and this side-dressing application can be split in two or three applications. The N rate applied in side-dressing usually is calculated based on soil analysis and yield expectation. However, soil texture, water availability, crop management and other factors can influence the calculation of the amount of N applied (FANCELLI, 2010).

According to Queiroz *et al.* (2011), when the N application exceeds the corn demand during the early stages of development, the ammonia volatilization losses and the greater mineral N immobilization can drastically reduce NUE. According to Rezende *et al.* (2015), the absence of adequate N availability between V4 and V6 stages may lead to decreased shoot and ear development, which negatively affects grain filling and grain yield. At V4 stage, the number of ovaries on corn ear is defined, and it is required at this stage, at least 25 kg N ha⁻¹ (RITCHIE *et al.*, 2003). In addition, it is between V4 and V12 stages, that occurs the definition of the number of rows and the size of the ears, which are important corn development components (VITTI *et al.*, 2001). Therefore, it is essential that N is available for these stages, and it is between 40 and 60 days after emergence that the most intense nutrient absorption occurs (ANDREWS *et al.*, 2018; GRIESHEIM *et al.*, 2019), corresponding to more than 50% of the total required by the corn plant (SOUZA; LOBATO, 2004). Also, at the grain establishment, initial formation

and grain filling, N plays a fundamental role in the maintenance of carbohydrate metabolism (MARSCHNER, 2012). The assimilation of N is closely related to the reactions of photosynthesis and carbohydrate metabolism, both by the need of energy to reduce nitrate to ammonia, and by the supply of carbon chains necessary for the incorporation of ammonia in the formation of amino acids (BREDEMEIER; MUNDSTOCK, 2000).

Different responses to N application rates in side-dressing in corn have been reported in Brazil. For example, Faria *et al.* (2019) reported greater grain yield varying between 60 and 76 kg N ha⁻¹ applied at V4 stage in corn second season. Silva *et al.* (2012) verified greater grain yield with application of 110 kg N ha⁻¹ applied at V3 stage in corn first season. Souza *et al.* (2011) verified greater grain yield with application of 150 kg N ha⁻¹ at V6 stage in corn second season. Mateus *et al.* (2019) reported greater corn grain yield with application of 113 kg N ha⁻¹ applied at V5 stage in corn first season. Lucas *et al.* (2019) verified greater grain yield with application of 155 kg N ha⁻¹ applied at V4 stage in corn first season. Queiroz *et al.* (2011) reported greater grain yield with 160 kg N ha⁻¹ applied at V5 stage in corn first season. Galindo *et al.* (2016) verified a positive response to N rates up to 200 kg N ha⁻¹ applied at V6 stage in corn first season. Caires; Milla (2016) obtained the maximum technical and economic efficiency with the application of 209 kg ha⁻¹ of N in V4 stage in corn first season. Mendes *et al.* (2013) verified greater grain yield with split application of 240 kg N ha⁻¹, applied at V4 (120 kg N ha⁻¹) and V6 (120 kg N ha⁻¹) stages in corn first season, respectively. Therefore, the side-dressing N application must be rational and optimized. The increased N rates application results in increased losses and less utilization by cropping systems, since plant nutritional demand is limited (GALINDO *et al.*, 2016).

2.2.2 Nitrogen fertilization in wheat crop under tropical conditions

Similarly to corn crop, current recommendations for side-dressing N fertilization are based on response curves, area history and expected yield and N application in wheat is recommended to be performed at least in two split applications. The first application is recommended to be applied at wheat planting (15 to 20 kg N ha⁻¹) next to

and below the seeds. The second application is suggested to be applied in side-dressing between wheat tillering and stem elongation (DE BONA *et al.*, 2016), and this side-dressing application can be split in two or three applications.

Excessive amounts of N are not recommended at planting, since the wheat plant demand in the initial growth phase is low, which implies on greater N losses (DE BONA *et al.*, 2016). The recommended rate to be applied in side-dressing can vary according to the soil organic matter, cultivar characteristics, solar radiation, planting time, water, nutrients, climatic condition and expected grain yield (DE BONA *et al.*, 2016). Generally, there is no reason to apply less than 30 kg N ha⁻¹ in side-dressing. The tillering takes place during an interval of about 30 days after wheat emergence, which coincides with the period between the emission from the 4th to the 8th leaf of the main stem. From this stage, the elongation of the stem begins, when the first training becomes apparent (DE BONA *et al.*, 2016). The N availability at the beginning of the peeling (4th leaf) defines the number of spikelets per spike and, in the final phase (7th leaf), determines the number of tines that will form fertile spikes (DE BONA *et al.*, 2016). In general, the alternative application of side-dressing N after the peeling phase does not increase grain yield. However, may increase N concentration and proteins in wheat grains, which can improve the technological quality of the flour (DE BONA *et al.*, 2016).

Under irrigated conditions, the most expensive cost in wheat crop production is with the purchase of fertilizers, in especial the N fertilizers (CÁNOVAS; SILVA, 2000). However, the N application in wheat is certainly one of the safest crop management practices in relation to the economic return (DE BONA *et al.*, 2016). Also, under irrigated conditions, which present greater grain yield potential, higher N rates are suggested to be applied. However, some cultivars are susceptible to plant bedding, which can lead to reductions in grain yield and quality (SILVA *et al.*, 1996).

Different responses to N rates in wheat applied in side-dressing have been reported in Brazil. According to De Bona *et al.* (2016), the amount of N to be applied in side-dressing in wheat crop generally varies from 60 to 120 kg N ha⁻¹. Under irrigated conditions in low altitude Cerrado region, Teixeira Filho *et al.* (2007) verified greater wheat grain yield, mass of 100 grains, N leaf concentration, number of spikes per meter, spike length and wheat grain yield with application of 70 kg N ha⁻¹. Similarly, under the

same conditions, Alves *et al.* (2017), Melero *et al.* (2013) and Galindo *et al.* (2017b) verified greater wheat grain yield with application of 120, 122 and 140 kg N ha⁻¹, respectively.

2.3 PLANT GROWTH PROMOTING BACTERIA: *Azospirillum brasilense*

The use of biological agents such as PGPB has increased significantly over the last decade in many different crops, mainly in Latin America (MARTINS *et al.*, 2018; PANKIEVICZ *et al.*, 2019) mainly in corn and wheat crops (MARKS *et al.*, 2015; GALINDO *et al.*, 2016, 2017b; SALVO *et al.*, 2018). Recently, several studies have showed the benefits of these bacteria in other non-leguminous crops, such as rice (TAN *et al.*, 2015), sugarcane (*Saccharum* sp.) (SCHULTZ *et al.*, 2012), sorghum (SANTOS *et al.*, 2017a) and pastures (*Urochloa* sp.) (HUNGRIA *et al.*, 2016). Due to the success obtained, research on inoculation with BPCPs has grown quickly, not only in grasses and legumes, but also in forest, ornamental, fruit and vegetable species (FUKAMI *et al.*, 2018a; TEIXEIRA FILHO; GALINDO, 2019). The BPCPs are epiphytic or endophytic residents, non-pathogenic, that act directly promoting growth or indirectly as agents of biological control of plant diseases and promoting plant growth in the rhizosphere and/or within plant tissues (MARIANO *et al.*, 2004).

The BPCPs have associative interactions with plants in two main types of association: 1) association between plants and free-living or rhizospheric bacteria and 2) association between plants and endophytic diazotrophic bacteria (TEIXEIRA FILHO; GALINDO, 2019). Several PGPB genera associate with different species of agricultural importance, such as *Azospirillum*, *Arthobacter*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Clostridium*, *Gluconacetobacter*, *Herbaspirillum*, *Pseudomonas*, *Rhizobium* and *Streptomyces* (ZEFFA *et al.*, 2018). Among the bacteria related to promote plant growth, the most studied are those of the genus *Azospirillum* (KANEKO *et al.*, 2010a; WISNIEWSKI-DYÉ *et al.*, 2011; FUKAMI *et al.*, 2018a; REIS *et al.*, 2018). The main characteristic of the *Azospirillum* genus is related to the ability to colonize plants in different ecosystems, from tropical regions to places with temperatures below 0° C, such as the Arctic (REED *et al.*, 2011). Additionally, *Azospirillum* is considered a

non-specific PGPB (BASHAN *et al.*, 2004), which aroused the interest of the scientific community in research with several plant species.

The genus *Spirillum* was first reported by Beijerinck (1925), and decades later reclassified as *Azospirillum*, because of its ability to fix N₂, discovered and reported by the group of Dr. Johanna Döbereiner in Brazil, in the 1970s (TARRAND *et al.* 1978; FUKAMI *et al.*, 2018a). This bacterium is characterized by the shape of rods, which are commonly uniflagellate, gram-negative, with characteristic vibratory movement and mixed flagellar standard (HALL; KRIEG, 1984). After the discovery that *Azospirillum* was diazotrophic, several studies evaluated its capacity to fix N₂ and to replace N-fertilizers when associated with grasses (OKON *et al.* 1983; FUKAMI *et al.*, 2018a), including sugarcane, grain crops such as corn, wheat and rice, pastures, among others (LIMA *et al.* 1987; MARKS *et al.* 2015; CASSÁN; DIAZ-ZORITA, 2016; FUKAMI *et al.* 2016; HUNGRIA *et al.* 2016; PEREG *et al.* 2016; FUKAMI *et al.*, 2018a). Twenty species of *Azospirillum* have been described so far, but *A. brasilense* and *A. lipoferum* have been the subjects of the highest numbers of physiological and genetic studies (BALDANI; BALDANI, 2005; KANEKO *et al.*, 2010a; WISNIEWSKI-DYÉ *et al.*, 2011; FIBACH-PALDI *et al.* 2012; FUKAMI *et al.*, 2018a).

Beneficial results have been obtained consistently with *Azospirillum* applied to a variety of crops (OKON; LABANDERA-GONZALEZ, 1994; BASHAN *et al.*, 2004; PEREG *et al.*, 2016; FUKAMI *et al.*, 2018a; HUNGRIA *et al.*, 2018; PANKIEVICZ *et al.*, 2019) in dozens of commercial inoculants worldwide (OKON *et al.* 2015). Although the Brazilian research group headed by Dr. Döbereiner contributed with studies related to *Azospirillum* benefits (DÖBEREINER; PEDROSA, 1987; REIS *et al.*, 2000; BALDANI; BALDANI, 2005), it was only in 2009 that the first commercial inoculant containing *A. brasilense* started to be commercialized in Brazil (HUNGRIA *et al.* 2010; HUNGRIA 2011). Currently, more than three million doses of inoculants are applied annually by farmers, for inoculation both of non-leguminous and for co-inoculation of legumes (FUKAMI *et al.*, 2018a).

Although the most prevalent reported benefit of *Azospirillum* sp. has been its capacity of fixing N₂, an increasing number of studies describe other properties that imply growth-promotion (FUKAMI *et al.*, 2018a, b, c). These bacteria can stimulate plant

growth by a series of mechanisms, including the production of phytohormones, such as indole-3-acetic acid (IAA), gibberellins, cytokinins and salicylic acid (TIEN *et al.*, 1979; BOTTINI *et al.*, 1989; PRINSEN *et al.*, 1993; STRZELCZYK *et al.*, 1994; COHEN *et al.*, 2008 BASHAN; DE-BASHAN, 2010; MEZA *et al.*, 2015; CASSÁN; DIAZ-ZORITA, 2016). Also, *Azospirillum* sp. has been related to increase nutrient availability (HUNGRIA *et al.*, 2010) and NUE (HUNGRIA *et al.*, 2016; GALINDO *et al.*, 2016), phosphate solubilization (PUENTE *et al.*, 2004), nitric oxide production (FIBACH-PALDI *et al.*, 2012), nitrate reductase activity (HUNGRIA *et al.*, 2010) and polyamines production, such as spermidine, spermine and cadaverine (CASSÁN *et al.*, 2009).

In addition, *Azospirillum* sp. has been reported to reduce biotic and abiotic stresses, for example, increase plant resistance to pathogens and diseases (RUSSO *et al.*, 2008; YASUDA *et al.*, 2009; BASHAN; DE-BASHAN, 2010; TORTORA *et al.*, 2011), increase the plant protection against excess of salt, drought and toxic soil components and reduce the damage by oxidative stress in plants (reduce the damage by reactive oxygen species) (CREUS *et al.*, 1997; CASSÁN; DIAZ-ZORITA, 2016; FUKAMI *et al.*, 2018a). Also, *Azospirillum* sp. has been reported to increase proline content in shoots and roots, improve water potential, increase apoplastic routes for water content, increase cell wall elasticity and chlorophyll content, increase photoprotection pigments and improve stomatal conductance (BASHAN; DE-BASHAN, 2010).

The positive response to inoculation has been described as the theory of multiple mechanisms, dependent on the inoculant-plant-environment interaction (BASHAN; DE-BASHAN, 2010; BASHAN *et al.*, 2014; SALVO *et al.*, 2018). In this context, the interaction between environment × genotype × strain (bacteria) is decisive in the operating mode of BPCPs, which use a range of simultaneous mechanisms to benefit plant development and growth (BASHAN *et al.*, 2014). Based on the multiple beneficial mechanisms of BPCPs, the plant growth promotion stands out, in which the greater root development can provide greater absorption of water and nutrients, reducing the stress caused by biotic and abiotic conditions, reflecting on increased development and yield (BASHAN; DE-BASHAN, 2010; BASHAN *et al.*, 2014; SALVO *et al.*, 2018).

Although the estimates of the contribution of FBN between diazotrophic bacteria and non-leguminous cultures are quite modest compared to the contribution with

legumes, ranging between 0 and 75 kg N ha⁻¹ year⁻¹, its importance in the energy balance transcends this value (BODDEY *et al.*, 2008). However, calculations of the contribution of N fixed on cereals are around 25 to 50 kg N ha⁻¹ year⁻¹, which is equivalent to the average supply of about 17% of the demands of these crops (MOREIRA *et al.*, 2010). Also, studies related to FBN in non-leguminous crops have shown that this association can also reach levels of economic importance (URQUIAGA *et al.*, 2012). Currently, the main role of inoculation on cereal crops is not specifically aimed at the total substitution of N applied via fertilizer by FBN. New studies have been performed aiming at increase fertilizer use efficiency, which could mainly reduce the amount of N-fertilizers applied to these crops and also increase grain yield (GALINDO *et al.*, 2016, 2017b, 2018a, 2019a). Inoculation with *A. brasilense* is a technology that is increasingly be used by farmers due to the ease of application and acquisition, low cost, and non-polluting technique in addition to provide increased NUE, grain yield, and profitability to agricultural activity, even when associated with high N rates (GALINDO *et al.*, 2017b, 2019a).

2.3.1 Inoculation with *Azospirillum brasilense* in corn and wheat associated with N application under tropical conditions

An analysis of field trials conducted worldwide for over 20 years, where various non-legume crops were inoculated with *Azospirillum* sp. under different weather and soil conditions, concluded that crop yield can increase up to 30% with inoculation (FUKAMI *et al.*, 2016). Longhini *et al.* (2016) studying inoculation with *A. brasilense* associated with N rates application (0, 30, 60, 90 and 120 kg N ha⁻¹) in corn, verified increased plant height and grain yield, regardless of N rate applied. Kappes *et al.* (2013) reported increased corn grain yield by 9.4% with application of 90 kg N ha⁻¹. Skonieski *et al.* (2019) studying inoculation with *A. brasilense* associated with N rates application (0, 60, 120, 240, and 480 kg N ha⁻¹) in corn, verified increased NUE and grain yield by 10.6 and 10.9%, respectively, regardless of N rate applied. Similarly, Galindo *et al.* (2018b) reported an increase up to 6% on corn grain yield with N application until 200 kg N ha⁻¹ in side-dressing. Due to the low cost of acquisition and application (on average, R\$

10.00 ha⁻¹) and the positive results on corn grain yield, inoculation with *A. brasilense* has been reported to provide greater profitability with corn production, with an average increase of 15% on obtained revenue (GALINDO *et al.*, 2017a, 2018b). Zeffa *et al.* (2018) performed a meta-analysis on the influence of side-dressing N application associated with *Azospirillum* sp. inoculation in corn and reported an average yield increase of 652 kg ha⁻¹. Galindo *et al.* (2016) verified increased NUE with seed inoculation with *A. brasilense* associated with N rates applied in side-dressing with an average increase in 12.53 kg grains kg N applied⁻¹ in corn crop. Also, Hungria *et al.* (2016) verified that *Azospirillum* sp. increased fertilizer use efficiency under different soil and climatic conditions.

Positive results in wheat crop with the use of *Azospirillum brasilense* were reported by Pereira *et al.* (2017), working with 60 kg N ha⁻¹ and seed inoculation with *A. brasilense*. According to the authors, inoculation resulted on an increase of 11.1% in wheat grain yield. Similar results were obtained by Nunes *et al.* (2015), where wheat yields were 7.6% higher with inoculation of *A. brasilense* when compared to control, even in an area with high N availability (the crops that preceded wheat were soybeans or carrots - soybean is a legume with a symbiotic association that allows the N₂ fixation, and the carrot, due to the high N amounts applied, leave high N residue in the soil and tend not to have the effect of N immobilization of the soil in the initial phases of succession crop). Alves *et al.* (2017) also verified increased wheat yield with seed inoculation with *A. brasilense* equivalent to 4.1%. Fukami *et al.* (2016) concluded that inoculation with *A. brasilense* can reduce by 25% the N-fertilizer application in wheat, without affecting grain yield. Galindo *et al.* (2017b), studying N rates in side-dressing (0, 50, 100, 150, and 200 kg N ha⁻¹) with and without inoculation with *A. brasilense* verified increased wheat grain yield as a function of inoculation equivalent to 3.3%, regardless the N rate applied. Additionally, Galindo *et al.* (2017b) reported an average NUE increase of 51.2% when the inoculation with *A. brasilense* was associated with N application rates varying between 50 and 200 kg ha⁻¹. Mumbach *et al.* (2017) reported an increase on wheat grain yield by 64.9% when associated with 70 kg N ha⁻¹ in side-dressing application. Munareto *et al.* (2019) verified the positive influence of side-dressing N application associated with *Azospirillum brasilense* seed inoculation in wheat

and reported an average yield increase of 36.8% and 13.5% when the inoculation was associated with N application rates of 70 and 140 kg ha⁻¹, respectively.

2.4 SILICON IN AGRICULTURE

Silicon is the second most abundant element in the earth's crust (about 28.8% of earth's crust) after oxygen (TUBANA *et al.*, 2016; NEU *et al.*, 2017). However, Si availability in tropical soils is limited mainly by the high levels of desilication of primary silicate minerals (EPSTEIN, 2009; HAYNES *et al.*, 2017; WALSH *et al.*, 2018; LINDEN *et al.*, 2019). In addition to desilication, chemical weathering of primary silicate and aluminosilicate minerals is accompanied by the release and leaching of basic cations and the development of low base saturation soils (KEEPING *et al.*, 2017). As part of this process, comparatively immobile ions of Al and Fe react with some of the Si to form secondary clay minerals dominated by Al and Fe sesquioxides and kaolinites (EPSTEIN, 2009; HAYNES, 2014; KEEPING *et al.*, 2017). These highly weathered soils are acids and as a result, may be high in soluble forms of Al (SANCHEZ, 1976; FAGERIA *et al.* 1988; KEEPING *et al.*, 2017), which can react with Si to form insoluble hydroxyaluminosilicates (DOUCET *et al.*, 2001; SCHNEIDER *et al.*, 2004; EXLEY, 2012). Loss of Si through these weathering processes is most severe in the tropics and subtropics, where rainfall and temperatures are high, and to a much lesser extent in temperate regions (KEEPING *et al.*, 2017).

In general, solutions of soils show liquid-phase Si levels varying between 2.8 and 16.8 mg dm⁻³, and the dynamic balance of the element in the soil depends on the pH (EPSTEIN, 2009). In addition, the main sources of Si in soil solutions are the decomposition of plant residues, dissociation of polymeric monosilicic acid (H₄SiO₄), release of Si from Fe and Al oxides and hydroxides, dissolution of non-crystalline and crystalline minerals, and addition of Si fertilizers and irrigation water (KORNDÖRFER *et al.*, 2004; MARAFON; ENDRES, 2013). Intensive cultivation removes Si from the soil, as it is estimated that annually 190-204 million Mg of Si is taken out from the world's arable soils (MEENA *et al.*, 2014). In cultivated areas, the removal of crops does not allow the recycling of Si by plants, and the biogeochemical cycle of Si is disturbed

(BAKHAT *et al.*, 2018). In this way, the decrease of bioavailable Si might have significant impacts on cereal yields if not properly restored (GUNTZER *et al.*, 2012).

Plants uptake Si directly from the soil solution. It is transported up to the roots mainly via mass flow (KORNDÖRFER *et al.*, 2004), and its absorption in grass occurs quickly. However, translocation varies among species, being able to be retained in the roots and little translocate in aerial part (ISA *et al.*, 2010; AGOSTINHO *et al.*, 2017). Silicon is transported by xylem and deposited on the cell wall in the form of hydrated amorphous silica or biogenic opals, also known as silica bodies or silica phytoliths ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$). Once deposited, it becomes virtually immobile and no longer redistributes to the plant (GUNTZER *et al.*, 2012). The transport of H_4SiO_4 inside the plant takes place in the same direction as the mass flow (transpiration). Thus, Si deposits occur more frequently in regions where water is lost in large amounts, for example in the leaf epidermis (ISA *et al.*, 2010). Given that the Si content in Typic Haplustox soils (Oxisols) under tropical conditions, such as the Brazilian Savannah, can be less than 1% due to the presence of extremely active desilication processes (FUJII *et al.*, 2018) and that some crops (mostly grasses, e.g., sugarcane, rice, wheat, and corn) can remove Si quickly and in great quantity (TUBANA *et al.*, 2016), studies with Si application aiming to increase NUE and N uptake in tropical conditions should be performed.

In recent years, the use of Si-fertilizers has been reported to improve plant growth and development with greater N uptake and NUE in cereal crops (NEU *et al.*, 2017; XU *et al.*, 2018; CONCEIÇÃO *et al.*, 2019; RAMÍREZ-OLVERA *et al.*, 2019). Silicon application has been associated with increased plant resistance to abiotic and biotic stresses, such as attacks from insects and pathogens, salinity, drought, flooding, and high and low temperatures (COSKUN *et al.*, 2016; CRUSCIOL *et al.*, 2018; RAMÍREZ-OLVERA *et al.*, 2019; VEGA *et al.*, 2019). Silicon enhances primary metabolism by decreasing the transpiration rate (CAMARGO *et al.*, 2017) and improving photosynthesis (ARAUJO *et al.*, 2019; VEGA *et al.*, 2019) and nutrient uptake (HAJIBOLAND *et al.*, 2018) as well as secondary metabolism by stimulating the production of phenolic compounds with either antioxidant (for example, flavonoids) or structural (for example, lignin and cellulose) functions (XU *et al.*, 2015; VEGA *et al.*, 2019). Silicon has also been reported to stimulate germination and increase root length

(TORABI *et al.*, 2012; RAMÍREZ-OLVERA *et al.*, 2019), the number of leaves and plant architecture (XU *et al.*, 2015; DORNELES *et al.*, 2018). Calcium and magnesium silicate as a liming source can correct soil acidity; increase soil pH, extractable levels of Si, Ca, Mg and P; and decrease the harmful effects of toxic heavy metals such as Al, Fe and Cd (ADREES *et al.*, 2015; SARTO *et al.*, 2015; CASTRO *et al.*, 2016; CRUSCIOL *et al.*, 2016; ANTONANGELO *et al.*, 2017).

Recently, Jang *et al.* (2018) concluded that Si application could induce higher levels of bioactive hormones that would participate in various physiological responses, especially with increased hormones involved in the mitigation of abiotic stress (e.g., active endogenous gibberellin and jasmonic acid). The Si can also stimulate plant growth and yield through the erectness of leaves, allowing better light transmittance through plant canopies and better plant architecture, which increase in the photosynthetic rate (TAMAI; MA, 2008; MA; YAMAJI, 2008; GONG; CHEN, 2012; XU *et al.*, 2015), with consequent reduction of bedding due to the greater structural rigidity of the tissues, and still presents another important benefit related to reduction in the transpiration rate (CAMARGO *et al.*, 2014a, 2014b). When accumulating in the cells of the epidermal layer, the Si can be a stable physical barrier in the penetration of some types of fungi (GUNTZER *et al.*, 2012; BAKHAT *et al.*, 2018).

It has been reported that Si affects the number of structural plant compounds like lignin and cellulose, shown for various species (SCHOELYNCK *et al.*, 2010). According to Schaller *et al.* (2012) Si can affect plant tissue cellulose, lignin, and phenol content, depending on the function of the tissue in the plant (photosynthesis or stabilization). Higher Si availability results in higher cellulose content in photosynthetic tissues (leaves) and less cellulose content in tissues with stabilization function (culm). In this way, Si could benefit the foliar architecture of wheat, making them more erect, improving the leaves angle and light interception, avoiding the excessive self-shading, delaying senescence, increasing the structural rigidity of the tissues, and improving photosynthesis and reducing lodging (MA; YAMAJI, 2008; GONG; CHEN, 2012; SARTO *et al.*, 2015). Moreover, Si might affect the global carbon turnover by changing the plant cellulose, lignin and phenol content in biomes, with grasses as the main vegetation. As a result, the effect of Si on the terrestrial and semiterrestrial part of the

global carbon turnover is a neglected research field and should be investigated (SCHALLER *et al.*, 2012).

Among plant species, rice and sugarcane contains H_4SiO_4 at about 10% of above-ground dry weight (ISA *et al.*, 2010; KEEPING *et al.*, 2017) and are capable to remove between 150 and 700 kg Si ha⁻¹ year⁻¹ (MEENA *et al.*, 2014; KEEPING, 2017; KEEPING *et al.*, 2017). Rice and sugarcane accumulates Si as silica opal and silica bodies in the shoots, and the roots and can absorb several times more Si than common nutritional minerals, such as N, P, and K. Studies have shown that accumulation of Si in rice is attributed to an efficient uptake system mediated by two Si transporters (MA *et al.*, 2006; ISA *et al.*, 2010; AGOSTINHO *et al.*, 2017). These transporters were characterized and identified as low-Si genes (Lsi1 and Lsi2), and are responsible for transporting Si from the soil solution to the root cells (influx, Lsi1) and from inside to the outside of the root cells (efflux, Lsi2) (MA *et al.*, 2006, 2007). Knockout of either Lsi1 or Lsi2 results in significant decrease in Si uptake (MA *et al.*, 2006, 2007). In addition, homologous Si transporters of Lsi1 and Lsi2 have also been identified from other Si-accumulator plants, including corn, wheat, barley and pumpking (*Cucurbita* sp.) (CHIBA *et al.*, 2009; MITANI *et al.*, 2009a, b; MITANI *et al.*, 2011; MITANI-UENO *et al.*, 2011; MONTPETIT *et al.*, 2012). Currently, three Si transporters (Lsi2, Lsi3, and Lsi6) were also identified in the rice node (YAMAJI *et al.*, 2015). While Si transporters in the roots facilitate uptake of the element by the plant, Si transporters in the node are involved in intervascular transfer, which is required for the preferential distribution of Si to the leaves and grains (YAMAJI *et al.*, 2015; AGOSTINHO *et al.*, 2017).

Researchers have documented the benefits of Si application principally when plants are under stress conditions (GUÉVEL *et al.*, 2007; AGOSTINHO *et al.*, 2017). For example, Guével *et al.* (2007) observed that wheat plant growth did not improve with foliar applications of three different Si products (Kasil, a standard potassium silicate solution, with 26.5% SiO_2 ; Silamol, a potassium silicate solution formulated for foliar applications, with 2% of SiO_2 and 46% of K_2O ; and MRD-250, a volcanic rock dust, high in silicon content, with 43.2% of SiO_2) in comparison to control (without Si application) under greenhouse conditions, except when the plants were infected by powdery mildew. The environment under greenhouse conditions is usually controlled, and plants are

usually exposed to minimal or no stress (SOUSA; KORNDÖRFER, 2010). Amin *et al.* (2018) observed that Si application (Ca silicate dissolved in KOH at 71°C) significantly increased plant height, stem diameter, number of leaves, cob length, number of grains, mass of 100 grains and corn grain yield under drought stress conditions. The Si benefits are more frequent in hyperaccumulator crops (CAMARGO *et al.*, 2014a, 2014b), which contain SiO₂ concentrations above 5% of shoot dry matter (PATI *et al.*, 2016; YANG *et al.*, 2019). According to Korndörfer *et al.* (2010) and Sarto *et al.* (2015), when the levels of soil available Si are greater than 10.0 mg kg⁻¹, an increased grain yield of grass species to the Si supply is unlikely.

Silicon application was also reported to increase rice (SOUSA; KORNDÖRFER, 2010), corn (CASTRO; CRUSCIOL, 2013), sugarcane (REIS *et al.*, 2013), wheat (PROVANCE-BOWLEY *et al.*, 2010; SARTO *et al.*, 2015), sorghum (FLORES *et al.*, 2018), sunflower (*Helianthus annuus* L.) (FLORES *et al.*, 2019), peanut (*Arachis hypogaea* L.), soybean and common bean (*Phaseolus vulgaris* L.) (CRUSCIOL *et al.*, 2013) yields. Recently, research on the interaction of Si application and PGPB inoculation has increased (ETESAMI, 2018; CHANDRAKALA *et al.*, 2019; REZAKHANI *et al.*, 2019a, b). Etesami (2018) suggests that the associated use of Si and PGPB can be a powerful and sustainable strategy to enhance plant growth under sub-optimal conditions. Rezakhani *et al.* (2019a, b) concluded that *Pseudomonas* sp. associated with Si application can improve wheat and sorghum development. Galindo *et al.* (2019c) studied inoculation methods associated with Ca and Mg silicate and reported an average wheat grain yield increase of 6.7% when seed inoculation and silicate application were performed.

Study i. CORN RESPONSE TO INOCULATION WITH *Azospirillum brasilense* ASSOCIATED WITH SILICON AND NITROGEN RATES

3 MATERIAL AND METHODS

3.1 FIELD SITE DESCRIPTION

The study was conducted under field conditions in Selvíria (Brazilian Cerrado – Savannah region), state of Mato Grosso do Sul, Brazil (20°22'S and 51°22'W, 335 m above sea level (a.s.l.)) (Figure 1), during the crop years of 2015/16 and 2016/17 (spring/summer season). The soil was classified as Latossolo Vermelho distrófico típico (SANTOS *et al.*, 2018), equivalent to Clayey Oxisol (Rhodic Haplustox) according to the Soil Survey Staff (2014). Soil chemical and granulometry were determined from soil samples collected prior to liming sources application (0-0.20 and 0.20-0.40m depth) and analysed according to Raji *et al.* (2001) and Teixeira *et al.* (2017), respectively. Twenty soil samples were collected, mixed and a random sub-sample was used to determine soil chemical attributes. After samples be collected and mixed, the sub-sample was dried in the shade and soil chemical attributes and granulometry were determined. Total nitrogen was determined by the semi-micro Kjeldahl method (BREMNER; BREITENBECK, 1983). Silicon was determined after extraction in Ca chloride (0.01 mol L⁻¹) according to the methodology of Korndörfer *et al.* (2004) (Table 1). The experimental area had been cultivated with annual cereal and legume crops for over 30 years. In addition, the area has been under no-tillage for the last 15 years. The crop sequence prior to corn in 2015/16 were corn (2014), soybean (2014/15) and corn (2015) and prior to corn in 2016/17 was wheat (2016). The 2015 corn crop received the application of 180 kg N ha⁻¹ on the entire experimental site. The maximum, average and minimum temperatures and rainfall observed during the study are presented in Figure 2.

Figure 1. Study area at the Selvíria, state of Mato Grosso do Sul, Brazil. Map created by using QGIS software and Google Earth program. QGIS Development Team (2019). QGIS Geographic Information System. Open Source Geospatial Foundation Project. <http://qgis.osgeo.org>. Image obtained in Google Earth program. Google company (2020).

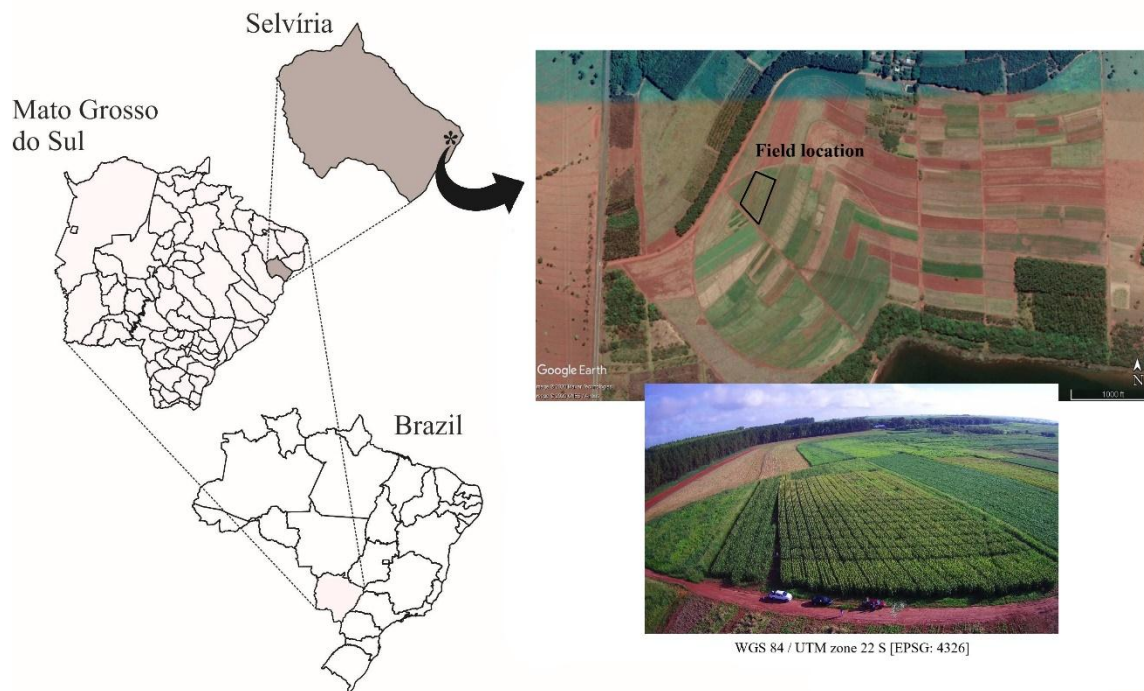
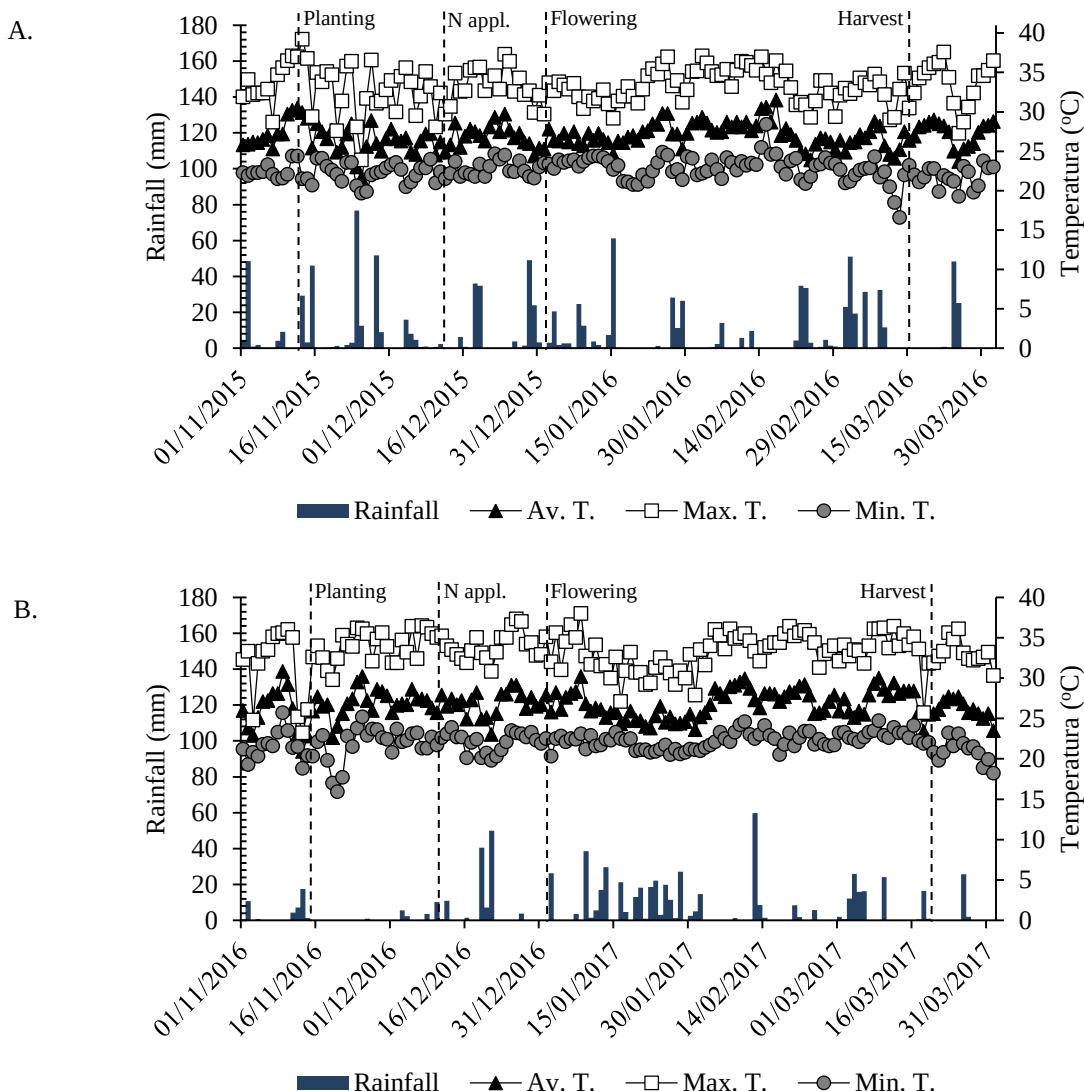


Table 1. Soil chemical attributes and granulometry in 0-0.20 m and 0.20-0.40 m layers before the application of liming sources.

Soil chemical attributes	0-0.20 m layer	0.20-0.40 m layer
Total N	1.04 g kg ⁻¹	0.81 g kg ⁻¹
Si (CaCl ₂)	9.4 mg dm ⁻³	10.2 mg dm ⁻³
P (resin)	19 mg dm ⁻³	17 mg dm ⁻³
S (SO ₄)	10 mg dm ⁻³	30 mg dm ⁻³
Organic matter	21 g dm ⁻³	16 g dm ⁻³
pH (CaCl ₂)	5.0	4.8
K	2.1 mmol _c dm ⁻³	1.2 mmol _c dm ⁻³
Ca	19.0 mmol _c dm ⁻³	11.0 mmol _c dm ⁻³
Mg	13.0 mmol _c dm ⁻³	8.0 mmol _c dm ⁻³
H+Al	28.0 mmol _c dm ⁻³	28.0 mmol _c dm ⁻³
Al	1.0 mmol _c dm ⁻³	2.0 mmol _c dm ⁻³
B (hot water)	0.17 mg dm ⁻³	0.11 mg dm ⁻³
Cu (DTPA)	3.1 mg dm ⁻³	2.1 mg dm ⁻³
Fe (DTPA)	20.0 mg dm ⁻³	10.0 mg dm ⁻³
Mn (DTPA)	27.2 mg dm ⁻³	10.7 mg dm ⁻³
Zn (DTPA)	0.8 mg dm ⁻³	0.2 mg dm ⁻³
Cation exchange capacity (pH 7.0)	62.1 mmol _c dm ⁻³	48.2 mmol _c dm ⁻³
Base saturation (%)	55	42
Granulometry	0-0.20 m layer	0.20-0.40 m layer
Sand	471 g kg ⁻¹	471 g kg ⁻¹
Silt	90 g kg ⁻¹	82 g kg ⁻¹
Clay	439 g kg ⁻¹	447 g kg ⁻¹

n = 20, DTPA = diethylenetriaminepentaacetic acid

Figure 2. Rainfall, maximum (Max. T.), average (Av. T.) and minimum (Min. T.) temperatures obtained from the weather station located in the Education and Research Farm of FE / UNESP during the corn cultivation in the period November 2015 to April 2016 (A) November 2016 to April 2017 (B).



3.2 EXPERIMENTAL DESIGN AND TREATMENTS

The experimental design was a randomized complete block design (RCBD), with four replicates, arranged in a $5 \times 2 \times 2$ factorial scheme. There were five N rates, as urea source, applied in side-dressing (0, 50, 100, 150, and 200 kg ha⁻¹). Two liming sources; Ca and Mg silicate, which was the Si source (the composition was 10.5% of Si, 25% CaO, and 6% MgO) with an effective neutralizing power (ENP) of 88%, and dolomitic limestone (the composition was 28% CaO and 20% MgO) with an ENP of 80%. Two

inoculations were used: with or without seed inoculation with *A. brasilense*. The experimental plots were six corn rows of five meters spaced at a distance of 0.45 m, and the useful area of the plot was the central four rows, excluding 0.5 m from each end.

Prior to the start of the study, liming sources were broadcast applied at the rate of 1.76 Mg ha⁻¹ for the silicate and 1.94 Mg ha⁻¹ for the limestone. 30 days before planting corn (2015/16) in one single application. No incorporation was performed as the area was under no-tillage. The amount of lime applied was based on the initial soil analysis (Table 1) and the amount needed to increase the base saturation to 80% based on the following equation 1.

$$LN = \frac{CEC (V2 - V1)}{10 \times ENP} \quad \text{Equation 1}$$

Where, LN = liming need, in Mg ha⁻¹, CEC = cation exchange capacity, V2 = base saturation to be achieved, V1 = current base saturation and ENP = effective neutralizing power.

The *A. brasilense* strains Ab-V5 Ab-V6 (CNPSO 2083 and CNPSO 2084, respectively) were inoculated at a rate of 300 mL of liquid inoculant per ha (equivalent to 300 mL of inoculant per 73,400 corn seeds planted - guarantee of 2×10⁸ colony forming unit [CFU] mL⁻¹). These are commercial strains used in Brazil with the brand name AzoTotal®. These strains, when used under similar conditions (Brazilian Savannah), have shown positive results on corn and wheat development (GALINDO *et al.*, 2016, 2017b, 2019a, c; ALVES *et al.*, 2017). Seeds were inoculated after the seed treatment with insecticide and fungicide when the seeds were completely dry, one hour before planting. The inoculation was performed by coating and mixing the inoculant and corn seeds in plastic bags, manually. The insecticide used was fipronil (62 g of a.i. per 100 kg of seed), and the fungicides used were thiophanate-methyl + pyraclostrobin (56 g + 6 g of a.i. per 100 kg of seed).

Nitrogen treatments (N application rates) were applied manually to evenly distribute the fertilizer on the soil surface without incorporation. The amount of fertilizer needed per plot was applied between the corn rows on December 13, 2015, and

December 10, 2016, respectively, when the plants were in the V6 stage (with six leaves completely unfolded). After the side-dressing application, the experimental area was irrigated with 14 mm of water to minimize losses by ammonia volatilization, in both crop years.

3.3 FIELD MANAGEMENT

Before planting (15 days before corn planting), weeds were controlled by the application of glyphosate (1800 g ha⁻¹ of the active ingredient [a.i.]) and 2,4-D (670 g ha⁻¹ of the a.i.). At planting, the straw remaining from the previous crop (corn 2015 crop year) was collected by removing the residue from 10 random points in the experimental area measuring 0.5 m². The residue sampled was used for chemical tests to determine nutrient accumulation and C/N ratio (Table 2).

Table 2. Nutrient accumulation and C/N ratio in corn straw (2015 corn - predecessor crop).

N	P	K	Ca	Mg	S	Si	B	Cu	Fe	Mn	Zn	C/N ratio
----- (kg ha ⁻¹) -----						----- (g ha ⁻¹) -----						
78.2	7.2	68.8	23.3	21.1	16.3	13.1	260	74.0	1018	710	185	38.3
<i>n</i> = 10												

The corn DOW 2B710 PW simple hybrid was mechanically planted on November 13 and November 11 for the 2015/16 and 2016/17 crops, respectively, at the planting density of 3.3 seeds per meter (7.3 plants m⁻²). Additionally, a fertilizer application of 375 kg ha⁻¹ of the granular fertilizer 08-28-16 (N-P₂O₅-K₂O – this fertilizer also contains 0.5% of B and 1% of Zn) was performed on the entire experimental site (planted furrows) to supply phosphorus (P) and potassium (K) based on the soil analysis and corn crop requirements (CANTARELLA *et al.*, 1997). During this nutrient application, 30 kg N ha⁻¹ was applied to the entire experimental area. Therefore, the total amount of N applied in each treatment was the amount of N applied in side-dressing (0 to 200 kg N ha⁻¹ as indicated above) in addition to the application of 30 kg N ha⁻¹. A split application of fertilizers (at planting and in side-dressing) is a general practice used by farmers growing cereals in Brazil.

Corn plants emerged five days after , on November 18, 2015, and November 16, 2016, respectively. The herbicides atrazine (1000 g ha^{-1} of a.i.) and tembotrione (84 g ha^{-1} of a.i.) associated with vegetable oil adjuvant (720 g ha^{-1} of a.i.) were used for post-emergence weed control on December 4, 2015 and December 2, 2016, respectively. The side-dressing N fertilization was performed on December 13, 2015, and December 10, 2016, as mentioned above. Insect control was made with triflumuron (24 g ha^{-1} a.i.) and methomyl (215 g ha^{-1} a.i.) on December 20, 2015, and December 17, 2016, respectively. Harvest occurred on March 15, 2016, and March 21, 2017, which was 117 and 125 days after corn emergence, respectively. Supplemental irrigation using a centre pivot sprinkling system was performed according to crop requirements, at a water depth of 14 mm.

3.4 MEASUREMENTS

3.4.1 In season evaluations

During the female flowering stage, leaf chlorophyll index (LCI) was indirectly measured in 10 plants using a portable non-destructive chlorophyll meter ClorofiLOG® model CFL-1030 (FALKER, 2008). Leaf NO_3^- , NH_4^+ , N and Si concentration were determined by collecting 20 main ear insertion leaves during the female flowering stage, from each plot, according to Cantarella *et al.* (1997). In addition, shoot and root NO_3^- , NH_4^+ , N and Si uptake were also quantified by collecting five plants per plot, calculating the uptake based on shoot and root biomass, in kg ha^{-1} (N and Si) and g ha^{-1} (NO_3^- and NH_4^+). Nitrogen analysis followed the methodology proposed by Malavolta *et al.* (1997), while NO_3^- , NH_4^+ and Si analysis followed the methodology proposed by Silva (2009).

3.4.2 Productive components, NUE, and grain yield

Plant height and stem diameter were measured at maturity in 10 plants per plot. Height was measured from the ground surface to the apex of the tassel, and stem diameter was measured in the second internode of the plant using a caliper. Ten ears were collected at harvest to determine ear diameter, also using a caliper; ear length,

determined from the base of the ear to the apex. Also, number of rows per ear, number of grains per row and number of grains per ear were determined by counting the number of rows per ear, grains per row and grains per each ear; mass of 100 grains determined in precision scale 0.01 g, at 13% (wet basis). Harvest index (HI), was calculated according to Hühn, (1990) (Equation 2). Nitrogen uptake (NUP – kg N accumulated kg N applied⁻¹), N utilization (NUT – kg grain kg N accumulated⁻¹) and NUE (kg grain kg N applied⁻¹) were calculated according to Moll *et al.* (1982) (Equations 3 to 5). Grain yield was determined by harvesting the useful experimental area, adjusted to 13% (wet basis) and extrapolated to kg ha⁻¹.

$$\text{HI, expressed as \%} = [\text{Grain yield} \div (\text{Grain yield} + \text{Shoot yield})] \times 100 \quad \text{Equation 2}$$

$$\text{NUP} = (\text{NT at Nf} - \text{NT at 0 kg N ha}^{-1}) \div \text{Nf} \quad \text{Equation 3}$$

$$\text{NUT} = (\text{GY at Nf} - \text{GY at 0 kg N ha}^{-1}) \div (\text{NT at Nf} - \text{NT at 0 kg N ha}^{-1}) \quad \text{Equation 4}$$

$$\text{NUE} = [(\text{GYF} - \text{GYW}) \div (\text{amount of N applied})] \quad \text{Equation 5}$$

Where, NT = Total N accumulated (kg ha⁻¹) in shoot (aerial part + grain) and Nf = is the rate applied as mineral fertilizer (kg ha⁻¹) (Equation 3); GY = grain yield (kg ha⁻¹), NT = Total N accumulated (kg ha⁻¹) in shoot (aerial part + grain) (Equation 4); GYF = Grain yield with fertilizer application and GYW = Grain yield without fertilizer application (Equation 5).

3.4.3 Nutrient uptake in corn straw and grain (uptake and removal)

Straw and grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake were quantified by collecting five plants per plot at harvest time, calculating the uptake based on straw and grain biomass, in kg ha⁻¹. Nitrogen, P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn analysis followed the methodology proposed by Malavolta *et al.* (1997), while Si analysis followed the methodology proposed by Silva (2009).

3.4.4 Soil chemical attributes after corn cultivation (0-0.20m depth)

Five soil samples per plot were collected between the corn rows soon after harvest time in 0-0.20m depth, mixed and a random sub-sample was used to determine

soil chemical attributes. After samples be collected and mixed, the sub-sample was dried in the shade and soil chemical attributes were determined according to Raji *et al.* (2001).

3.4.5 Economic analysis

For the economic analysis, the structure based on the total operating production costs (TOC) used by the Institute of Agricultural Economics (IEA) was adopted, according to Matsunaga *et al.* (1976), consisting of the sum of operating expenses: operations performed, inputs (e.g., liming sources, fertilizers, seeds, inoculant, pesticides), labor, machinery, and irrigation, named effective operating costs (EOC). In this study, besides the TOC, other operating expenses and interests were included, considering 5% of the EOC (MATSUNAGA *et al.*, 1976), thus resulting in the total operating cost (TOC), which was extrapolated to one ha. This methodology has been already used in several studies on economic evaluation in crops such as Kaneko *et al.* (2010b), Gitti *et al.* (2012), Kaneko *et al.* (2015), and Galindo *et al.* (2017a).

To determine the profitability of the treatments, profitability analyses were carried out following Martin *et al.* (1998) methodology. The following variables were determined: gross revenue (GR) (in R\$), as the product of the amount produced (in number of 60-kg sacks) by the average sale price (in R\$); operating profit (OP), as the difference between the gross revenue and total operating cost; accumulated operating profit (AOP), as the sum of the OP obtained in the two years of study; profitability index (PI), understood as the ratio between operating profit (OP) and the net revenue (NR), in percent; equilibrium price, given a certain total operating production cost, as the minimum price calculated to cover this cost, considering the average yield obtained; and equilibrium yield, given a certain total operating production cost, as the minimum yield to cover this cost, considering the average price paid. The average prices were quoted in the region of Selvíria, state of Mato Grosso do Sul - where the experimental site was located (average of three years - 2015, 2016 and 2017). In this study, simulations were performed as if each experimental treatment represented commercial crops. The cost of the corn 60-kg sack for the region of Selvíria (average of 2015, 2016 and 2017) was R\$

28.63 per unit produced. The cost with urea fertilizer was R\$ 1,750.00 per Mg. As regards the acidity correctives sources, the price paid by farmers was R\$ 85.00 and R\$ 115.00 per Mg for dolomitic limestone and Ca and Mg silicate, respectively. For the *A. brasilense* inoculant, the expenditure was around R\$ 10.00 per dose.

3.5 STATISTICAL ANALYSIS

All data were initially tested for normality using the Shapiro; Wilk (1965) test and Levene's homoscedasticity test ($p \leq 0.05$) (LEVENE, 1960), which showed the data to be normally distributed ($W \geq 0.90$). The data was analysed by ANOVA in a 3-way factorial design with liming sources, inoculation or not with *A. brasilense* and N rates, and their interactions considered fixed effects in the model using ExpDes package. Mean separation was done when significant factors or interactions were observed using the Tukey test ($p \leq 0.05$). Regression analysis was used to discern whether there was a linear or non-linear response to N rates using R software (R CORE TEAM, 2015). The heatmap was developed by calculating the Pearson correlation ($p \leq 0.05$) using the corrplot package to evaluate the relationship among the corn nutritional, productive components, NUE, grain yield and soil chemical attributes parameters using R software (R CORE TEAM, 2015).

4 RESULTS AND DISCUSSION

4.1 IN SEASON EVALUATIONS

Leaf chlorophyll index (LCI) in corn crop was significantly affected by the main effect of inoculation, in addition to the interaction between N rates $\times$ liming sources in both years (Appendix A). Leaf NH_4^+ concentration was significantly affected by the interaction between N rates $\times$ liming sources in both years, also by the main effect of inoculation in 2016/17 (Appendix A). Leaf N concentration was significantly affected by the main effect of N rates in 2015/16, also by the interactions between N rates $\times$ inoculation and liming sources $\times$ inoculation in 2016/17 (Appendix A). Leaf Si concentration was significantly affected by the main effect of liming sources in both years, also by the main effect of N rates in 2016/17 (Appendix A).

Shoot NO_3^- uptake was significantly affected by the main effect of N rates in 2016/17 (Appendix A). Shoot N uptake was significantly affected by the interaction between N rates $\times$ inoculation in 2015/16, also by the main effect of N rates in 2016/17 (Appendix A). Shoot Si uptake was significantly affected by the main effect of liming sources in both years, also by the main effect of N rates in 2016/17 (Appendix A). Root NO_3^- uptake was significantly affected by the main effect of N rates in both years (Appendix A). Root NH_4^+ uptake was significantly affected by the main effect of N rates in both years, also by the main effect of liming sources in 2015/16 (Appendix A). Root N uptake was significantly affected by the interactions between N rates $\times$ liming sources and N rates $\times$ inoculation in both years (Appendix A). Root Si uptake was significantly affected by the main effects of N rates and liming sources in both years (Appendix A). Leaf NO_3^- concentration and shoot NH_4^+ uptake were not significantly affected by N rates, liming sources, inoculation and their interactions (Appendix A).

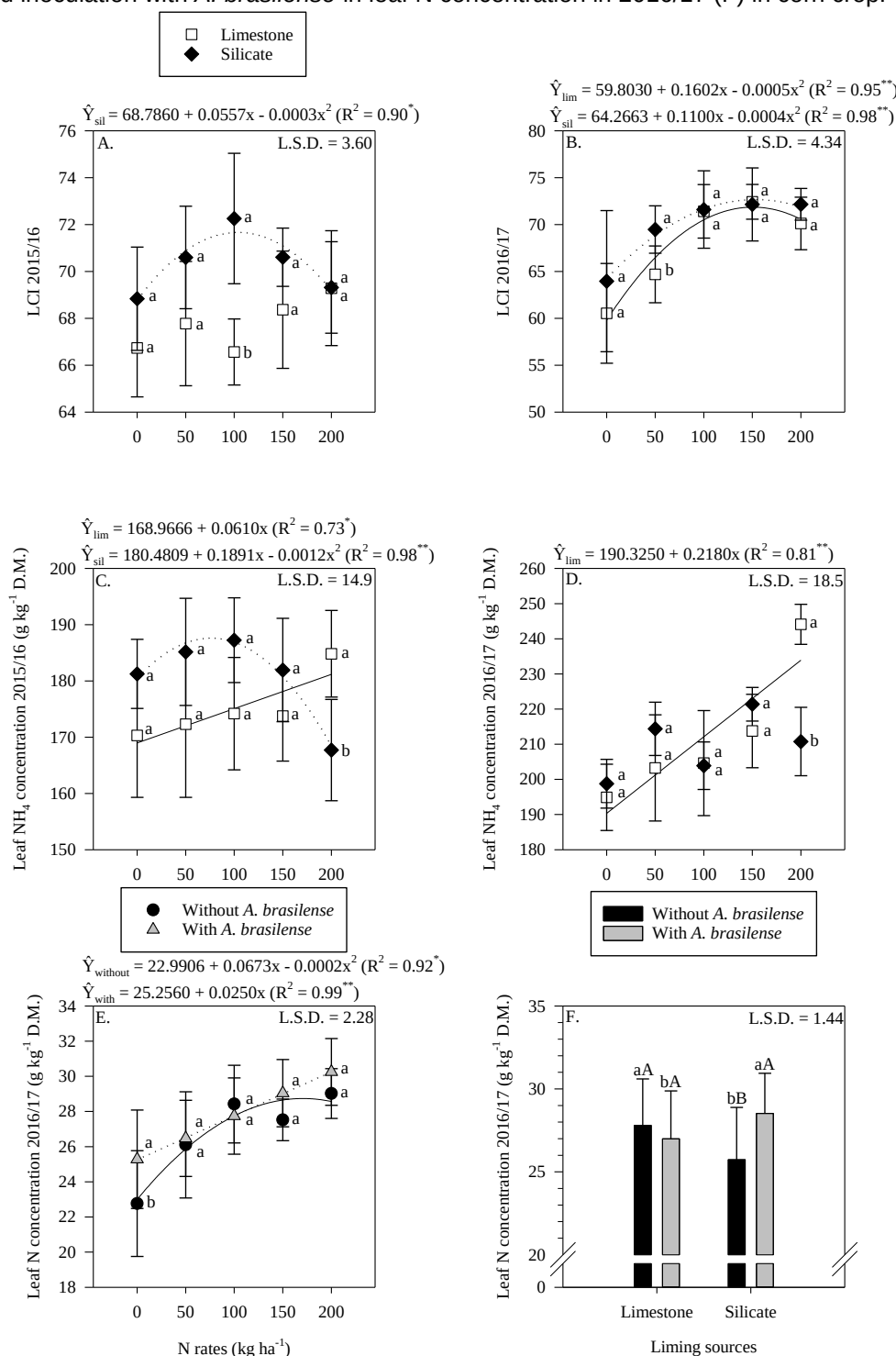
Leaf chlorophyll index was greater in inoculated plants in both years (Table 3). Furthermore, silicate application provided greater LCI than limestone with application of 100 kg N ha^{-1} in 2015/16 and 50 kg N ha^{-1} in 2016/17, respectively (Figures 3A and 3B). In both years, LCI was found to respond non-linearly to increasing N rates when silicate was applied (up to 93 and 137 kg N ha^{-1} , respectively) (Figures 3A and 3B). Similarly, in 2016/17, LCI was found to respond non-linearly to increasing N rates when limestone was applied (up to 160 kg N ha^{-1}) (Figure 3B).

Table 3. Leaf chlorophyll index (LCI), leaf NO_3^- , NH_4^+ , N and Si concentration in corn dry matter (DM) as a function of N rates, liming sources and inoculation with *A. brasilense*.

N rate	LCI		Leaf NO_3^- concentration		Leaf NH_4^+ concentration	
			(mg kg ⁻¹ DM)			
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	67.8	62.3	148	150	176	168
50	69.2	67.1	159	158	179	169
100	69.4	71.5	156	154	181	172
150	69.5	72.3	160	153	178	170
200	69.3	71.2	161	160	176	171
Liming source						
Dolomitic limestone	67.8	67.8	155 a	153 a	175	167
Ca and Mg silicate	70.3	69.9	159 a	157 a	181	173
Inoculation						
Without <i>A. brasilense</i>	67.9 b†	67.7 b	153 a	151 a	175 a	165 b
With <i>A. brasilense</i>	70.1 a	70.0 a	161 a	159 a	181 a	174 a
L.S.D. (5%)	1.61	1.94	8.6	10.5	6.7	4.95
C.V. (5%)	4.46	5.4	10.5	10.2	7.2	4.41
Overall Mean	69.0	68.9	157	155	178	170
N rate	Leaf N concentration		Leaf Si concentration			
			(g kg ⁻¹ DM)			
	2015/16	2016/17	2015/16	2016/17		
0	28.4	24.0	12.0		16.5	
50	28.4	26.3	12.1		17.7	
100	28.6	28.1	11.8		17.2	
150	29.7	28.3	12.2		18.2	
200	29.6	29.6	11.7		18.0	
Liming source						
Dolomitic limestone	29.0 a	27.4	11.2 b		16.9 b	
Ca and Mg silicate	28.8 a	27.1	12.7 a		18.1 a	
Inoculation						
Without <i>A. brasilense</i>	28.9 a	26.8	12.3 a		17.4 a	
With <i>A. brasilense</i>	29.0 a	27.8	11.6 a		17.6 a	
L.S.D. (5%)	0.78	1.02	0.79		0.87	
C.V. (5%)	5.2	7.2	12.6		9.5	
Overall Mean	28.9	27.3	12.0		17.5	

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Figure 3. Interaction between N rates and liming sources in leaf chlorophyll index (LCI) in 2015/16 (A) and 2016/17 (B), leaf NH_4^+ concentration in 2015/16 (C) and 2016/17 (D), interaction between N rates and inoculation with *A. brasilense* in leaf N concentration in 2016/17 (E) and interaction between liming sources and inoculation with *A. brasilense* in leaf N concentration in 2016/17 (F) in corn crop.



Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). Uppercase letters indicate difference between inoculation or not with *A. brasilense*, and lowercase letters indicate differences between liming sources, respectively. L.S.D. = Least significant difference. Error bars indicate the standard deviation ($n = 4$).

Leaf NH_4^+ concentration in corn was greater with limestone application when associated with 200 kg N ha^{-1} compared to silicate application in both years (Figures 3C and 3D). Also, in both years, leaf NH_4^+ concentration was found to respond linearly to increasing N rates when limestone was applied (Figures 3C and 3D). Differently, in 2015/16, leaf NH_4^+ concentration was found to respond non-linearly to increasing N rates when silicate was applied (up to 79 kg N ha^{-1}) (Figure 3C). In addition, in 2016/17, leaf NH_4^+ concentration was greater in inoculated plants compared to non-inoculated plants (Table 3).

Leaf N concentration was found to respond linearly to increasing N rates in 2015/16 (Table 4). In 2016/17, leaf N concentration was greater in inoculated plants compared to non-inoculated plants in the absence of N fertilization (0 kg N ha^{-1}). Leaf N concentration was found to respond linearly and non-linearly (up to 168 kg N ha^{-1}) to increasing N rates in inoculated and non-inoculated plots, respectively (Figure 3E). Furthermore, also in 2016/17, leaf N concentration was greater when limestone was applied in the absence of inoculation compared to silicate application (Figure 3F). However, leaf N concentration was greater when silicate was applied associated with inoculation with *A. brasilense* compared to limestone application (Figure 3F). Silicate application provided greater leaf N concentration when associated with inoculation with *A. brasilense* compared to non-inoculated plants (Figure 3F).

Table 4. Leaf N and Si concentration in corn dry matter (DM), shoot NO_3^- , N and Si uptake and root NO_3^- , NH_4^+ and Si uptake as a function of N rates in corn crop.

Independent variable	2015/16 crop season
Leaf N concentration (g kg^{-1} DM)	$\hat{Y} = 28.1655 + 0.0077x$ ($R^2 = 0.84^{**}$)
Root NO_3^- uptake (g ha^{-1})	$\hat{Y} = 285.6632 + 1.1113x$ ($R^2 = 0.87^{**}$)
Root NH_4^+ uptake (g ha^{-1})	$\hat{Y} = 112.0000 + 0.9985x - 0.0027x^2$ ($R^2 = 0.96^*$ and $\text{PM} = 185 \text{ kg ha}^{-1}$)
Root Si uptake (kg ha^{-1})	$\hat{Y} = 5.1999 + 0.0683x - 0.0002x^2$ ($R^2 = 0.99^{**}$ and $\text{PM} = 171 \text{ kg ha}^{-1}$)
	2016/17 crop season
Leaf Si concentration (g kg^{-1} DM)	$\hat{Y} = 16.8081 + 0.0070x$ ($R^2 = 0.65^*$)
Shoot NO_3^- uptake (g ha^{-1})	$\hat{Y} = 1559.3455 + 3.1177x$ ($R^2 = 0.77^*$)
Shoot N uptake (kg ha^{-1})	$\hat{Y} = 152.5325 + 0.3496x$ ($R^2 = 0.99^{**}$)
Shoot Si uptake (kg ha^{-1})	$\hat{Y} = 24.5345 + 0.0707x$ ($R^2 = 0.96^{**}$)
Root NO_3^- uptake (g ha^{-1})	$\hat{Y} = 380.2805 + 1.2212x$ ($R^2 = 0.82^{**}$)
Root NH_4^+ uptake (g ha^{-1})	$\hat{Y} = 166.9760 + 0.5258x$ ($R^2 = 0.84^{**}$)
Root Si uptake (kg ha^{-1})	$\hat{Y} = 6.9308 + 0.0146x$ ($R^2 = 0.54^*$)

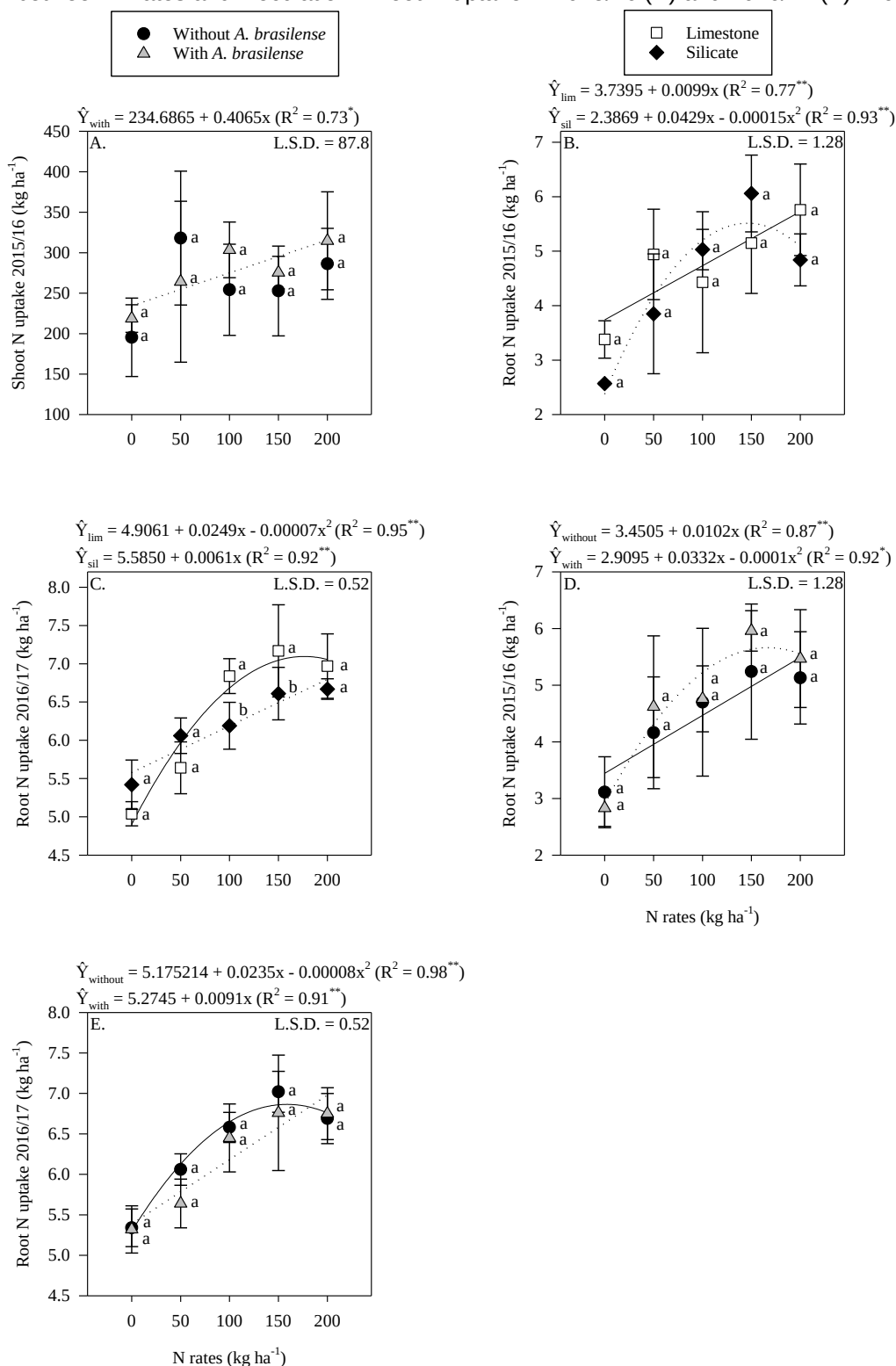
** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Leaf Si concentration was greater when silicate was applied in both years (Table 3). In 2016/17, leaf Si concentration was found to respond linearly to increasing N rates (Table 4).

Shoot NO_3^- uptake by corn was found to respond linearly to increasing N rates in 2016/17 (Table 4). Shoot N uptake was found to respond linearly to increasing N rates only in non-inoculated plots in 2015/16 (Figure 4A), and responded linearly to increasing N rates, regardless of inoculation and liming source in 2016/17 (Table 4). Shoot Si uptake was greater when silicate was applied in both years (Table 5). Also, shoot Si uptake was found to respond linearly to increasing N rates in 2016/17 (Table 4).

Root NO_3^- uptake was found to respond linearly to increasing N rates in both years (Table 4). Root NH_4^+ was found to respond non-linearly (up to 185 kg N ha⁻¹) and linearly to increasing N rates, respectively, in 2015/16 and 2016/17 (Table 4). Also, in 2015/16, root NH_4^+ was greater when limestone was applied (Table 5). Root N uptake was found to respond linearly and non-linearly (up to 143 kg N ha⁻¹) to increasing N rates when limestone and silicate were applied, respectively, in 2015/16 (Figure 4B). In 2016/17, root N uptake was greater when limestone was applied with 100 and 150 kg N ha⁻¹ compared to silicate application (Figure 4C). Root N uptake responded linearly and non-linearly (up to 178 kg N ha⁻¹) to increasing N rates when silicate and limestone were applied, respectively (Figure 4C). Regarding the interaction between N rates and inoculation, root N uptake was found to respond linearly and non-linearly (up to 166 kg N ha⁻¹) to increasing N rates without inoculation and with *A. brasilense* inoculation, respectively, in 2015/16 (Figure 4D). Differently, in 2016/17, root N uptake responded linearly and non-linearly (up to 147 kg N ha⁻¹) to N rates with and without inoculation, respectively (Figure 4E). Root Si uptake was found to respond non-linearly (up to 171 kg N ha⁻¹) and linearly to increasing N rates in 2015/16 and 2016/16, respectively (Table 4). Also, root Si uptake was greater when silicate was applied in both years (Table 5).

Figure 4. Interaction between N rates and inoculation with *A. brasilense* in shoot N uptake in 2015/16 (A), interaction between N rates and liming sources in root N uptake in 2015/16 (B), and 2016/17 (C), interaction between N rates and inoculation in root N uptake in 2015/16 (D) and 2016/17 (E) in corn crop.



Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference. Error bars indicate the standard deviation ($n = 4$).

Table 5. Shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

N rate	Shoot NO_3^- uptake		Shoot NH_4^+ uptake		Shoot N uptake		Shoot Si uptake	
	(g ha ⁻¹)		(g ha ⁻¹)		(kg ha ⁻¹)		(kg ha ⁻¹)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	1445	1479	2024	2072	207	151	12.5	24.0
50	2536	1894	3048	2268	291	172	17.6	29.4
100	2028	1843	2569	2387	279	187	16.3	30.2
150	1878	1869	2371	2347	264	204	15.6	36.2
200	2282	2271	2747	2704	300	223	17.1	38.3
Liming source								
Dolomitic limestone	2065 a†	1952 a	2652 a	2539 a	278 a	199 a	14.8 b	27.9 b
Ca and Mg silicate	2003 a	1790 a	2451 a	2173 a	258 a	176 a	16.8 a	35.3 a
Inoculation								
Without <i>A. brasilense</i>	2043 a	1899 a	2541 a	2314 a	261	183 a	15.5 a	32.6 a
With <i>A. brasilense</i>	2025 a	1843 a	2562 a	2397 a	275	192 a	16.1 a	30.6 a
L.S.D. (5%)	327	437	418	549	39.3	36.8	1.96	7.0
C.V. (5%)	24.2	29.2	24.8	29.2	22.1	29.6	18.8	15.0
Overall Mean	2034	1871	2552	2356	268	187	15.8	31.6
N rate	Root NO_3^- uptake		Root NH_4^+ uptake		Root N uptake		Root Si uptake	
	(g ha ⁻¹)		(g ha ⁻¹)		(kg ha ⁻¹)		(kg ha ⁻¹)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	259	402	111	176	2.98	5.2	5.2	7.1
50	363	463	162	201	4.40	5.9	8.1	8.6
100	398	445	173	202	4.73	6.5	9.5	7.4
150	495	528	209	225	5.6	6.9	10.6	8.0
200	470	674	201	295	5.3	6.8	9.8	11.0
Liming source								
Dolomitic limestone	414 a	517 a	186 a	231 a	4.73	6.3	7.6 b	6.6 b
Ca and Mg silicate	380 a	488 a	156 b	208 a	4.47	6.2	9.7 a	10.1 a
Inoculation								
Without <i>A. brasilense</i>	430 a	541 a	177 a	225 a	4.47	6.3	8.7 a	8.6 a
With <i>A. brasilense</i>	364 a	464 a	165 a	214 a	4.73	6.2	8.6 a	8.2 a
L.S.D. (5%)	72.7	114	20.8	45.8	0.58	0.24	0.90	1.62
C.V. (5%)	27.7	29.2	18.4	28.5	18.9	5.7	15.7	29.1
Overall Mean	397	502	171	220	4.60	6.3	8.7	8.4

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Applied N was absorbed as evidenced by increased leaf N concentration, shoot and root N uptake and LCI and favored Si absorption, with increase in leaf Si concentration and shoot and root Si uptake. Nitrogen is the nutrient that is most demanded by corn plants and directly affects crop development and yield. The higher N availability as a function of the N application likely favored the development of a well-established root system leading to improved uptake of water and nutrients, providing increased N uptake in shoot (GALINDO *et al.*, 2016). Increased root biomass can positively influence root scavenging, which is important for the interception of N and Si in crop systems (GALINDO *et al.*, 2019a).

Silicate application as a Si source was effective to make available Si to corn plants as verified by the increased leaf Si concentration and shoot Si uptake, mainly in 2016/17, with greater values for Si uptake (on average, an increase of 100% in shoot Si uptake in 2016/17, compared to 2015/16). The use of Si associated to N rates favored LCI, however, it reduced root N uptake when associated with 100 and 150 kg N ha⁻¹ and leaf NH₄⁺ concentration when associated with 200 kg N ha⁻¹. The main sources of Si in soil solutions are cover crops residues, release of Si from Fe and Al oxides and hydroxides, dissociation of polymeric H₄SiO₄, dissolution of non-crystalline and crystalline minerals, irrigation water and application of Si fertilizers (KORNDÖRFER *et al.*, 2004; MARAFON; ENDRES, 2013). In addition, the dynamic balance of Si is dependent of soil pH. On average, soils solutions present Si levels varying between 2.8 and 16.8 mg dm⁻³ (EPSTEIN, 2009). The increase on pH after Ca and Mg silicate application associated with straw decomposition may provide a greater availability of Si in the second year, justifying greater Si uptake in 2016/17. According to Alcarde; Rodella (2003), silicate is 6.78 times more soluble than Ca carbonate (CaCO₃ = 0.014 g L⁻¹ and CaSiO₃ = 0.095 g L⁻¹), therefore, silicate may have an increased potential to correct soil acidity in the initial periods after application due to the greater reaction time of limestone.

In this study, most of the Si taken up by the plant accumulated in the leaf tissue because Si deposits are known to occur more frequently in tissues where water is lost in large amounts due to plant transpiration (ISA *et al.*, 2010; MCLARNON *et al.*, 2017). Plants uptake Si directly from soil solution. It is transported up to the roots mainly via

mass flow (KORNDÖRFER *et al.*, 2004), and its absorption in grass occurs quickly (ISA *et al.*, 2010; AGOSTINHO *et al.*, 2017). Silicon is transported by xylem and deposited on the cell wall in the form of hydrated amorphous silica or biogenic opals, also known as silica bodies or silica phytoliths ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$). Once deposited, it becomes virtually immobile and no longer redistributes to the plant (GUNTZER *et al.*, 2012). The transport of H_4SiO_4 inside the plant takes place in the same direction as the mass flow (transpiration). Thus, Si deposits occur more frequently in regions where water is lost in large amounts, for example in the leaf epidermis (ISA *et al.*, 2010), which explains the high leaf Si concentration, followed by shoot Si uptake.

Some grass species, such as corn, can take up and redistribute large amounts of Si in the aboveground tissue due to specific Si transporters (MA; YAMAJI, 2015). For example, rice and sugarcane accumulates Si as silica opal and silica bodies in the shoots, and the roots and can absorb several times more Si than common nutritional minerals, such as N, P, and K. Studies have shown that accumulation of Si in rice is attributed to an efficient uptake system mediated by two Si transporters (MA *et al.*, 2006; ISA *et al.*, 2010; AGOSTINHO *et al.*, 2017). These transporters were characterized and identified as low-Si genes (Lsi1 and Lsi2), and are responsible for transporting Si from the soil solution to the root cells (influx, Lsi1) and from inside to the outside of the root cells (efflux, Lsi2) (MA *et al.*, 2006, 2007). Knockout of either Lsi1 or Lsi2 results in significant decrease in Si uptake (MA *et al.*, 2006, 2007). In addition, homologous Si transporters of Lsi1 and Lsi2 have also been identified from other Si-accumulator plants, including corn, wheat, barley and pumpking (*Cucurbita* sp.) (CHIBA *et al.*, 2009; MITANI *et al.*, 2009a, b; MITANI *et al.*, 2011; MITANI-UENO *et al.*, 2011; MONTPETIT *et al.*, 2012), which may justify how some grasses species such as corn can absorb and translocate large amounts of Si in shoot.

The amount of Si accumulated in the roots was greater than the amount of N accumulated in the roots. The N/Si ratio in the leaf tissue and shoot averaged 1.9 and 9.6, respectively, in both seasons, while the N/Si ratio in the root accumulation was 0.64. In the second season, Si appeared to be more soluble, or a residual effect was observed, and greater uptake was detected, which affected the N/Si ratio in leaves, shoot, and root. The N/Si ratio decreased from 2.4 (2015/16) to 1.6 (2016/17) in leaves

and 17.0 (2015/16) to 5.9 (2016/17) in shoot, however, the N/Si ratio in roots increased from 0.53 (2015/16) to 0.75 (2016/17). The Si concentration in tissue (11.95 and 17.51 g Si kg⁻¹) was near the suitable concentration range for K (17-35 g kg⁻¹ D.M.) and above the suitable concentration for Ca (2.5-8.0 g kg⁻¹ of D.M.), which are the second and third nutrients most absorbed by corn according to Cantarella *et al.* (1997).

The *A. brasilense* inoculation increased LCI and when associated with silicate favored leaf N concentration. When *A. brasilense* inoculation was associated with N rates, it favored N uptake, providing greater leaf N concentration, shoot and root N uptake with increasing N rates. In 2016/17, in the absence of N fertilization, leaf N concentration was greater when inoculation was performed. *Azospirillum brasilense* distribution is characteristic of an early-endophytic bacterium (MERCADO-BLANCO; PRIETO, 2012) also colonized primary and secondary roots in the interior of hairs with intact cell walls (SANTOS *et al.*, 2017a). The broadly favorable results obtained by *Azospirillum* sp. inoculation in plants is well characterized (HUNGRIA *et al.*, 2010; SANTOS *et al.*, 2017a) and occur, among other factors, because BNF (PANKIEVICZ *et al.*, 2015), increased nitrate reductase activity when they grow endophytically in plants (HUNGRIA *et al.*, 2010) and is able to produce phytohormones, such as indol-3-acetic acid (IAA) (MEZA *et al.*, 2015). The *A. brasilense* synthesizes IAA by the tryptophan-dependent way (DUCA *et al.*, 2014). The tryptophan synthesis requires a great quantity of ammonium (GÜNES *et al.*, 2014). Therefore, N accumulation in plant must be increased so that it is possible to synthesize tryptophan properly and to produce hormones that will promote greater development of the crop, with a reflection on grain yield. In the present study, although shoot NH₄⁺ and N uptake were not statistically affected by *A. brasilense* inoculation, on the average of both years, *A. brasilense* provided an increase of 2.2 and 5.4% in shoot NH₄⁺ and N uptake, respectively.

4.2 PRODUCTIVE COMPONENTS, NUE, AND GRAIN YIELD

Shoot biomass was significantly affected by the main effect of N rates in 2016/17 (Appendix A). Root biomass was significantly affected by the interaction between N rates × liming sources in 2015/16, also by the main effect of N rates in 2016/17 (Appendix A). Plant height was significantly affected by the main effect of N rates in

2016/17 (Appendix A). Stem diameter was significantly affected by the main effect of liming sources in both years, also by the main effects inoculation in 2015/16 and N rates in 2016/17 (Appendix A). Ear length was significantly affected by the main effect of N rates in both years (Appendix A). Ear diameter was significantly affected by the main effects of N rates and liming sources in 2016/17 (Appendix A). Number of rows per ear was significantly affected by the main effect of inoculation in 2016/17 (Appendix A).

Number of grains per row was significantly affected by the main effect of N rates in 2016/17 (Appendix A). Number of grains per ear was significantly affected by the interaction between N rates $\times$ inoculation in 2015/16, also by the main effects of N rates and inoculation in 2016/17 (Appendix A). Mass of 100 grains was significantly affected by the main effect of N rates in both years, also by the main effects of liming sources and inoculation in 2015/16 (Appendix A). Straw biomass was significantly affected by the main effect of N rates in both years (Appendix A). Harvest index was significantly affected by the main effects of N rates and inoculation in both years, also by the main effect of liming sources in 2015/16 (Appendix A). Grain yield was significantly affected by the interactions between N rates $\times$ liming sources and N rates $\times$ inoculation (Appendix A).

Nitrogen uptake was significantly affected by the main effects of N rates and liming sources in both years, also by the main effect of inoculation in 2016/17 (Appendix A). Nitrogen utilization was significantly affected by the main effect of liming source in 2015/16, also by the main effect of N rates in 2016/17 (Appendix A). Nitrogen use efficiency was significantly affected by the main effect of N rates in both years, also by the main effect of inoculation in 2015/16 (Appendix A).

Shoot biomass was found to respond linearly to increasing N rates in 2016/17 (Table 6). Root biomass was greater with limestone application when associated with 50 kg N ha⁻¹ compared to silicate application in 2015/16 (Figure 5A). Root biomass was found to respond non-linearly when limestone (up to 138 kg N ha⁻¹) and silicate were applied (up to 142 kg N ha⁻¹) (Figure 5A). Also, root biomass responded linearly to N rates in 2016/17, regardless of inoculation and liming source (Table 6). Plant height was found to respond non-linearly to increasing N rates (up to 190 kg N ha⁻¹) in 2016/17 (Table 6). Stem diameter was greater when silicate was applied in both years (Table 7).

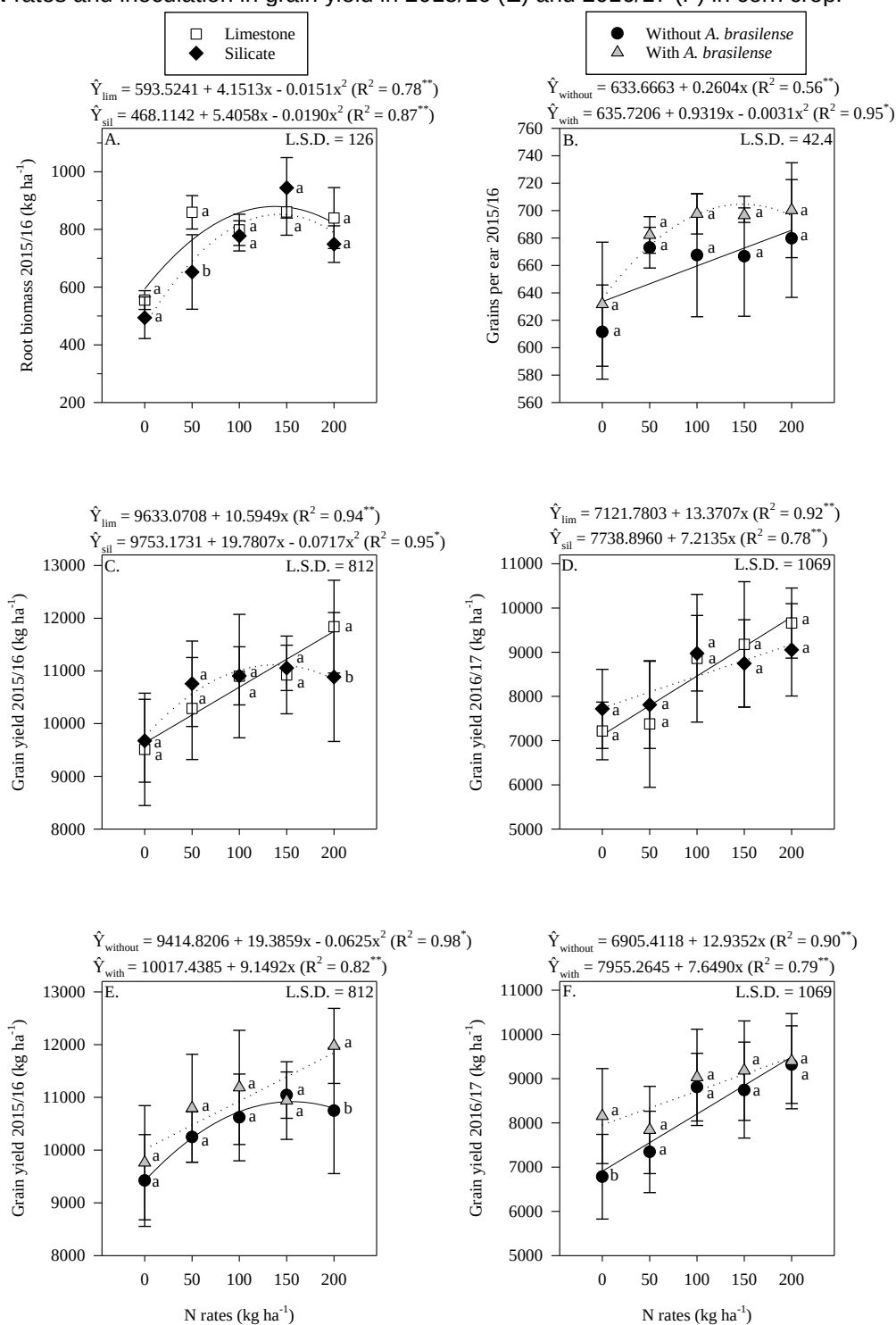
In addition, stem diameter responded non-linearly (up to 140 kg N ha⁻¹) to N rates (Table 6). Ear length was found to respond linearly to increasing N rates, in both years (Table 6). Ear diameter also responded linearly to N rates, in addition, ear diameter was greater when limestone was applied, both in 2016/17 (Tables 6 and 7). Also in 2016/17, number of rows per ear was greater in inoculated plants (Table 7).

Table 6. Shoot and root biomass, plant height, stem diameter, ear length, ear diameter, number of rows per ear, grains per row and grains per ear, mass of 100 grains, straw biomass and harvest index as a function of N rates in corn crop.

Independent variable	2015/16 crop season
Ear length (cm)	$\hat{Y} = 16.8721 + 0.0043x$ ($R^2 = 0.71^{**}$)
Mass of 100 grains (g)	$\hat{Y} = 30.3150 + 0.0079x$ ($R^2 = 0.98^{**}$)
Straw biomass (kg ha ⁻¹)	$\hat{Y} = 11860.1668 + 8.7775x$ ($R^2 = 0.68^*$)
Harvest index (%)	$\hat{Y} = 41.0715 + 0.0236x$ ($R^2 = 0.55^*$)
	2016/17 crop season
Shoot biomass (kg ha ⁻¹)	$\hat{Y} = 10123.4143 + 15.0346x$ ($R^2 = 0.96^*$)
Root biomass (kg ha ⁻¹)	$\hat{Y} = 768.6313 + 1.2807x$ ($R^2 = 0.73^*$)
Plant height (m)	$\hat{Y} = 2.4589 + 0.0019x - 0.000005x^2$ ($R^2 = 0.95^{**}$ and PM = 190 kg ha ⁻¹)
Stem diameter (cm)	$\hat{Y} = 1.9180 + 0.0028x - 0.00001x^2$ ($R^2 = 0.86^{**}$ and PM = 140 kg ha ⁻¹)
Ear length (cm)	$\hat{Y} = 15.3472 + 0.0126x$ ($R^2 = 0.92^{**}$)
Ear diameter (cm)	$\hat{Y} = 5.4497 + 0.0014x$ ($R^2 = 0.78^{**}$)
Grains per row	$\hat{Y} = 32.9052 + 0.0243x$ ($R^2 = 0.92^{**}$)
Grains per ear	$\hat{Y} = 605.9887 + 0.3856x$ ($R^2 = 0.89^{**}$)
Mass of 100 grains (g)	$\hat{Y} = 30.0132 + 0.0141x$ ($R^2 = 0.97^{**}$)
Straw biomass (kg ha ⁻¹)	$\hat{Y} = 11415.1016 + 10.0818x$ ($R^2 = 0.82^{**}$)
Harvest index (%)	$\hat{Y} = 42.2123 + 0.0269x$ ($R^2 = 0.79^{**}$)

^{**} and ^{*}: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Figure 5. Interaction between N rates and liming sources in root biomass in 2015/16 (A), interaction between N rates and inoculation with *A. brasilense* in number of grains per ear in 2015/16 (B), interaction between N rates and liming sources on corn grain yield in 2015/16 (C) and 2016/17 (D), interaction between N rates and inoculation in grain yield in 2015/16 (E) and 2016/17 (F) in corn crop.



Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference. Error bars indicate the standard deviation ($n = 4$).

Table 7. Shoot and root biomass, plant height, stem diameter, ear length, ear diameter and number of rows per ear as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

Rows per ear as a function of N rates, liming sources and inoculation with <i>A. brasilense</i> in corn crop.								
	Shoot biomass		Root biomass		Plant height		Stem diameter	
	----- (kg ha ⁻¹) -----				(m)		(cm)	
N rate	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	10825	10376	525	758	2.69	2.45	2.15	1.92
50	14874	10521	756	903	2.66	2.57	2.11	2.01
100	13715	11698	788	846	2.68	2.59	2.11	2.11
150	13413	12287	902	894	2.68	2.62	2.06	2.03
200	14314	13252	794	1082	2.69	2.65	2.08	2.00
Liming source								
Dolomitic limestone	13549 a†	12178 a	783	909 a	2.67 a	2.58 a	2.03 b	1.94 b
Ca and Mg silicate	13308 a	11076 a	724	884 a	2.69 a	2.57 a	2.17 a	2.09 a
Inoculation								
Without <i>A. brasilense</i>	13160 a	11495 a	739 a	874 a	2.69 a	2.58 a	2.04 b	2.01 a
With <i>A. brasilense</i>	13696 a	11759 a	768 a	920 a	2.67 a	2.57 a	2.15 a	2.02 a
L.S.D. (5%)	1652	2164	56.6	176	0.02	0.04	0.08	0.07
C.V. (5%)	18.6	28.1	11.3	29.7	1.50	3.00	7.3	6.7
Overall Mean	13428	11627	753	897	2.68	2.57	2.10	2.02
Ear length			Ear diameter		Rows per ear			
	----- (cm) -----							
N rate	2015/16	2016/17	2015/16	2016/17	2015/16		2016/17	
0	16.8	15.1	5.6	5.5	18.2		18.3	
50	17.3	16.3	5.5	5.5	18.6		18.4	
100	17.1	16.6	5.6	5.6	18.2		18.2	
150	17.4	17.5	5.6	5.8	18.5		18.2	
200	17.9	17.6	5.6	5.7	18.3		18.1	
Liming source								
Dolomitic limestone	17.2 a	16.7 a	5.6 a	5.7 a	18.5 a		18.4 a	
Ca and Mg silicate	17.4 a	16.5 a	5.6 a	5.5 b	18.2 a		18.1 a	
Inoculation								
Without <i>A. brasilense</i>	17.4 a	16.5 a	5.6 a	5.6 a	18.3 a		18.0 b	
With <i>A. brasilense</i>	17.2 a	16.8 a	5.6 a	5.6 a	18.4 a		18.5 a	
L.S.D. (5%)	0.33	0.49	0.07	0.10	0.44		0.44	
C.V. (5%)	4.20	5.7	2.61	3.47	5.3		4.58	
Overall Mean	17.3	16.6	5.6	5.6	18.4		18.2	

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Number of grains per row was found to respond linearly to increasing N rates in 2016/17 (Table 6). Number of grains per ear responded linearly and non-linearly (up to 150 kg N ha⁻¹) to increasing N rates without inoculation and with *A. brasilense* inoculation, respectively, in 2015/16 (Figure 5B). In 2016/17, grains per ear responded linearly to N rates, regardless of inoculation and liming source (Table 6). In addition, also in 2016/17, grains per ear was greater in inoculated plants compared to non-inoculated plants (Table 8). Mass of 100 grains was found to respond linearly to increasing N rates in both years (Table 6). Also, mass of 100 grains was greater when silicate was applied compared to limestone application and was greater in inoculated

plants compared to non-inoculated plants in 2015/16 (Table 8). Straw biomass was found to respond linearly to increasing N rates in both years (Table 6). Similarly, harvest index responded linearly to N rates in both years (Table 6). In addition, harvest index was greater in inoculated plants in both years (Table 8).

Table 8. Number of grains per row, grains per ear, mass of 100 grains, straw biomass, harvest index and grain yield as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

N rate	Grains per row		Grains per ear		Mass of 100 grains (g)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	35.0	32.4	622	596	30.2	30.1
50	36.8	34.7	678	637	30.9	30.7
100	36.4	35.2	683	642	31.1	31.2
150	36.2	37.2	682	675	31.5	32.5
200	36.4	37.2	690	673	31.9	32.7
Liming source						
Dolomitic limestone	35.9 a	35.5 a	669 a	650 a	30.8 b	31.7 a
Ca and Mg silicate	36.4 a	35.2 a	672 a	639 a	31.5 a	31.1 a
Inoculation						
Without <i>A. brasilense</i>	36.2 a	34.9 a	660	627 b	30.8 b	31.3 a
With <i>A. brasilense</i>	36.1 a	35.7 a	682	662 a	31.4 a	31.6 a
L.S.D. (5%)	1.05	1.25	18.9	28.3	0.53	0.68
C.V. (5%)	6.5	6.8	6.3	8.4	3.80	4.14
Overall Mean	36.2	35.3	671	645	31.1	31.4
N rate	Straw biomass (kg ha ⁻¹)		Harvest index (%)		Grain yield (kg ha ⁻¹)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	11267	10988	39.5	42.5	9592	7468
50	12844	12307	42.7	42.2	10521	7592
100	13040	12552	45.6	46.6	10904	8919
150	13311	13214	45.4	45.8	10991	8961
200	13228	13055	44.0	47.5	11362	9357
Liming source						
Dolomitic limestone	12782 a	12610 a	45.2 a	44.1 a	10693	8459
Ca and Mg silicate	12694 a	12237 a	41.6 b	45.7 a	10656	8460
Inoculation						
Without <i>A. brasilense</i>	12455 a	12212 a	41.6 b	42.8 b	10416	8199
With <i>A. brasilense</i>	13020 a	12634 a	45.3 a	47.1 a	10932	8720
L.S.D. (5%)	918	907	3.3	2.80	363.2	478
C.V. (5%)	10.9	11.0	11.3	9.4	7.6	12.6
Overall Mean	12738	12423	43.4	44.9	10674	8460

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

The greater N uptake, together with the increased LCI provided by increasing N rates, favored root development leading to a greater shoot development, plant height and stem diameter. In addition, N rates provided better ear development and grain filling, with greater ear length, ear diameter, number of grains per row and grain per ear,

mass of 100 grains and harvest index. Several studies have been reported the benefit of N fertilization in cereals, for example as corn (COYOS *et al.*, 2018; ICHAMI *et al.*, 2019), wheat (YU *et al.*, 2018; OMARA *et al.*, 2020), rice (JIA *et al.*, 2019; TATSUMI *et al.*, 2019), sorghum (MAW *et al.*, 2016; SAINJU *et al.*, 2018), barley (YOUSIF; EVANS, 2018; KASSIE; TESFAYE, 2019) and oats (MA *et al.*, 2017; ALLWOOD *et al.*, 2018). This nutrient plays important roles in plant nutrition and development (LOLLATO *et al.*, 2019) such as synthesis and production of phytohormones, coenzymes, nucleic acids, secondary metabolites, chlorophyll and proteins (MARSCHNER, 2012). The positive Pearson's correlations verified between the leaf N concentration and shoot and root N uptake with the productive components and grain yield (Appendix C) support this hypothesis.

Corn grain yield was greater with limestone application when associated with 200 kg N ha⁻¹ compared to silicate application in 2015/16 (Figure 5C). Grain yield was found to respond linearly and non-linearly (up to 138 kg N ha⁻¹) to increasing N rates when limestone and silicate were applied, respectively (Figure 5C). In 2016/17, grain yield responded linearly to N rates when limestone and silicate were applied (Figure 5D). Regarding the interaction between N rates and inoculation, corn grain yield was greater in inoculated plants when associated with 200 kg N ha⁻¹ compared to non-inoculated plants in 2015/16 (Figure 5E). Grain yield was found to respond linearly and non-linearly (up to 155 kg N ha⁻¹) to increasing N rates with *A. brasilense* and without inoculation, respectively (Figure 5E). In 2016/17, grain yield was greater in inoculated plots without N fertilization in side-dressing compared to non-inoculated plots (Figure 5F). Grain yield responded linearly to N rates for both inoculated and non-inoculated plants (Figure 5F).

Different responses to side-dressing N application rates in corn have been reported in Brazil. For example, Faria *et al.* (2019) reported greater grain yield varying between 60 and 76 kg N ha⁻¹ applied at V4 stage in corn second season. Silva *et al.* (2012) verified greater grain yield with application of 110 kg N ha⁻¹ applied at V3 stage in corn first season. Souza *et al.* (2011) verified greater grain yield with application of 150 kg N ha⁻¹ at V6 stage in corn second season. While Mateus *et al.* (2019) reported greater corn grain yield with application of 113 kg N ha⁻¹ applied at V5 stage in corn first season. Lucas *et al.* (2019) verified greater grain yield with application of 155 kg N ha⁻¹

applied at V4 stage in corn first season. Queiroz *et al.* (2011) reported greater grain yield with 160 kg N ha⁻¹ applied at V5 stage in corn first season. Galindo *et al.* (2016) verified a positive response to N rates up to 200 kg N ha⁻¹ applied at V6 stage in corn first season. Caires; Milla (2016) obtained the maximum technical and economic efficiency with the application of 209 kg ha⁻¹ of N in the V4 stage in corn first season. Mendes *et al.* (2013) verified greater grain yield with split application of 240 kg N ha⁻¹, applied at V4 (120 kg N ha⁻¹) and V6 (120 kg N ha⁻¹) stages in corn first season, respectively. Therefore, the side-dressing N application must be rational and optimized.

The use of Si had little to no quantifiable effect in corn development and grain yield. The Si benefits are more frequent in hyperaccumulator crops (CAMARGO *et al.*, 2014a, 2014b), which contain SiO₂ concentrations above 5% of shoot biomass (PATI *et al.*, 2016; YANG *et al.*, 2019). In addition, increased grain yield is unlikely when available Si in soil is above 10.0 mg kg⁻¹ (SARTO *et al.*, 2015; KORNDÖRFER *et al.*, 2010). In this study, the available soil Si contents were close to this range (9.4 mg kg⁻¹ at 0-0.20 m and 10.2 at 0.20-0.40 m). In addition, more Si became available as straw decomposed during the growing season (13.1 kg ha⁻¹ Si, and 38.3 of C/N ratio, Table 2). Some studies have reported Si can be beneficial to corn growth under biotic and abiotic conditions (GUÉVEL *et al.*, 2007; AGOSTINHO *et al.*, 2017). For example, Latef; Tran (2016) concluded that Si plays a central role in alleviating the negative effects of alkaline stress on corn growth by improving water status, enhancing photosynthetic pigments, accumulating osmoprotectants rather than proline, activating the antioxidant machinery, and maintaining the balance of K/Na ratio. Similarly, Khan *et al.* (2018) observed that Si addition alleviated both osmotic and oxidative stress in corn crop by improving the performance of defensive machinery and water use efficiency under salinity stress. Amin *et al.* (2018) verified that Si application to drought stressed corn plants improved the growth and yield which could be attributed to improved photosynthetic rate and lowered transpiration. The fact that adequate amounts of Si were present in the soil used for this study and the lack of a positive response to added Si suggest that little to no biotic or abiotic stress was present during the growing seasons studied.

Besides that, the Pearson's correlations showed that with the increase in the leaf Si concentration and shoot and root Si uptake, some important nutritional, biometric and components production are benefited, like root and shoot biomass, LCI, leaf N concentration, shoot and root N uptake, stem diameter, mass of 100 grains, grains per ear, harvest index, NUE and corn grain yield for both non-inoculated and *A. brasilense* inoculated treatments increased, demonstrating that the Si uptake and accumulation may be beneficial to corn nutrition and development (Appendix C).

Inoculation with *A. brasilense* provided greater corn development as verified by the increased stem diameter, number of rows per ear, grains per ear, mass of 100 grains and harvest index, leading to an increase of 5.6% (518 kg ha^{-1}) on corn grain yield, on average of both years. The positive Pearson's correlations verified between important productive components such as shoot and root biomass, grains per ear, mass of 100 grains and harvest index, grain yield and NUE in inoculated plants (Appendix C) support this hypothesis. Under high N application rates (200 kg N ha^{-1}), inoculation provided an increase of 11.4% (1229 kg ha^{-1}) in grain yield, in 2015/16. In the absence of N fertilization, inoculation provided an increase of 20.2% (1371 kg ha^{-1}) in grain yield, in 2016/17. Therefore, our results showed an additive effect of N rates on *A. brasilense* inoculation. In the presence and absence of N application, *A. brasilense* benefited corn development and yield.

Similar results were obtained by Galindo et al. (2016), working with N doses application in side-dressing (0, 50, 100, 150 and 200 kg ha^{-1}) with and without inoculation with *A. brasilense* verified greater corn grain yield as a function of inoculation in 1012 kg ha^{-1} , equivalent to 17.7%. Kappes et al. (2013), reported that corn inoculated with *A. brasilense* had an increased yield of 9.4%. Cavallet et al. (2000), reported a 17% increase on corn yield when the seeds were inoculated with *Azospirillum* sp. Similar results were obtained by Müller et al. (2016), with an increase of 3.8% on corn grain yield with seed inoculation of *A. brasilense*, with an average grain yield above 11000 kg ha^{-1} . Hungria et al. (2010) also obtained increased corn grain yield in 27%, corresponding to an increase of 743 kg ha^{-1} . Zeffa et al. (2018) performed a meta-analysis on the influence of side-dressing N application associated with *Azospirillum* sp. inoculation in corn and reported an average yield increase of 652 kg ha^{-1} .

¹ when associated with low (> 0 and ≤ 50 kg N ha⁻¹), moderate (> 50 and ≤ 100 kg N ha⁻¹) and high (> 100 kg N ha⁻¹) N rates.

Plant and soil bacteria participate in several molecular signaling events that establish specific endophytic, symbiotic or associative relationships (SANTOS *et al.*, 2017a). Such relationships differ according to plant genotypes, bacterial strains, ability to improve plant growth and soil types (PHILIPPOT *et al.*, 2013). *Azospirillum* sp. is one of the most studied genera of PGPB at present due its capacity to colonize many plant species (CASSÁN; DIAZ-ZORITA, 2016). The plant growth-promotion by *Azospirillum* is mainly associated with its ability to fix N in grasses (PANKIEVICZ *et al.*, 2015; SANTOS *et al.*, 2017a) but apparently, BNF does not play a major role in plant growth promotion in most cropping systems evaluated (FIBACH-PALDI *et al.*, 2012). On the other hand, *Azospirillum* sp. are able to produce and secrete phytohormones such as AIA, gibberellins and cytokinins, nitric oxide (FIBACH- PALDI *et al.*, 2012), which likely are key signals and components of plant growth promotion effects (BASHAN; DE-BASHAN, 2010) and can increase the use of applied N in grasses, as verified in this study.

Nitrogen uptake (NUP) by corn was found to decrease with increasing N rates in both years (Table 9). Also, in both years, NUP was greater when limestone was applied (Table 10). In addition, NUP was greater in inoculated plants in 2016/17 (Table 10). Nitrogen utilization was found to decrease with increasing N rates in 2016/17 (Table 9). In 2015/16, NUT was greater when silicate was applied (Table 10). Nitrogen use efficiency (NUE) was found to decrease to increasing N rates in both years (Table 9). In 2015/16, NUE was greater in inoculated plants (Table 10).

Table 9. Nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use efficiency (NUE) as a function of N rates in corn crop.

Independent variable	2015/16 crop season
NUP (kg N accumulated kg N applied ⁻¹)	$\hat{Y} = 0.6375 - 0.0014x$ ($R^2 = 0.89^{**}$)
NUE (kg grain kg N applied ⁻¹)	$\hat{Y} = 20.7759 - 0.0592x$ ($R^2 = 0.87^{**}$)
	2016/17 crop season
NUP (kg N accumulated kg N applied ⁻¹)	$\hat{Y} = 0.5618 - 0.0010x$ ($R^2 = 0.75^{**}$)
NUT (kg grain kg N accumulated ⁻¹)	$\hat{Y} = 47.7650 - 0.0788x$ ($R^2 = 0.91^{**}$)
NUE (kg grain kg N applied ⁻¹)	$\hat{Y} = 26.2053 - 0.0787x$ ($R^2 = 0.94^{**}$)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively.

Table 10. Nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use efficiency (NUE) as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

N rate	NUP (kg N accumulated kg N applied ⁻¹)		NUT (kg grain kg N accumulated ⁻¹)		NUE (kg grain kg N applied ⁻¹)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	-	-	-	-	-	-
50	0.56	0.49	35.6	42.5	19.2	21.6
100	0.52	0.49	24.4	40.9	12.8	19.9
150	0.38	0.35	31.2	37.7	11.8	13.1
200	0.37	0.36	28.7	30.5	9.7	10.8
Liming source						
Dolomitic limestone	0.51 a†	0.46 a	27.7 b	36.5 a	13.4 a	17.0 a
Ca and Mg silicate	0.40 b	0.38 b	32.3 a	39.3 a	13.3 a	15.7 a
Inoculation						
Without <i>A. brasilense</i>	0.43 a	0.39 b	28.4 a	38.3 a	12.0 b	15.2 a
With <i>A. brasilense</i>	0.48 a	0.46 a	31.6 a	37.5 a	14.8 a	17.5 a
L.S.D. (5%)	0.05	0.06	3.94	5.7	2.04	3.47
C.V. (5%)	22.4	30.5	26.1	29.6	30.3	20.6
Overall Mean	0.46	0.42	30.0	37.9	13.4	16.4

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

The decrease in NUE as a function of increased N rates can be attributed to the loss of N, as clearly described in the literature. Higher N rates application result in increased losses and less utilization by cropping systems since plant nutritional demand is limited (GALINDO *et al.*, 2016). Plants are able to absorb a certain quantity of nutrients as a function of time; the N that is applied and is not uptake may be lost, decreasing the fertilization efficiency with higher N rates, as stated in the literature as the principle of diminishing returns.

Although the exact mechanisms underlying the PGPB effect on N acquisition by corn was not evaluated in the present study, it is possible that the greater NUE observed in inoculated plants (an increase of 19.1% on average of both years) was due to its ability to promote plant growth (GALINDO *et al.*, 2016; FUKAMI *et al.*, 2017; MARTINS *et al.*, 2018; SALVO *et al.*, 2018). Specifically, it has been demonstrated in draft genome sequences that the strains Ab-V5 and Ab-V6 of *A. brasilense*, carry similar *nif* and *fix* genes that confer their ability to fix atmospheric N (HUNGRIA *et al.*, 2018). Although the strains differ in their capacity to synthesize phytohormones (CASSÁN; DIAZ-ZORITA, 2016; FUKAMI *et al.*, 2018b), both share the same genes related to the synthesis of auxins. This growth promotion mechanism might have improved the ability of plants to explore the soil more efficiently, as indicated in previous

studies using *A. brasilense* (MARTINS *et al.*, 2018; FUKAMI *et al.*, 2018a, b; ZEFFA *et al.*, 2019). Also, according to Cormier *et al.* (2013), two strategies may be devised for N use efficiency improvement: increasing the yield at a constant N supply and/or maintaining high yield when reducing N supply. Therefore, *A. brasilense* inoculation could be a powerful technique to improve N management in corn crop.

4.3 NUTRIENT UPTAKE IN CORN STRAW AND GRAIN (UPTAKE AND REMOVAL)

Straw N uptake was significantly affected by the main effect of N rates in both years, also by the main effect of inoculation in 2016/17 (Appendix A). Straw P uptake was significantly affected by the main effect of liming sources in 2015/16, also by the main effect of N rates in 2016/17 (Appendix A). Straw K uptake was significantly affected by the main effects of liming sources and inoculation in 2015/16 (Appendix A). Straw Ca uptake was significantly affected by N rates in both years, also by the main effects of liming sources and inoculation in 2015/16 (Appendix A). Straw Mg uptake was significantly affected by the main effect of N rates in both years (Appendix A). Straw S uptake was significantly affected by the main effect of N rates in both years, also by the main effect of liming sources in 2016/17 (Appendix A).

Straw B uptake was significantly affected by the main effect of N rates in both years, also by the main effects of liming sources in 2015/16 and inoculation in 2016/17 (Appendix A). Straw Fe uptake was significantly affected by the main effect of N rates in both years, also by the main effect of liming sources in 2015/16 (Appendix A). Straw Mn uptake was significantly affected by the main effect of N rates in both years (Appendix A). Straw Zn uptake was significantly affected by the main effect of N rates in 2016/17 (Appendix A). Straw Si uptake was significantly affected by the main effect of liming sources in both years, also by the main effects of N rates and inoculation in 2016/17 (Appendix A). Straw Cu uptake was not significantly affected by N rates, liming sources, inoculation and their interactions (Appendix A).

Grain N uptake was significantly affected by the interaction between N rates × inoculation in 2015/16, also by the main effect of N rates in 2016/17 (Appendix A). Grain P uptake was significantly affected by the main effect of N rates in both years (Appendix A). Grain K uptake was significantly affected by the main effect of N rates in 2016/17

(Appendix A). Grain Ca uptake was significantly affected by the main effect of N rates in both years, also by the main effect of liming source in 2015/16 (Appendix A). Grain Mg uptake was significantly affected by the main effect of N rates in both years (Appendix A). Grain S uptake was significantly affected by the interaction between N rates $\times$ inoculation in 2015/16, also by the main effect of N rates in 2016/17 (Appendix A).

Grain B uptake was significantly affected by the main effect of N rates in 2016/17 (Appendix A). Grain Cu uptake was significantly affected by the main effect of inoculation in 2015/16 (Appendix A). Grain Fe uptake was significantly affected by the main effects of N rates and liming sources in 2016/17 (Appendix A). Grain Mn uptake was significantly affected by the main effect of N rates in both years (Appendix A). Grain Zn uptake was significantly affected by the interaction between N rates $\times$ inoculation in 2015/16 (Appendix A). Grain Si uptake was significantly affected by the main effects of N rates in both years, also by the main effect of liming sources in 2016/17 (Appendix A).

Straw N uptake was found to respond linearly to increasing N rates in both years (Table 11). Also, straw N uptake was greater in inoculated plants in 2016/17 (Table 12). Straw P uptake was greater when silicate was applied in 2015/16 (Table 12). Also, straw P uptake was found to respond linearly to N increasing rates in 2016/17 (Table 11). Straw K uptake was greater when silicate was applied in 2015/16 (Table 12). In addition, also in 2015/16, straw K uptake was greater in inoculated plants (Table 12). Straw Ca uptake was found to respond linearly to increasing N rates in both years (Table 11). Also, straw Ca uptake was greater with silicate application compared to limestone and greater in inoculated plants compared to non-inoculated plants, both in 2015/16 (Table 12). Straw Mg uptake was found to respond linearly to increasing N rates in both years (Table 11). Similarly, straw S uptake responded linearly to N rates in both years (Table 11). In addition, straw S uptake was greater when silicate was applied in 2016/17 (Table 12).

Straw B uptake was found to respond linearly to increasing N application rates in both years (Table 11). Also, straw B uptake was greater when silicate was applied compared to limestone application in 2015/16 and was greater in inoculated plants compared to non-inoculated plants in 2016/17 (Table 12). Straw Fe uptake was found to respond non-linearly (up to 121 kg N ha⁻¹) and linearly to increasing N rates in 2015/16

and 2016/17, respectively (Table 11). Also, straw Fe uptake was greater when limestone was applied in 2015/16 (Table 12). Straw Mn uptake was found to respond linearly to increasing N rates in both years (Table 11). Straw Zn uptake was found to respond linearly to increasing N rates in 2016/17 (Table 11). Straw Si uptake was greater when silicate was applied in both years (Table 12). Straw Si uptake also responded linearly to N rates in 2016/17 (Table 11). In addition, straw Si uptake was greater in inoculated plants in 2016/17 (Table 12).

Table 11. Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates in corn crop.

Independent variable	2015/16 crop season
Straw N uptake (kg ha ⁻¹)	$\hat{Y} = 98.3462 + 0.1718x$ ($R^2 = 0.87^{**}$)
Straw Ca uptake (kg ha ⁻¹)	$\hat{Y} = 37.2705 + 0.0579x$ ($R^2 = 0.89^{**}$)
Straw Mg uptake (kg ha ⁻¹)	$\hat{Y} = 20.7010 + 0.0335x$ ($R^2 = 0.84^{**}$)
Straw S uptake (kg ha ⁻¹)	$\hat{Y} = 16.4358 + 0.0159x$ ($R^2 = 0.94$)
Straw B uptake (g ha ⁻¹)	$\hat{Y} = 137.2950 + 0.3281x$ ($R^2 = 0.90^{**}$)
Straw Fe uptake (g ha ⁻¹)	$\hat{Y} = 2507.7416 + 28.6662x - 0.1186x^2$ ($R^2 = 0.87^*$ and PM = 121 kg ha ⁻¹)
Straw Mn uptake (g ha ⁻¹)	$\hat{Y} = 1745.0270 + 2.6948x$ ($R^2 = 0.78^{**}$)
	2016/17 crop season
Straw N uptake (kg ha ⁻¹)	$\hat{Y} = 64.0873 + 0.1860x$ ($R^2 = 0.99^{**}$)
Straw P uptake (kg ha ⁻¹)	$\hat{Y} = 6.4122 + 0.0123x$ ($R^2 = 0.85^{**}$)
Straw Ca uptake (kg ha ⁻¹)	$\hat{Y} = 35.3618 + 0.0559x$ ($R^2 = 0.87^{**}$)
Straw Mg uptake (kg ha ⁻¹)	$\hat{Y} = 13.2480 + 0.014x$ ($R^2 = 0.77^*$)
Straw S uptake (kg ha ⁻¹)	$\hat{Y} = 9.4482 + 0.0220x$ ($R^2 = 0.98^{**}$)
Straw B uptake (g ha ⁻¹)	$\hat{Y} = 126.6725 + 0.2720x$ ($R^2 = 0.97^{**}$)
Straw Fe uptake (g ha ⁻¹)	$\hat{Y} = 1920.6740 + 4.6024x$ ($R^2 = 0.79^{**}$)
Straw Mn uptake (g ha ⁻¹)	$\hat{Y} = 609.1902 + 1.8325x$ ($R^2 = 0.94^{**}$)
Straw Zn uptake (g ha ⁻¹)	$\hat{Y} = 187.7920 + 0.5023x$ ($R^2 = 0.95^{**}$)
Straw Si uptake (kg ha ⁻¹)	$\hat{Y} = 28.11285 + 0.0386x$ ($R^2 = 0.67^*$)

^{**} and ^{*}: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Table 12. Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

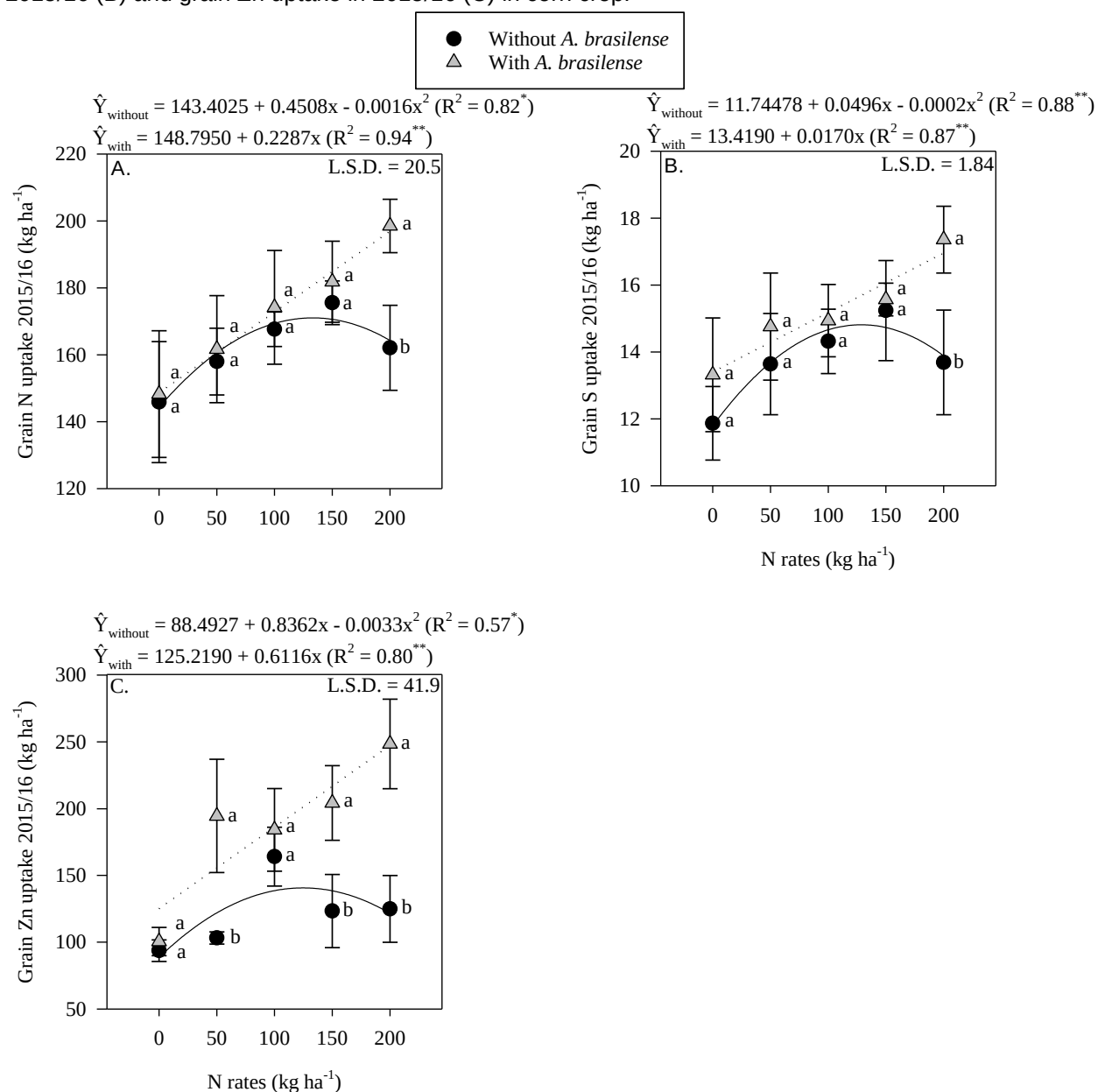
	Straw N uptake		Straw P uptake		Straw K uptake		Straw Ca uptake		Straw Mg uptake		Straw S uptake	
	----- (kg ha ⁻¹) -----											
N rate	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	94.0	62.7	9.6	5.9	109	90.4	35.3	33.5	19.3	12.6	16.0	9.3
50	110	74.7	11.4	7.5	130	110	42.7	40.6	23.5	14.7	17.6	10.6
100	123	84.0	11.6	7.9	120	106	43.4	40.5	25.2	14.5	18.3	11.6
150	119	90.9	11.5	8.4	123	116	45.7	44.5	25.8	15.9	18.9	13.1
200	132	101	11.8	8.5	134	107	48.3	45.6	26.5	15.6	19.4	13.6
Liming source												
Dolomitic limestone	118 a†	83.8 a	10.0 b	7.4 a	116 b	108 a	40.3 b	42.7 a	24.6 a	14.9 a	18.0 a	11.1 b
Ca and Mg silicate	113 a	81.6 a	12.3 a	7.9 a	130 a	104 a	45.9 a	39.3 a	23.5 a	14.4 a	18.0 a	12.2 a
Inoculation												
Without <i>A. brasilense</i>	112 a	77.4 b	10.8 a	7.4 a	114 b	99.2 a	41.2 b	40.0 a	22.7 a	14.2 a	17.4 a	11.4 a
With <i>A. brasilense</i>	119 a	88.0 a	11.6 a	7.9 a	132 a	113 a	44.9 a	42.0 a	25.5 a	15.1 a	18.7 a	12.0 a
L.S.D. (5%)	12.6	9.3	1.64	1.00	11.5	16.6	3.56	5.3	3.13	1.70	1.80	1.00
C.V. (5%)	16.4	16.8	22.2	19.9	14.1	23.6	12.5	19.6	19.7	17.6	15.2	13.1
Overall Mean	116	82.7	11.2	7.6	123	106	43.1	41.0	24.1	14.7	18.0	11.7
	Straw B uptake		Straw Cu uptake		Straw Fe uptake		Straw Mn uptake		Straw Zn uptake		Straw Si uptake	
	----- (g ha ⁻¹) -----											
N rate	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	140	126	228	183	2532	1986	1128	920	301	176	15.3	25.5
50	150	138	279	233	3705	2040	1391	953	324	224	15.0	33.1
100	163	158	275	257	3863	2266	1460	1077	310	246	15.7	32.6
150	201	170	277	257	4510	2911	1550	1318	395	263	17.5	34.1
200	197	177	272	245	3362	2701	1520	1392	363	282	16.0	34.6
Liming source												
Dolomitic limestone	156 b	158 a	276 a	232 a	4054 a	2445 a	1363 a	1171 a	326 a	249 a	14.9 b	28.3 b
Ca and Mg silicate	184 a	149 a	256 a	238 a	3134 b	2317 a	1457 a	1094 a	351 a	227 a	16.9 a	35.6 a
Inoculation												
Without <i>A. brasilense</i>	158 a	139 b	263 a	215 a	3297 a	2377 a	1363 a	1124 a	335 a	229 a	15.4 a	29.4 b
With <i>A. brasilense</i>	182 a	169 a	269 a	255 a	3891 a	2385 a	1457 a	1141 a	342 a	248 a	16.4 a	34.6 a
L.S.D. (5%)	25.9	15.5	38.7	49.1	753	393	150	172	61.0	39.7	1.77	4.86
C.V. (5%)	23.0	15.3	22.0	29.6	29.7	24.9	16.1	23.0	27.2	25.2	16.8	23.0
Overall Mean	170	154	266	235	3594	2381	1410	1132	339	238	15.9	32.0

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Grain N uptake was greater in inoculated plants when associated with 200 kg N ha⁻¹ compared to non-inoculated plants in 2015/16 (Figure 6A). Grain N uptake was found to respond linearly and non-linearly (up to 141 kg N ha⁻¹) to increasing N rates with *A. brasilense* and without inoculation, respectively (Figure 6A). In 2016/17, grain N uptake responded linearly to N rates, regardless of inoculation and liming source (Table 13). Grain P uptake was found to respond linearly to increasing N rates in both years (Table 13). Similarly, grain K uptake responded linearly to N rates in 2016/17 (Table 13). Grain K uptake was found to respond linearly to increasing N rates in both years (Table 13). Also, grain Ca uptake was greater when silicate was applied in 2015/16 (Table 14). Grain Mg was found to respond linearly to increasing N rates in both years. Grain S uptake was greater in inoculated plants when associated with 200 kg N ha⁻¹ compared to non-inoculated plants in 2015/16 (Figure 6B). Grain S uptake was found to respond linearly and non-linearly (up to 124 kg N ha⁻¹) to increasing N rates with *A. brasilense* and without inoculation, respectively (Figure 6B). Also, in 2016/17, grain S uptake responded linearly to N rates, regardless of inoculation and liming source (Table 13).

Grain B uptake was found to respond linearly to increasing N rates in 2016/17 (Table 13). Grain Cu uptake was greater in inoculated plants in 2015/16 (Table 14). Grain Fe uptake was found to respond linearly to increasing N rates in 2016/17 (Table 13). In addition, also in 2016/17, grain Fe was greater when limestone was applied (Table 14). Grain Mn was found to respond linearly to increasing N rates in both years (Table 13). Grain Zn uptake was greater in inoculated plants when associated with 50, 150 and 200 kg N ha⁻¹ compared to non-inoculated plants (Figure 6C). Grain Zn was found to respond linearly and non-linearly (up to 127 kg ha⁻¹) to increasing N rates with *A. brasilense* and without inoculation, respectively (Figure 6C). Grain Si uptake was found to respond linearly to increasing N rates in both years (Table 13). In addition, in 2016/17, grain Si uptake was greater when silicate was applied (Table 14).

Figure 6. Interaction between N rates and inoculation in grain N uptake in 2015/16 (A), grain S uptake in 2015/16 (B) and grain Zn uptake in 2015/16 (C) in corn crop.



Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference. Error bars indicate the standard deviation ($n = 4$).

Table 13. Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates in corn crop.

Independent variable	2015/16 crop season
Grain P uptake (kg ha ⁻¹)	$\hat{Y} = 41.4194 + 0.0393x$ ($R^2 = 0.91^{**}$)
Grain K uptake (kg ha ⁻¹)	$\hat{Y} = 114.5989 + 0.1000x$ ($R^2 = 0.57^{**}$)
Grain Ca uptake (kg ha ⁻¹)	$\hat{Y} = 4.1101 + 0.0119x - 0.00004x^2$ ($R^2 = 0.84^*$ and PM = 149 kg ha ⁻¹)
Grain Mg uptake (kg ha ⁻¹)	$\hat{Y} = 13.7654 + 0.0160x$ ($R^2 = 0.96^{**}$)
Grain Mn uptake (g ha ⁻¹)	$\hat{Y} = 116.2365 + 0.1203x$ ($R^2 = 0.98^{**}$)
Grain Si uptake (kg ha ⁻¹)	$\hat{Y} = 1.0900 + 0.0026x$ ($R^2 = 0.77^{**}$)
	2016/17 crop season
Grain N uptake (kg ha ⁻¹)	$\hat{Y} = 84.952 + 0.2045x$ ($R^2 = 0.89^{**}$)
Grain P uptake (kg ha ⁻¹)	$\hat{Y} = 32.4492 + 0.0580x$ ($R^2 = 0.76^{**}$)
Grain K uptake (kg ha ⁻¹)	$\hat{Y} = 87.9947 + 0.1633x$ ($R^2 = 0.71^{**}$)
Grain Ca uptake (kg ha ⁻¹)	$\hat{Y} = 5.3263 + 0.0083x$ ($R^2 = 0.72^{**}$)
Grain Mg uptake (kg ha ⁻¹)	$\hat{Y} = 9.6777 + 0.0159x$ ($R^2 = 0.65^{**}$)
Grain S uptake (kg ha ⁻¹)	$\hat{Y} = 10.8525 + 0.0212x$ ($R^2 = 0.81^{**}$)
Grain B uptake (g ha ⁻¹)	$\hat{Y} = 49.5825 + 0.0970x$ ($R^2 = 0.59^{**}$)
Grain Fe uptake (g ha ⁻¹)	$\hat{Y} = 201.2072 + 0.4196x$ ($R^2 = 0.66^{**}$)
Grain Mn uptake (g ha ⁻¹)	$\hat{Y} = 39.1802 + 0.1556x$ ($R^2 = 0.89^{**}$)
Grain Si uptake (kg ha ⁻¹)	$\hat{Y} = 0.6665 + 0.0020x$ ($R^2 = 0.89^{**}$)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Table 14. Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

com crop.	Grain N uptake		Grain P uptake		Grain K uptake		Grain Ca uptake		Grain Mg uptake		Grain S uptake	
	(kg ha ⁻¹)											
N rate	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	147	86.4	40.4	32.6	114	89.4	4.12	5.2	13.5	9.8	15.1	9.3
50	160	88.0	44.0	32.4	116	85.8	4.50	5.5	14.8	9.3	17.0	9.0
100	171	114	46.3	42.0	135	116	5.0	6.9	15.7	12.7	17.6	11.8
150	179	114	47.9	42.0	122	114	4.69	6.5	16.0	12.1	18.5	11.5
200	180	124	48.2	42.3	136	116	4.67	6.8	16.9	12.4	18.6	12.5
Liming source	0											
Dolomitic limestone	169 a†	104 a	44.5 a	38.6 a	121 a	106 a	4.25 b	6.1 a	15.5 a	11.4 a	17.1 a	11.0 a
Ca and Mg silicate	166 a	107 a	46.2 a	37.9 a	129 a	103 a	4.95 a	6.2 a	15.3 a	11.1 a	17.6 a	10.6 a
Inoculation												
Without <i>A. brasilense</i>	163	104 a	44.33 a	38.03 a	122 a	103 a	4.49 a	5.8 a	15.0 a	11.2 a	16.6	10.7 a
With <i>A. brasilense</i>	172	107 a	46.36 a	38.48 a	128 a	105 a	4.70 a	6.5 a	15.7 a	11.4 a	18.2	10.9 a
L.S.D. (5%)	9.2	11.2	2.67	4.50	17.3	10.7	0.33	0.69	0.86	1.31	0.99	1.09
C.V. (5%)	8.3	16.0	8.9	17.8	21.0	15.4	10.9	16.7	8.5	17.6	8.6	15.4
Overall Mean	167	105	45.4	38.3	125	104	4.60	6.2	15.4	11.3	17.4	10.8
Grain B uptake		Grain Cu uptake		Grain Fe uptake		Grain Mn uptake		Grain Zn uptake		Grain Si uptake		
(g ha ⁻¹)												
N rate	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	82.7	48.9	53.1	52.2	335	181	115	41.8	97.0	202	0.99	0.70
50	109	48.1	53.8	50.5	379	224	123	42.8	149	201	1.24	0.67
100	100	69.8	47.7	65.0	375	282	128	58.3	174	250	1.46	0.92
150	100	64.6	56.2	60.8	403	259	136	57.4	164	242	1.58	0.98
200	103	65.0	55.0	63.7	373	269	139	73.4	187	232	1.47	1.06
Liming source												
Dolomitic limestone	99.8 a	62.9 a	52.6 a	57.8 a	378.2 a	280.8 a	129 a	57.8 a	158 a	232 a	1.27 a	0.72 b
Ca and Mg silicate	98.6 a	55.7 a	53.7 a	59.0 a	367.5 a	205.6 b	128 a	51.7 a	151 a	219 a	1.42 a	1.01 a
Inoculation												
Without <i>A. brasilense</i>	94.6 a	58.9 a	46.6 b	57.8 a	356 a	242 a	126 a	53.4 a	122	224 a	1.35 a	0.81 a
With <i>A. brasilense</i>	104 a	59.7 a	59.7 a	59.0 a	390 a	245 a	130 a	56.1 a	186	226 a	1.34 a	0.92 a
L.S.D. (5%)	15.8	8.8	9.9	12.0	40.8	42.4	10.7	9.1	18.7	29.3	0.23	0.16
C.V. (5%)	24.0	22.5	28.2	29.1	16.6	26.3	12.6	25.1	18.4	19.7	26.8	28.4
Overall Mean	99.2	59.3	53.2	58.4	373	243	128	54.7	154	225	1.35	0.86

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

The N application rates positive influenced nutrient uptake as verified by increased straw N, P, Ca, Mg, S, B, Fe, Mn, Zn and Si uptake and grain N, P, K, Ca, Mg, S, B, Fe, Mn, Zn and Si uptake. As mentioned before, N is the nutrient that is most demanded by corn plants and directly affects crop development and yield. The higher N availability as a function of the N application likely favoured the development of a well-established root system leading to improved uptake of water and nutrients. Increased root biomass can positively influence root scavenging that is important for the interception of nutrients in crop systems. Based on two years of cultivation, the average nutrient uptake in the corn straw and grain in descending order was $K > N > Ca > Si > Mg > S > P > Fe > Mn > Zn > Cu > B$ for straw; and $N > K > P > S > Mg > Ca > Si > Fe > Zn > Mn > B > Cu$ for grains.

The silicate application improved nutrient uptake as verified by greater straw Si uptake in both years and grain Si uptake in 2016/17, also, greater straw P, K, Ca, and B uptake in 2015/16 and straw S uptake in 2016/17. In addition, silicate application increased grain Ca uptake in 2015/16, however, reduced straw Fe uptake in 2015/16 and grain Fe uptake in 2016/17. The positive Pearson's correlations verified between the shoot and grain Si uptake and the other nutrients uptake, mainly in straw (Appendix C) support this hypothesis. Also, Si was the fourth and seventh element most absorbed in corn straw and grain respectively, reinforcing the importance of this element on corn nutrition.

The Si application can affect the amount of structural plant compounds such as cellulose and lignin for different species (SCHOELYNCK *et al.*, 2010). According to Schaller *et al.* (2012), Si effect on phenol content, cellulose and lignin depending on the function of the tissue in the plant (stabilization tissue or photosynthetic tissue). Higher Si availability results in higher cellulose content in photosynthetic tissues, such as leaves and less cellulose content in tissues with stabilization function, such as culm. In this way, Si could benefit corn foliar architecture, promote erectness, improving the leaves angle and light interception, avoiding the excessive self-shading, reducing lodging, delaying senescence, increasing the structural rigidity of the tissues (MA; YAMAJI, 2008; GONG; CHEN, 2012; SARTO *et al.*, 2015), leading to a greater stem diameter and improve the photosynthetic rate. Although both straw biomass and grain yield were

not greatly affected by silicate application, stem diameter increased with silicate application, which may positive influenced nutrient uptake, as verified in this study.

Inoculation with *A. brasilense* also positive influenced nutrient uptake as verified by increased straw K and Ca uptake in 2015/16, straw N, B and Si uptake in 2016/17, grain Cu uptake in 2015/16, grain N and S uptake when associated with 200 kg N ha⁻¹ and grain Zn uptake with 50, 150 and 200 kg N ha⁻¹, both in 2015/16, respectively. The greater N uptake provided by *A. brasilense* inoculation (an increase of 9.5 and 4.5% on straw and grain uptake, in 2015/16 and 2016/17, respectively) positively influenced corn shoot development, leading to a greater grain filling and grain yield, as mentioned before. With an increased shoot development, corn plant may also improve nutrient uptake to promote greater shoot development and grain filling. In addition, both S and Zn are related to C and N metabolism (NIE *et al.*, 2018; JOBE *et al.*, 2019). Therefore, it should be expected that with an increased N uptake and corn development, nutrients that are directly involved with C and N metabolism would be increased taken up by corn.

4.4 SOIL CHEMICAL ATTRIBUTES AFTER CORN CULTIVATION (0-0.20M DEPTH)

Soil P-resin content was significantly affected by the main effects of N rates and inoculation in 2015/16, also by the main effect of liming sources in 2016/17 (Appendix A). Soil organic matter (OM) was significantly affected by the main effect of N rates in 2015/16 (Appendix A). Soil K content was significantly affected by the main effect of inoculation in both years, also by the main effect of liming sources in 2015/16 (Appendix A). Soil Ca content was significantly affected by the main effect of N rates in 2015/16 (Appendix A). Soil Mg content was significantly affected by the main effect of inoculation in 2015/16, also by the main effect of liming sources in 2016/17 (Appendix A). Soil sum of bases (SB) was significantly affected by the main effect of N rates in 2015/16 (Appendix A). Soil S content was significantly affected by the main effect of liming sources in 2016/17 (Appendix A). Soil cation exchange capacity (CEC) was significantly affected by the main effect of N rates in 2015/16 (Appendix A).

Soil H+Al content was significantly affected by the main effect of inoculation in 2016/17 (Appendix A). Soil B content was significantly affected by the main effects of

liming sources and inoculation in 2016/17 (Appendix A). Soil Cu content was significantly affected by the main effect of inoculation in 2016/17 (Appendix A). Soil Mn content was significantly affected by the main effect of liming sources in 2016/17 (Appendix A). Soil Zn content was significantly affected by the main effects of N rates and inoculation in both years, also by the main effect of liming source in 2015/16 (Appendix A). Soil Si content was significantly affected by the main effects of N rates, liming sources and inoculation in both years (Appendix A). Soil pH, base saturation (V), Al and Fe content were not significantly affected by N rates, liming sources, inoculation and their interactions (Appendix A).

Soil P-resin content was found to respond non-linearly ($P_{min} = 125 \text{ kg N ha}^{-1}$) to increasing N rates in 2015/16 (Table 15). Also in 2015/16, P-resin was greater in non-inoculated plots (Table 16). In addition, P-resin was greater when limestone was applied in 2016/17 (Table 16). Soil OM was found to respond non-linearly ($P_{min} = 165 \text{ kg N ha}^{-1}$) to increasing N rates in 2015/16 (Table 15). Soil K content was greater in inoculated plants in both years (Table 16). In addition, soil K content was greater when silicate was applied in 2015/16 (Table 16). Soil Ca content was found to respond non-linearly ($P_{min} = 102 \text{ kg N ha}^{-1}$) to increasing N rates in 2015/16 (Table 15). Soil Mg content was greater in inoculated plants in 2015/16 (Table 16). Also, soil Mg was greater when limestone was applied in 2016/17 (Table 16). Soil SB was found to respond non-linearly ($P_{min} = 95 \text{ kg N ha}^{-1}$) to increasing N rates in 2015/16 (Table 15). Soil S content was greater when limestone was applied in 2016/17 (Table 16). Soil CEC was found to respond non-linearly ($P_{min} = 93 \text{ kg N ha}^{-1}$) to increasing N rates in 2015/16 (Table 15).

Table 15. Soil P-resin, organic matter (OM), Ca, sum of bases (SB), cation exchange capacity (CEC), Zn and Si content in 0-0.20m depth as a function of N rates in corn crop.

Independent variable	2015/16 crop season
P-resin (mg dm^{-3})	$\hat{Y} = 33.1560 - 0.1494x + 0.0006x^2$ ($R^2 = 0.68^*$ and $P_{min} = 125 \text{ kg ha}^{-1}$)
OM (g dm^{-3})	$\hat{Y} = 23.0071 - 0.0330x + 0.0001x^2$ ($R^2 = 0.88^*$ and $P_{min} = 165 \text{ kg ha}^{-1}$)
Ca ($\text{mmol}_c \text{ dm}^{-3}$)	$\hat{Y} = 30.7142 - 0.0818x + 0.0004x^2$ ($R^2 = 0.92^*$ and $P_{min} = 102 \text{ kg ha}^{-1}$)
SB ($\text{mmol}_c \text{ dm}^{-3}$)	$\hat{Y} = 52.3214 - 0.1333x + 0.0007x^2$ ($R^2 = 0.77^*$ and $P_{min} = 95 \text{ kg ha}^{-1}$)
CEC ($\text{mmol}_c \text{ dm}^{-3}$)	$\hat{Y} = 87.0571 - 0.1299x + 0.0007x^2$ ($R^2 = 0.67^*$ and $P_{min} = 93 \text{ kg ha}^{-1}$)
Zn (mg dm^{-3})	$\hat{Y} = 1.4817 - 0.0073x + 0.00003x^2$ ($R^2 = 0.77^{**}$ and $P_{min} = 122 \text{ kg ha}^{-1}$)
Si (mg dm^{-3})	$\hat{Y} = 12.9475 - 0.0193x + 0.0001x^2$ ($R^2 = 0.99^*$ and $P_{min} = 97 \text{ kg ha}^{-1}$)
	2016/17 crop season
Zn (mg dm^{-3})	$\hat{Y} = 1.3007 + 0.0033x - 0.00002x^2$ ($R^2 = 0.74^{**}$ and $PM = 83 \text{ kg ha}^{-1}$)
Si (mg dm^{-3})	$\hat{Y} = 12.8000 - 0.0067x$ ($R^2 = 0.78^*$)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. P_{min} = point of minimum response to N increasing rates. PM = point of maximum response to N increasing rates.

Table 16. Soil P-resin, organic matter (OM), pH, K, Ca, Mg, sum of bases (SB), S-SO₄, cation exchange capacity (CEC), base saturation (V) in 0-0.20m depth as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

N rate	P-resin (mg dm ⁻³)		OM (g dm ⁻³)		pH (CaCl ₂)		K ----- (mmol _c dm ⁻³) -----		Ca -----	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	33.8	29.3	22.9	22.5	5.2	4.99	1.74	1.98	30.5	25.4
50	25.1	24.0	22.0	21.4	5.1	5.1	1.56	1.80	28.0	25.5
100	28.5	28.5	22.0	21.3	5.2	4.99	1.95	1.56	27.9	23.6
150	24.5	26.9	21.9	21.6	5.1	5.0	1.55	1.79	27.9	24.3
200	31.7	20.3	24.5	21.6	5.2	4.91	1.39	1.64	33.5	22.5
Liming source										
Dolomitic limestone	28.3 a†	28.4 a	22.2 a	21.9 a	5.1 a	5.0 a	1.42 b	1.82 a	29.4 a	24.8 a
Ca and Mg silicate	29.1 a	23.2 b	23.2 a	21.5 a	5.2 a	4.99 a	1.86 a	1.69 a	29.8 a	23.7 a
Inoculation										
Without <i>A. brasilense</i>	32.8 a	26.6 a	23.3 a	21.9 a	5.1 a	4.95 a	1.48 b	1.60 b	29.2 a	24.6 a
With <i>A. brasilense</i>	24.6 b	25.0 a	22.0 a	21.5 a	5.2 a	5.1 a	1.80 a	1.91 a	30.0 a	24.0 a
L.S.D. (5%)	5.6	4.75	1.40	1.63	0.13	0.18	0.23	0.24	2.87	4.52
C.V. (5%)	29.7	27.9	9.3	11.4	3.81	5.6	20.9	20.7	14.7	28.1
Overall Mean	28.7	25.8	22.7	21.7	5.2	5.0	1.64	1.75	29.6	24.3
N rate	Mg ----- (mmol _c dm ⁻³) -----		SB		S-SO ₄ (mg dm ⁻³)		CEC (mmol _c dm ⁻³)		V (%)	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	19.1	15.3	51.4	44.9	4.75	3.88	85.5	79.2	59.6	55.3
50	19.5	18.3	49.1	44.7	4.88	3.00	85.6	78.4	56.5	56.4
100	17.1	16.0	47.2	37.5	4.88	3.25	80.8	78.4	57.9	53.3
150	16.5	15.5	45.7	43.4	4.75	3.25	80.6	77.4	56.8	55.4
200	20.8	15.9	55.9	40.1	4.75	3.50	90.8	75.7	61.1	52.1
Liming source										
Dolomitic limestone	19.9 a	17.9 a	50.8 a	44.3 a	4.60 a	4.05 a	85.2 a	81.2 a	58.9 a	55.9 a
Ca and Mg silicate	17.4 a	14.5 b	49.0 a	39.9 a	5.0 a	2.70 b	84.2 a	74.4 a	57.9 a	53.1 a
Inoculation										
Without <i>A. brasilense</i>	16.9 b	16.7 a	48.0 a	41.8 a	5.0 a	3.25 a	83.3 a	80.2 a	57.6 a	53.6 a
With <i>A. brasilense</i>	20.3 a	15.7 a	51.7 a	42.4 a	4.60 a	3.50 a	86.1 a	75.5 a	59.2 a	55.4 a
L.S.D. (5%)	3.11	2.53	5.9	7.9	0.55	0.60	5.1	7.6	3.92	5.5
C.V. (5%)	25.3	23.7	17.7	28.4	17.2	26.7	9.1	14.7	10.2	15.3
Overall Mean	18.6	16.1	49.9	42.1	4.80	3.38	84.7	77.8	58.4	54.5

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Soil H+Al content was greater in non-inoculated plots in 2016/17 (Table 17). Soil B content was greater when silicate was applied in 2016/17 (Table 17). Also in 2016/17, soil B was greater in non-inoculated plants (Table 17). Soil Cu content was greater in non-inoculated plants in 2016/17 (Table 17). Soil Mn content was greater when limestone was applied in 2016/17 (Table 17). Soil Zn content was found to respond non-linearly to increasing N rates in both years (2015/16 – $P_{min} = 122 \text{ kg N ha}^{-1}$, 2016/17 – $P_{min} = 83 \text{ kg N ha}^{-1}$) (Table 17). Also in both years, soil Zn was greater in non-inoculated plots (Table 17). In addition, soil Zn was greater when limestone was applied in 2015/16 (Table 17). Soil Si content was found to respond non-linearly ($P_{min} = 97 \text{ kg N ha}^{-1}$) and decreased linearly with increasing N rates in 2015/16 and 2016/17, respectively (Table 16).

Table 17. Soil H+Al, Al, B, Cu, Fe, Mn, Zn and Si content as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

N rate	H + Al		Al		B		Cu	
	----- (mmol _c dm ⁻³) -----		----- (mg dm ⁻³) -----					
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	34.1	34.8	0.50	1.00	0.19	0.20	3.46	3.64
50	36.5	33.8	1.00	1.00	0.18	0.20	3.59	3.49
100	33.6	36.4	0.50	1.20	0.23	0.22	3.49	3.68
150	34.9	34.0	1.00	1.10	0.20	0.19	3.56	3.48
200	34.9	35.6	0.50	1.50	0.21	0.20	3.49	3.63
Liming source								
Dolomitic limestone	34.4 a†	35.3 a	0.70 a	1.08 a	0.20 a	0.19 b	3.57 a	3.60 a
Ca and Mg silicate	35.2 a	34.6 a	0.70 a	1.24 a	0.21 a	0.22 a	3.47 a	3.56 a
Inoculation								
Without <i>A. brasilense</i>	35.3 a	36.7 a	0.65 a	1.32 a	0.21 a	0.22 a	3.55 a	3.68 a
With <i>A. brasilense</i>	34.4 a	33.1 b	0.75 a	1.00 a	0.20 a	0.18 b	3.49 a	3.48 b
L.S.D. (5%)	2.91	3.29	0.43	0.55	0.04	0.03	0.13	0.17
C.V. (5%)	12.6	14.2	27.9 [#]	29.4 [#]	29.4	20.8	5.7	7.1
Overall Mean	34.8	34.9	0.70	1.16	0.20	0.20	3.52	3.58
N rate	Fe		Mn		Zn		Si	
	----- (mg dm ⁻³) -----							
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
0	25.4	29.3	41.4	34.8	1.50	1.35	13.0	12.6
50	25.1	25.1	44.5	38.3	1.13	1.33	12.3	12.8
100	25.9	25.7	41.1	38.8	1.26	1.32	12.3	11.9
150	24.6	25.3	49.3	39.1	1.13	1.40	13.1	12.1
200	25.6	26.4	43.8	38.6	1.55	0.90	14.4	11.3
Liming source								
Dolomitic limestone	26.1 a	27.1 a	45.0 a	40.5 a	1.50 a	1.29 a	12.3 b	11.3 b
Ca and Mg silicate	24.6 a	25.6 a	43.1 a	35.4 b	1.13 b	1.23 a	13.8 a	13.0 a
Inoculation								
Without <i>A. brasilense</i>	25.3 a	26.0 a	43.9 a	36.0 a	1.48 a	1.90 a	12.7 a	12.7 a
With <i>A. brasilense</i>	25.4 a	26.7 a	44.1 a	39.8 a	1.15 b	0.63 b	12.4 b	11.6 b
L.S.D. (5%)	4.45	2.98	4.55	4.16	0.19	0.14	1.11	0.76
C.V. (5%)	26.5	17.1	15.6	16.6	22.1	17.6	12.9	9.5
Overall Mean	25.3	26.3	44.0	37.9	1.31	1.26	13.0	12.1

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

With an increased nutrient uptake both in straw and grains provided by increasing N rates leading to a greater corn shoot development and grain filling, mainly in 2015/16, it should be expected that the nutrients would be greater absorbed, reducing soil fertility as verified by decreased soil P-resin, OM, Ca, SB, CEC, Zn and Si content until N application between 93 to 165 kg N ha⁻¹. As mentioned before, higher N rates application result in increased losses and less utilization by cropping systems since plant nutritional demand is limited (GALINDO *et al.*, 2016). Plants are able to absorb a certain quantity of nutrients as a function of time; the N that is applied and is not uptake may be lost, decreasing the fertilization efficiency with higher N rates and nutrient

uptake. Therefore, the application side-dressing N application must follow best management practices developed for each region. Differently, in 2016/17, increasing N rates provided greater Zn and Si content in soil. Probably, the nutrient cycling that occurs as a function of straw decomposition (corn 2015/16 – straw biomass = 12738 kg ha⁻¹, C/N ratio = 38.2 and wheat 2016 – straw biomass = 5781 kg ha⁻¹, C/N ratio = 34.6) and the fact that the study was conducted under no-tillage for the last 15 years positive influenced in Zn and Si availability.

Also, with an increased nutrient uptake provided by silicate application, mainly in straw, it should be expected that the nutrients would be greater absorbed, reducing soil fertility as verified by decreased soil Zn in 2015/16, P-resin, Mg, S and Mn content in 2016/17. However, K in 2015/16 and B content in 2016/17 increased as a function of silicate application, which could be explained because K does not form compounds in plant tissue, therefore is quickly released to the soil and because one the main source of B to plants is the OM decomposition. Thus, with a greater straw biomass production as a function of silicate application, mainly in wheat crop in 2016, it should be expected that the B availability could increase in 2016/17.

Although soil acidity components (pH, H+Al and Al) were not influenced by silicate application after corn cropping, negative Pearson's correlation between pH, H+Al and Al with soil nutrient availability, OM, SB, CEC and V reinforce the importance of liming application in agricultural systems. Also, the positive Pearson's correlation between soil Si content and corn grain yield, pH, V, SB, CEC, OM, K, Ca and Zn reinforce the need for further studies with silicate application under tropical conditions.

Similar behavior observed with silicate application was verified in soil chemical attributes as a function of inoculation with *A. brasilense*, with reduction in soil Zn and Si content in both years, P-resin in 2015/16, H+Al and B in 2016/17 and increase of K in both years and Mg in 2015/16. The increased nutrient uptake, corn shoot development and grain filling as a function of inoculation followed an increased nutrient absorption, reducing the soil fertility. In addition, soil pH is one of the main limiting factors for microorganisms survivor (WANG *et al.*, 2019). Some species of PGPB can tolerate acidity better than others, and this tolerance can vary between strains of the same species (GLICK, 2012). However, the majority of PGPB growth in an ideal pH range

between 6.0 and 7.0, and few microorganisms can grow well at a pH below 5.0 (RUFINI *et al.*, 2011). Inoculation with *A. brasilense* can break down soil pollutants, stimulate P solubilization, indole acetic acid production, 1-aminocyclopropane-1-carboxylate deaminase activity, and exudation of organic acid anions and siderophores (D' ANGIOLI *et al.*, 2017; MORA *et al.*, 2017). The produced carboxylates could form soluble complex with metallic cations (BALDOTTO; BALDOTTO, 2014). Therefore, this bacterium could alleviate Al stress by forming Al-siderophore complexes, reducing H+Al and regulating pH to close to the ideal survival range, increasing also P uptake by plant, as verified in this study.

4.5 ECONOMIC ANALYSIS

Table 18 shows the total operating costs (TOC) structure for corn crop in Selvíria, state of Mato Grosso do Sul, describing the treatment with dolomitic limestone application, in the absence of N fertilization (0 kg N ha^{-1}) and without inoculation with *A. brasilense*. This TOC structure model was used in all treatments. On average, the expenses with fertilizers, followed by mechanized operations, represented the highest costs, corresponding to 30.8 and 28.7% of the TOC, respectively. This outcome is in line with Kaneko *et al.* (2010b), who studied the corn crop viability for the northeast region of São Paulo State, in the 2007/2008 and 2008/2009 years, in a no-till system and N management up to the dose of 120 kg N ha^{-1} and found greater expenditures with fertilizers and mechanized operations, corresponding to 32.74 and 30.91% TOC, with yields ranging from 85.9 and 146.1 of 60-kg sc ha^{-1} , respectively.

Table 18. Total operating cost (TOC) structure model of corn crop for the control treatment (0 kg N ha⁻¹ in side-dressing), with limestone application and without inoculation with *A. brasilense* per hectare.

Description	Specification ¹	Times	Coefficient	Unitary Value (R\$)	Total Value (R\$)
A. OPERATIONS					
Liming sources application	HM	1.00	0.30	85.0	25.5
Weed control	HM	1.00	0.50	85.0	42.5
Hoeing (triton)	HM	1.00	0.50	85.0	42.5
Planting	HM	1.00	1.00	110	110
Pulverization	HM	2.00	0.60	85.0	102
Side-dressing	HM	1.00	0.50	85.0	42.5
Harvest	HM	1.00	0.60	118	70.8
Irrigation (pivot)	mm	1.00	120	2.50	300
Subtotal A					736
B - AGRICULTURAL INPUT					
Dolomitic limestone	Mg	1.00	0.49	85.0	41.2
Fertilizer 08-28-16	Mg	1.00	0.38	1998	749
Urea	Mg	1.00	0.00	1750	0.00
Inoculant (<i>A. brasilense</i>)	L	1.00	0.00	10.0	0.00
Corn seed DOW 2B710 PW	sc 20 kg	1.00	1.22	500	610
Glyphosate	L	1.00	4.00	14.5	58.0
2,4-D	L	1.00	1.00	13.2	13.2
Tembotrione	L	1.00	0.20	403	80.6
Atrazine	L	1.00	2.00	14.5	29.0
Vegetable oil adjuvant	L	1.00	0.78	8.4	6.6
Metomyl	L	1.00	1.00	20.6	20.6
Triflumuron	L	1.00	0.05	203	10.2
Seed treatment pyraclostrobin + thiophanate-methyl + fipronil	L	1.00	0.05	350	17.5
Subtotal B					1636
Effective operating costs (EOC)					2372
Other expenses					119
Interest cost					77.1
Total operating cost (TOC)					2568

HM = hour machine, sc = sack. 2016 and 2017 average exchange rate: R\$3.32 = U\$1.00.

Similar results were also reported by Galindo *et al.* (2017a), studying corn crop viability in Brazilian Cerrado region in 2013/2014 and 2014/2015 years with N rates and sources (0, 50, 100, 150 and 200 kg ha⁻¹, with the sources urea and urea with NBPT), with and without inoculation with *A. brasilense*. These authors found greater expenditures with fertilizers and mechanized operations, corresponding to 31.3 and

28.9% TOC, with yields ranging from 92.6 and 153.6 of 60-kg sc ha⁻¹. It should be noted that with the elevation of N rates and change of analyzed liming source, the percentage of expenses in relation to the TOC of the fertilizers tends to increase. Garcia *et al.* (2012) studying corn crop in the region of Selvíria, with different intercrops with forages, verified greater expenses related to corn seeds inputs (46.46%), followed by costs with fertilization (39.01%) and greater operating costs with irrigation via center pivot (45.28%), with yields varying from 105 to 137 of 60-kg sc ha⁻¹, using urea as the source at the N dose of 100 kg ha⁻¹.

The costs with N side-dressing application, as a function of increasing N rates, ranged from 9.2 to 23.9% of the TOC. Working with N rates (0, 45, 90, 135, and 180 kg ha⁻¹ in side-dressing) with and without inoculation with *A. brasilense*, Kaneko *et al.* (2015) verified costs with fertilization at planting in the first and second crops of 31.80 and 24.16% of the TOC, respectively, with yields ranging from 119 to 191 corn 60-kg sc ha⁻¹. Galindo *et al.* (2017a) working with N rates (0, 50, 100, 150 and 200 kg ha⁻¹) with and without inoculation with *A. brasilense* obtained costs with fertilization at planting ranged from 7.6 to 24.8% of the TOC as a function of the increasing N rates. For the costs with liming sources, the values ranged (in both crops) between 1.9 and 2.6% (limestone) and 2.1 and 2.9% (silicate).

Regarding TOC and yield (Table 19), the highest cost for the liming sources was found in the treatment with silicate application associated with 200 kg N ha⁻¹ and inoculation with *A. brasilense*. The lowest cost in TOC corresponded to the treatments with limestone application, in the absence of N fertilization in side-dressing (0 kg N ha⁻¹) and without inoculation with *A. brasilense* (Table 19). For grain yield, the highest values were verified in the treatment with limestone application associated with 200 kg N ha⁻¹ and with inoculation with *A. brasilense* in 2015/16, and with dolomitic limestone associated with 200 kg N ha⁻¹ without inoculation in 2016/17, with average yields of 208 and 164 of 60-kg sc ha⁻¹, respectively (Table 19).

Concerning gross revenue (GR) (Table 19), being the price of corn constant, the GR of the treatments followed the same trend as the yields (Table 19); that is, the increases in revenue are due to the increases in grain yield. This result is in agreement with Duete *et al.* (2009), according to these authors, the yield is a primordial factor to

ensure good profitability to the producer. Also according to Duete *et al.* (2009), even in regions where producers obtain good corn prices, if their yield is low, profitability is compromised. Thus, investment in management practices, such as balanced N fertilization, elevates the grain yield and the gross margin with corn cultivation.

Table 19. Total operating costs (TOC), grain yield and gross revenue (GR) as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

Without <i>Azospirillum brasilense</i>										
----- Dolomitic limestone -----					----- Ca and Mg silicate -----					
	2015/16	2016/17	2015/16	2016/17		2015/16	2016/17	2015/16	2016/17	
N rates	TOC	YIELD	GR		TOC	YIELD	GR			
	R\$	60-kg sc ha ⁻¹	----- (R\$) -----			60-kg sc ha ⁻¹	R\$			
0	2568	148	120	4237	3436	2578	166	106	4756	3037
50	2778	164	125	4688	3584	2788	178	120	5092	3426
100	2989	168	151	4806	4332	2999	186	142	5329	4074
150	3199	185	158	5300	4532	3209	183	133	5238	3810
200	3410	186	164	5335	4698	3420	172	147	4922	4195
Mean	2989	170	144	4873	4116	2999	177	130	5067	3708
With <i>Azospirillum brasilense</i>										
----- Dolomitic limestone -----					----- Ca and Mg silicate -----					
	2015/16	2016/17	2015/16	2016/17		2015/16	2016/17	2015/16	2016/17	
N rates	TOC	YIELD	GR		TOC	YIELD	GR			
	R\$	60-kg sc ha ⁻¹	----- (R\$) -----			60-kg sc ha ⁻¹	R\$			
0	2600	169	121	4839	3452	2610	156	151	4480	4329
50	2811	179	121	5129	3456	2821	181	141	5173	4026
100	3021	196	144	5598	4126	3031	177	157	5080	4492
150	3232	179	148	5125	4226	3242	186	158	5315	4536
200	3442	208	158	5965	4521	3452	191	155	5466	4445
Mean	3021	186	138	5331	3956	3031	178	152	5085	4366

*Average corn trading price R\$ 28.63 per 60-kg sc according to IEA (2017).

The operating profit (OP) was positive for all the evaluated treatments, regardless of N rate, liming sources, and inoculation (Table 20), agreeing with the results obtained by Galindo *et al.* (2017a), who obtained positive OP in 2013/2014 year working with N rates and inoculation with *A. brasilense* in corn crop under Savannah conditions. In 2015/16, the greater OP was obtained with limestone application associated with 100 kg N ha⁻¹ and inoculation with *A. brasilense* (R\$ 2577) (Table 20). For 2016/17, the greater OP (R\$1719) was obtained by using silicate in the absence of N fertilization and with inoculation with *A. brasilense* (Table 20). Even with N fertilization in side-dressing in high N doses up to 200 kg ha⁻¹ (which would increase costs, possibly reducing the OP), corn growing would be viable because of the high yield obtained; this reinforces the importance of N fertilization management to achieve high yields and consequently high

financial return.

Regarding the profitability index (PI), in 2015/16 the highest value was obtained with limestone application in the absence of N fertilization and with *A. brasilense* providing a PI of 46.27 % (Table 20). In 2016/17 crop, the highest value was obtained with application of silicate in the absence of N fertilization and with inoculation with *A. brasilense* provided a PI of 35.71% (Table 20). Similarly, Kaneko *et al.* (2015), obtained an average PI varying between 49.92 and 37.58, and 48.30 and 36.56 with urea source and without and with inoculation of *A. brasilense*, respectively, as a function of the N rates of 0, 45, 90, 135 and 180 kg ha⁻¹.

Table 20. Operating profit (OP) and profitability index (PI) as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

Without <i>Azospirillum brasilense</i>								
----- Dolomitic limestone -----					----- Ca and Mg silicate -----			
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
N rates	OP R\$		PI ----- (%)-----		OP R\$		PI ----- (%)-----	
0	1669	869	39.4	25.3	2178	459	45.8	15.1
50	1910	805	40.7	22.5	2303	637	45.2	18.6
100	1817	1344	37.8	31.0	2330	1075	43.7	26.4
150	2101	1333	39.6	29.4	2029	601	38.7	15.8
200	1926	1288	36.1	27.4	1502	775	30.5	18.5
Mean	1885	1128	38.7	27.1	2068	709	40.8	18.9
With <i>Azospirillum brasilense</i>								
----- Dolomitic limestone -----					----- Ca and Mg silicate -----			
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
N rates	OP R\$		PI ----- (%)-----		OP R\$		PI ----- (%)-----	
0	2239	852	46.3	24.7	1870	1719	41.7	39.7
50	2318	645	45.2	18.7	2352	1206	45.5	29.9
100	2577	1105	46.0	26.8	2049	1461	40.3	32.5
150	1893	995	36.9	23.5	2073	1294	39.0	28.5
200	2522	1079	42.3	23.9	2013	993	36.8	22.3
Mean	2310	935	43.4	23.5	2071	1335	40.7	30.6

Evaluating the accumulated operating profit (AOP), in the absence of inoculation, the greater AOP was obtained with application of 150 kg N ha⁻¹ associated with limestone (Table 21), providing R\$ 3434. When the treatments were inoculated with *A. brasilense*, the highest AOP was obtained with application of 100 kg N ha⁻¹ associated with dolomitic limestone, providing R\$ 3681 (Table 21). Analyzing the liming sources application, the greater AOP was obtained with limestone application associated with 100 kg N ha⁻¹ and with inoculation with *A. brasilense*, providing R\$ 3681 (Table 21).

Concerning the silicate application, the highest AOP was obtained in the absence of N fertilization and with inoculation with *A. brasilense*, providing R\$ 3589 (Table 21). An expressive increase in AOP was observed when inoculation with *A. brasilense* was performed, with an increase of 7.2% from the highest obtained AOP without inoculation (R\$ 3434 – 150 kg N ha⁻¹ with limestone application and without inoculation) to the treatment that provided the highest AOP with inoculation (R\$ 3681 – 100 kg N ha⁻¹ with limestone and *A. brasilense* inoculation) (Table 21). In turn, Galindo *et al.* (2017a) verified that *A. brasilense* inoculation associated with 100 kg N ha⁻¹ in side-dressing promoted greater profitability with the corn crop under the same field conditions.

Table 21. Accumulated operating profit (AOP), equilibrium price (EP) and equilibrium yield (EY) as a function of N rates, liming sources and inoculation with *A. brasilense* in corn crop.

Without <i>Azospirillum brasilense</i>								
----- Dolomitic limestone -----					----- Ca and Mg silicate -----			
		2015/16	2016/17				2015/16	2016/17
N rates	AOP R\$	EP R\$ sc ⁻¹		EY 60 kg ha ⁻¹ sc	AOP R\$	EP R\$ sc ⁻¹		EY 60 kg ha ⁻¹ sc
0	2538	17.4	21.4	89.7	2637	15.5	24.3	90.0
50	2715	17.0	22.2	97.0	2941	15.7	23.3	97.4
100	3161	17.8	19.8	104	3405	16.1	21.1	104.7
150	3434	17.3	20.2	112	2629	17.5	24.1	112.1
200	3214	18.3	20.8	119	2277	19.9	23.3	119.5
Mean	3012	17.5	20.9	104	2778	17.0	23.2	104.7
With <i>Azospirillum brasilense</i>								
----- Limestone -----					----- Silicate -----			
		2015/16	2016/17				2015/16	2016/17
N rates	AOP R\$	EP R\$ sc ⁻¹		EY 60 kg ha ⁻¹ sc	AOP R\$	EP R\$ sc ⁻¹		EY 60 kg ha ⁻¹ sc
0	3091	15.4	21.6	90.8	3589	16.7	17.3	91.2
50	2963	15.7	23.3	98.2	3558	15.6	20.1	98.5
100	3681	15.5	21.0	106	3510	17.1	19.3	106
150	2888	18.1	21.9	113	3367	17.5	20.5	113
200	3601	16.5	21.8	120	3006	18.1	22.2	121
Mean	3245	16.2	21.9	106	3406	17.0	19.9	106

The values (in R\$) of the 60-kg corn sc for the equilibrium price (EP) (minimum price to cover the TOC) are shown in Table 21. Analyzing the liming sources, the application of limestone provided lower EP compared to silicate application in both years (average of R\$16.88 and R\$21.4 for limestone and R\$16.97 and R\$21.6 for silicate, respectively) (Table 21). Concerning the N rates, in 2016 the lower EP was verified in the absence of N fertilization associated with limestone application, when inoculation with *A. brasilense* was performed (R\$ 15.5), and in 2017, the lower EP was

obtained also in the absence of N fertilization associated with silicate and inoculation with *A. brasilense* (R\$ 15.3) (Table 21). In 2016, the greater EP was verified with application of 200 kg N ha⁻¹ associated with silicate and without inoculation (R\$ 19.9) (Table 21). In 2017, the greater EP was verified in the absence of N fertilization associated with silicate and without inoculation (R\$ 24.3), respectively (Table 21). Kaneko *et al.* (2015), reported an average EP of ranging from R\$ 13.66 to R\$ 16.86 and R\$ 13.96 to R\$ 17.13 for the treatments with and without inoculation, respectively, as a function of N rates, values that are somewhat lower than those verified in the present study. However, according to Galindo *et al.* (2017a), the average EP ranged from R\$ 20.81 to R\$ 32.80 and R\$ 19.28 to R\$ 26.61 for the treatments with and without inoculation, respectively, as a function of increasing N rates, similarly to the reported results in this study.

The greater equilibrium yield (EY) (minimum yield to cover the costs) was verified with application of 200 kg N ha⁻¹ associated with silicate application and *A. brasilense* inoculation (Table 21) (121 of 60-kg sc ha⁻¹), while the lowest EY were observed in the absence of N fertilization (89.7 and 90.0 of 60-kg sc ha⁻¹ without and with *A. brasilense* inoculation, respectively), to cover the production costs. Although the lowest EY was obtained, in general, in the absence of N fertilization, it is noteworthy that if N is not properly replenished, a lack of N supply in agricultural soils will negatively affect the success of agricultural activity over time. Also, we would like to mention that little variation was observed between non-inoculated and inoculated treatments in EY, regardless of N rates and liming sources applied, which is due to the low cost of the inoculation (R\$ 10.00 per rate), which is one of the main advantages of this technique. For Kaneko *et al.* (2015), the average equilibrium yield ranged at approximated 79 of 60-kg sc ha⁻¹ with application of urea without and with inoculation, respectively, at the N rates of 0, 45, 90, 135, and 180 kg ha⁻¹. However, it is worth mentioning that a great fluctuation of prices and yields in corn producing regions as a function of the agricultural crops.

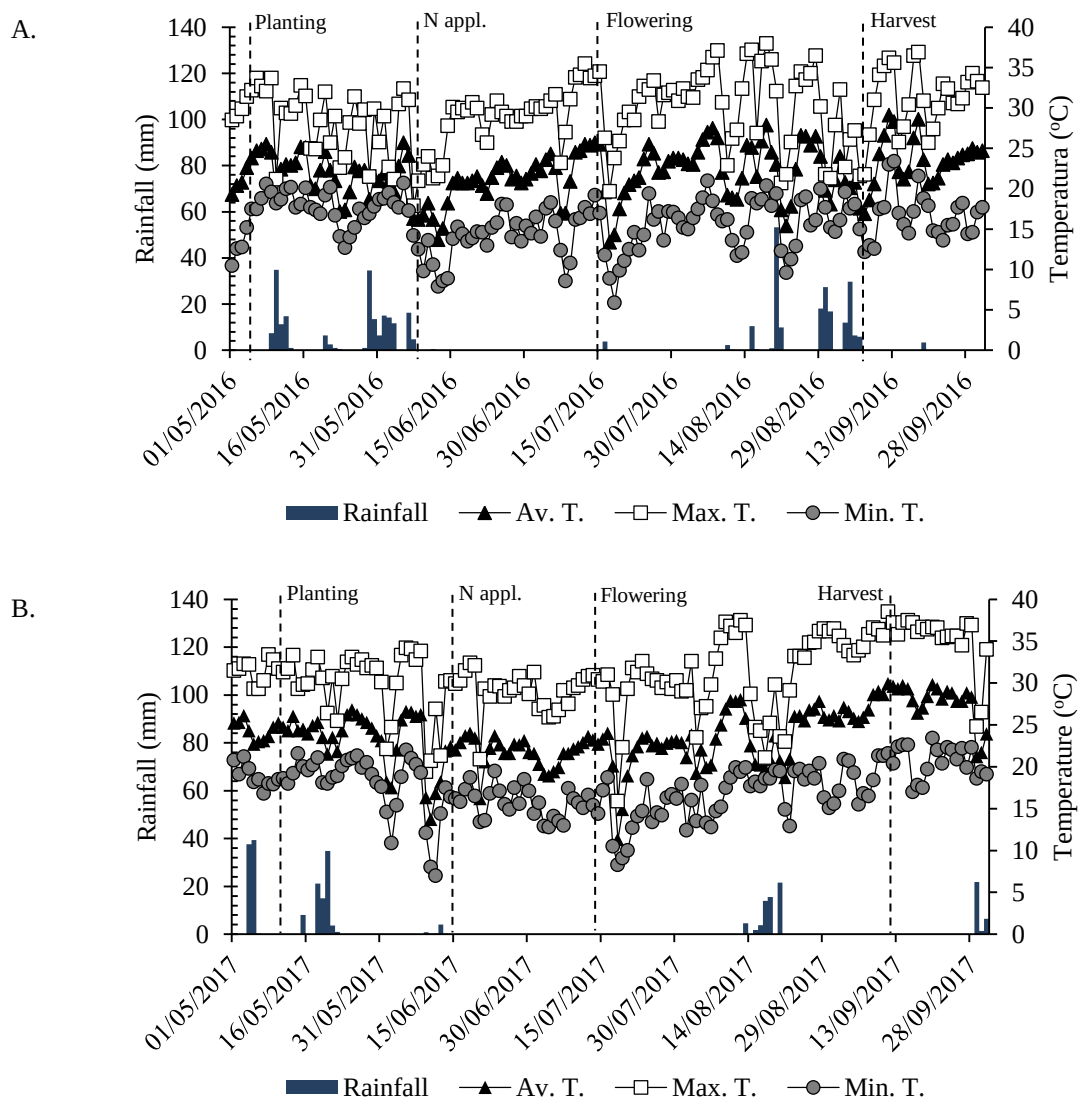
Study ii. WHEAT RESPONSE TO INOCULATION WITH *Azospirillum brasilense* ASSOCIATED WITH SILICON AND NITROGEN RATES

3 MATERIAL AND METHODS

3.1 FIELD SITE DESCRIPTION

The wheat study was conducted during the crop years of 2016 and 2017 (winter season), at the same location of corn study (same experimental plots), after corn cultivation (2015/16 and 2016/17 crop years). The maximum, average and minimum temperatures and rainfall observed during the study are presented in Figure 7.

Figure 7. Rainfall, maximum (Max. T.), average (Av. T.) and minimum (Min. T.) temperatures obtained from the weather station located in the Education and Research Farm of FE / UNESP during the wheat cultivation in the period May 2016 to October 2016 (A) May 2017 to October 2017 (B).



3.2 EXPERIMENTAL DESIGN AND TREATMENTS

The experimental design was a randomized complete block design (RCBD), with four replicates, arranged in a $5 \times 2 \times 2$ factorial scheme. There were five N rates, as urea source, applied in side-dressing (0, 50, 100, 150, and 200 kg ha⁻¹). Two liming sources; Ca and Mg silicate, which was also the Si source (the composition was 10.5% of Si, 25% CaO, and 6% MgO) with an effective neutralizing power (ENP) of 88%, and dolomitic limestone (the composition was 28% CaO and 20% MgO) with an ENP of

80%. Two inoculations were used: with or without seed inoculation with *A. brasilense*. The experimental plots were 12 wheat rows of five meters spaced at a distance of 0.17 m, and the useful area of the plot was the central eight rows, excluding 0.5 m from each end.

As mentioned before, liming sources were broadcast applied at the rate of 1.76 Mg ha⁻¹ for the silicate and 1.94 Mg ha⁻¹ for the limestone 30 days before planting corn (2015/16 - predecessor crop, seven months before wheat planting), in one single application. No incorporation was performed as the area was under no-tillage. The amount of lime applied was based on the initial soil analysis and the amount needed to increase the base saturation to 80% (Equation 1).

The *A. brasilense* strains Ab-V5 Ab-V6 were inoculated at a rate of 300 mL of liquid inoculant per 50-kg sack of seeds planted (guarantee of 2×10^8 CFU mL⁻¹). Seeds were inoculated after the seed treatment with insecticide and fungicide when the seeds were completely dry, one hour before planting. The inoculation was performed by coating and mixing the inoculant and wheat seeds in plastic bags, manually. The insecticide used was fipronil (50 g of a.i. per 100 kg of seed), and the fungicides used were thiophanate-methyl + pyraclostrobin (45 g + 5 g of a.i. per 100 kg of seed).

Nitrogen treatments (N application rates) were applied manually to evenly distribute the fertilizer on the soil surface without incorporation. The amount of fertilizer needed per plot was applied between the wheat rows on June 8, 2016, and June 15, 2017, when the plants were in the tillering - decimal growth stage GS21 (ZADOKS *et al.*, 1974). After the side-dressing N application, the experimental area was irrigated with 14 mm of water, by system center pivot sprinkler irrigation, to minimize ammonia volatilization.

3.3 FIELD MANAGEMENT

Before planting (15 days before wheat planting), weeds were controlled by the application of glyphosate (1800 g ha⁻¹ of the a.i.) and 2,4-D (670 g ha⁻¹ of the a.i.). At planting, the straw remaining from the previous crop (corn 2015/16 crop year) was collected by removing the residue from 10 random points in the experimental area

measuring 0.5 m². The residue sampled was used for chemical tests to determine nutrient accumulation (Table 22).

Table 22. Nutrient accumulation and C/N ratio in corn straw (2015/16 corn - predecessor crop).

N	P	K	Ca	Mg	S	Si	B	Cu	Fe	Mn	Zn	C/N ratio
----- (kg ha ⁻¹) -----						----- (g ha ⁻¹) -----						
116	11.1	91.0	61.0	30.0	15.0	10.3	136	266	2380	1132	239	38.2

n = 10

The wheat CD 1104 cultivar was mechanically planted on May 3 and May 10 for the 2016 and 2017 crops, respectively, at a planting density of 70 seed per meter (412 plants m⁻²). Additionally, a fertilizer application of 275 kg ha⁻¹ of the granular fertilizer 08-28-16 (N-P₂O₅-K₂O – this fertilizer also contains 0.5% of B and 1% of Zn) was performed on the entire experimental site (planted furrows) to supply phosphorus and potassium based on the soil analysis and wheat crop requirements (CANTARELLA *et al.*, 1997). During this nutrient application, 22 kg N ha⁻¹ was applied to the entire experimental area. Therefore, the total amount of N applied in each treatment was the amount of N applied in side-dressing (0 to 200 kg N ha⁻¹ as indicated above) in addition to the application of 22 kg N ha⁻¹.

Wheat plants emerged five days after planting, on May 8, 2016, and May 15, 2017, respectively. The herbicide metsulfuron-methyl (3 g ha⁻¹ of a.i.) associated with a vegetable oil adjuvant (720 g ha⁻¹ of a.i.) were used for post-emergence weed control on May 28, 2016, and June 6, 2017, respectively. The side-dressing N fertilization was performed on June 8, 2016, and June 15, 2017, as mentioned above. Harvest occurred on September 8, 2016, and September 12, 2017, which was 120 and 117 days after wheat emergence, respectively. Supplemental irrigation using a centre pivot sprinkling system was performed at intervals of approximately 72 hours during the experiment following the crop needs, at a water depth of 14 mm.

3.4 MEASUREMENTS

3.4.1 In season evaluations

During the flowering stage - decimal growth stage GS61 (ZADOKS *et al.*, 1974), Leaf chlorophyll index (LCI) was indirectly measured in 10 plants using a portable non-destructive chlorophyll meter ClorofiLOG® model CFL-1030 (FALKER, 2008). Leaf NO_3^- , NH_4^+ and N and Si concentration were determined by collecting 30 flag leaves during the flowering stage from each plot, according to Cantarella *et al.* (1997). In addition, shoot and root NO_3^- , NH_4^+ , N and Si uptake were also quantified by collecting 0.17 m² of plants per plot (1 m × 0.17 m), calculating the uptake based on shoot and root biomass, in kg ha⁻¹ (N e Si) and g ha⁻¹ (NO_3^- and NH_4^+). Nitrogen analysis followed the methodology proposed by Malavolta *et al.* (1997), while NO_3^- , NH_4^+ and Si analysis followed the methodology proposed by Silva (2009).

3.4.2 Productive components, NUE, and grain yield

Plant height was measured at maturity for 10 plants per plot. Height was measured from the ground surface to the apex of the spike. The number of spikes per meter was measured at maturity by counting spikes in 0.17 m² (0.17 m × 1.0 m) per experimental plot. At the maturity stage, 10 wheat spikes also were collected in each experimental plot for the following evaluations: spike length, distance from the apex to the base of the spike; number of defective grains, by counting the number of undeveloped grains per spike; number of spikelets, by counting all spikelets with grains; number of grains per spikelet, by counting the number of grains in each spikelet; number of grains per spikes, by counting the number of grains in each spike; hectoliter mass, corresponding to the mass of wheat grains in a 100-L container determined on a ¼ scale after adjusting the water content of the grains to 13% (wet basis); mass of 1000 grains (g), determined in precision scale 0.01 g, at 13% (wet basis); Harvest index (HI), nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use efficiency (NUE), calculated with the equations 2 to 5 respectively, and grain yield determined by

collecting the useful experimental plot area, adjusted to 13% (wet basis) and extrapolated to kg ha^{-1} .

3.4.3 Nutrient uptake in wheat straw and grain (uptake and removal)

Straw and grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake were quantified by collecting 0.17 m^2 ($0.17 \text{ m} \times 1.0 \text{ m}$) of plants per plot at harvest time, calculating the uptake based on straw and grain biomass, in kg ha^{-1} . Nitrogen, P, K, Ca, Mg, S, B, Cu, Fe, Mn and Zn analysis followed the methodology proposed by Malavolta *et al.* (1997), while Si analysis followed the methodology proposed by Silva (2009).

3.4.4 Soil chemical attributes after wheat cultivation (0-0.20m depth)

Five soil samples per plot were collected between the wheat rows soon after harvest time, in the 0-0.20m depth mixed and a random sub-sample was used to determine soil chemical attributes. After samples be collected and mixed, the sub-sample was dried in the shade and soil chemical attributes were determined according to Rajj *et al.* (2001).

3.4.5 Economic analysis

Similarly to corn, for the wheat economic analysis, the structure based on the total operating production costs (TOC) used by the IEA was adopted, according to Matsunaga *et al.* (1976). To determine the profitability of the treatments, profitability analyses were carried out following Martin *et al.* (1998). The following variables were determined: gross revenue (GR) (in R\$), operating profit (OP), accumulated operating profit (AOP), profitability index (PI), equilibrium price, and equilibrium yield. The average prices were quoted in the region of Selvíria - where the experimental site was located (average of 3 years - 2015, 2016 and 2017). In this study, simulations were performed as if each experimental treatment represented commercial crops. The cost of the wheat 60-kg sack for the region of Selvíria (average of 2015, 2016 and 2017) was R\$ 40.67

per unit produced. The cost with urea fertilizer was R\$ 1,750.00 per Mg. As regards the acidity correctives sources, the price paid by farmers was R\$ 85.00 and R\$ 115.00 per Mg for dolomitic limestone and Ca and Mg silicate in the region, respectively. For the *A. brasilense* inoculant, the expenditure was around R\$ 10.00 per dose.

3.5 STATISTICAL ANALYSIS

All data were initially tested for normality using the Shapiro; Wilk (1965) test and Levene's homoscedasticity test ($p \leq 0.05$) (LEVENE, 1960), which showed the data to be normally distributed ($W \geq 0.90$). The data was analysed by ANOVA in a 3-way factorial design with liming sources, inoculation or not with *A. brasilense* and N rates, and their interactions considered fixed effects in the model using ExpDes package. Mean separation was done when significant factors or interactions were observed using the Tukey test ($p \leq 0.05$). Regression analysis was used to discern whether there was a linear or non-linear response to N rates using R software (R CORE TEAM, 2015). The heatmap was developed by calculating the Pearson correlation ($p \leq 0.05$) using the corrplot package to evaluate the relationship among the wheat nutritional, productive components, NUE, grain yield and soil chemical attributes parameters using R software (R CORE TEAM, 2015).

4 RESULTS AND DISCUSSION

4.1 IN SEASON EVALUATIONS

Leaf chlorophyll index (LCI) was significantly affected by the main effect of inoculation, in both years, also by the main effect of N rates in 2016 and the interaction between N rates $\times$ liming sources in 2017 (Appendix B). Leaf NO_3^- concentration was significantly affected by the main effects of liming sources and inoculation in 2016, also by the interactions between N rates $\times$ liming sources and liming sources $\times$ inoculation in 2017 (Appendix B). Leaf NH_4^+ concentration years was significantly affected by the main effects of N rates in both years, and liming sources and inoculation in 2016 (Appendix B). Leaf N concentration was significantly affected by the interaction between N rates $\times$ inoculation in both years, also by the interaction between liming sources $\times$ inoculation in 2016 (Appendix B). Leaf Si was significantly affected by the main effect of liming sources in both years, also by the interaction between N rates $\times$ inoculation in 2017 (Appendix B).

Shoot NO_3^- uptake was significantly affected by the main effect of N rates in 2016, also by the main effect of liming sources in 2017 (Appendix B). Shoot NH_4^+ uptake was significantly affected by the main effect of N rates in both years, also by the main effects of inoculation in 2016 and liming sources in 2017 (Appendix B). Shoot N uptake was significantly affected by the interaction between N rates $\times$ inoculation in 2016, also by the main effect of N rates and liming sources in 2017 (Appendix B). Shoot Si uptake was significantly affected by the main effect of N rates and liming sources in both years (Appendix B). Root NO_3^- uptake was significantly affected by the main effect of N rates and liming sources in 2016 (Appendix B). Root NH_4^+ uptake was significantly affected by the main effect of N rates in 2017 (Appendix B). Root N uptake was significantly affected by the main effect of N rates in 2016 (Appendix B). Root Si uptake was significantly affected by the main effects of N rates in 2016 and liming sources in 2017 (Appendix B).

Leaf chlorophyll index was greater in inoculated plants in both years (Table 23). In 2016, LCI was found to respond linearly to increasing N rates (Table 24). In 2017, LCI was greater with silicate application when associated with 50 and 100 kg N ha⁻¹ compared to limestone application (Figure 8A). Leaf chlorophyll index was found to

respond linearly and non-linearly (up to 138 kg N ha⁻¹) to increasing N rates when limestone and silicate were applied, respectively (Figure 8A).

Table 23. Leaf chlorophyll index (LCI), leaf NO₃⁻, NH₄⁺, N and Si concentration in wheat dry matter (DM) as a function of N rates, liming sources and inoculation with *A. brasilense*.

N rate	LCI		Leaf NO ₃ ⁻ concentration		Leaf NH ₄ ⁺ concentration	
	2016	2017	2016	2017	2016	2017
0	58.4	45.5	179	158	207	197
50	58.8	50.3	174	166	222	209
100	59.8	52.5	158	160	215	204
150	60.5	52.3	170	161	228	218
200	62.1	52.4	174	164	239	227
Liming source						
Dolomitic limestone	59.1 a†	50.0	179 a	162	231 a	212 a
Ca and Mg silicate	60.7 a	51.2	163 b	161	213 b	210 a
Inoculation						
Without <i>A. brasilense</i>	58.8 b	49.9 b	180 a	165	228 a	209 a
With <i>A. brasilense</i>	61.0 a	51.3 a	162 b	159	216 b	213 a
L.S.D. (5%)	1.96	1.44	11.7	5.5	8.8	8.3
C.V. (5%)	6.3	5.4	10.4	5.1	6.0	5.9
Overall Mean	59.9	50.6	171	162	222	211
Leaf N concentration						
(g kg ⁻¹ DM)						
N rate	2016	2017	2016	2017	2016	2017
0	37.7	37.4	5.7	7.9	5.7	7.9
50	38.0	36.2	5.9	7.6	5.9	7.6
100	39.8	38.0	6.0	8.0	6.0	8.0
150	40.4	39.4	5.9	8.5	5.9	8.5
200	42.2	40.6	6.0	8.5	6.0	8.5
Liming source						
Dolomitic limestone	40.0	38.3 a	5.7 b	7.9 b	5.7 b	7.9 b
Ca and Mg silicate	39.3	38.3 a	6.0 a	8.3 a	6.0 a	8.3 a
Inoculation						
Without <i>A. brasilense</i>	39.7	37.3	5.9 a	7.6	5.9 a	7.6
With <i>A. brasilense</i>	39.5	39.3	5.9 a	8.6	5.9 a	8.6
L.S.D. (5%)	0.68	0.62	0.16	0.24	0.16	0.24
C.V. (5%)	3.30	2.46	5.2	4.41	5.2	4.41
Overall Mean	39.6	38.3	5.9	8.1	5.9	8.1

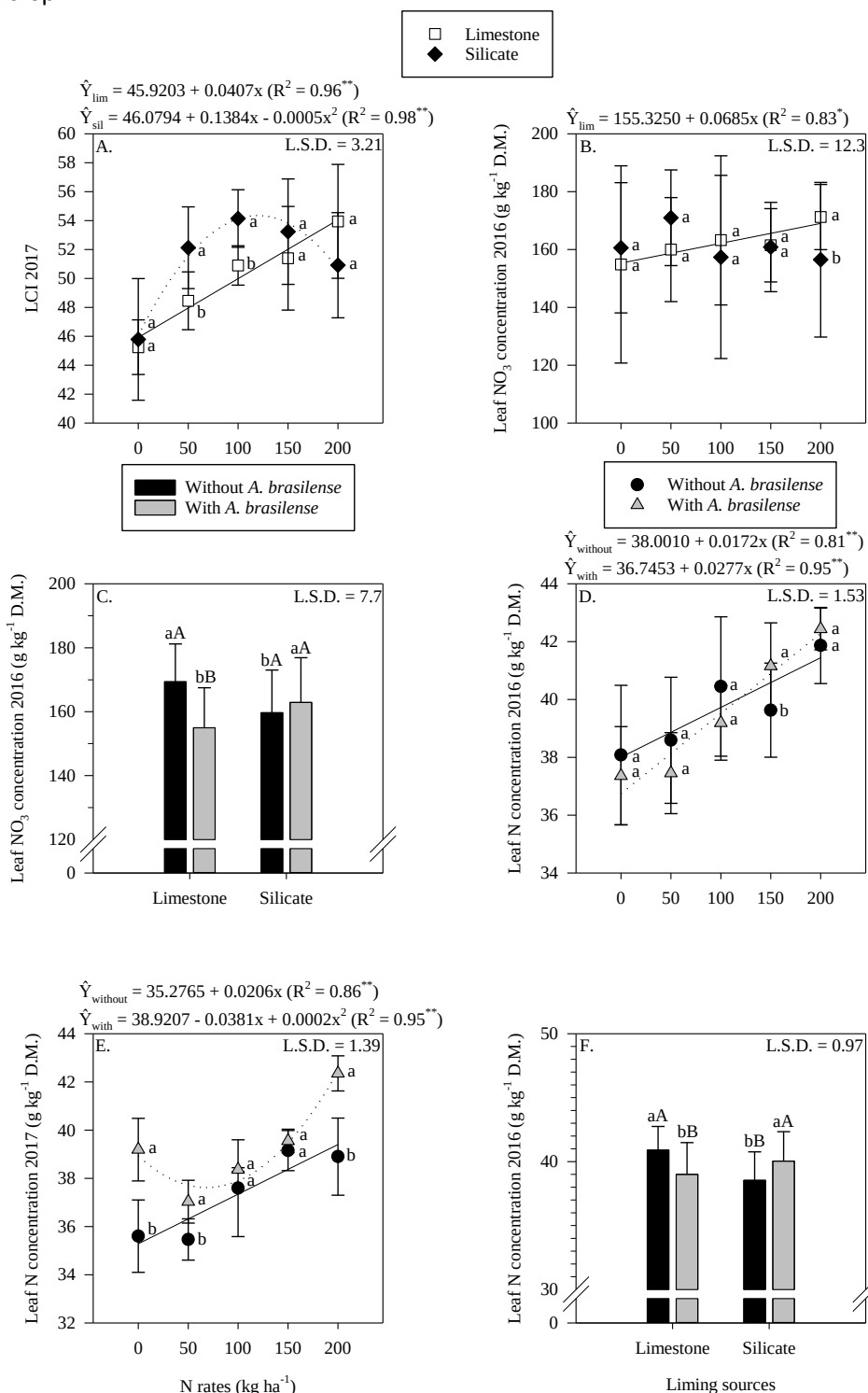
† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Table 24. Leaf chlorophyll index (LCI), leaf NH_4^+ concentration, shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rates in wheat crop.

Independent variable	2016 crop season
LCI	$\hat{Y} = 58.0986 + 0.0180x$ ($R^2 = 0.96^{**}$)
Leaf NH_4^+ concentration (mg kg^{-1})	$\hat{Y} = 208.0820 + 0.1409x$ ($R^2 = 0.80^{**}$)
Shoot NO_3^- uptake (g ha^{-1})	$\hat{Y} = 1271.7094 + 9.1193x - 0.0284x^2$ ($R^2 = 0.97^{**}$ and $\text{PM} = 161 \text{ kg ha}^{-1}$)
Shoot NH_4^+ uptake (g ha^{-1})	$\hat{Y} = 910.4210 + 2.6239x$ ($R^2 = 0.88^{**}$)
Shoot Si uptake (kg ha^{-1})	$\hat{Y} = 35.1210 + 0.0770x$ ($R^2 = 0.82^{**}$)
Root NO_3^- uptake (g ha^{-1})	$\hat{Y} = 132.8695 + 0.3891x$ ($R^2 = 0.81^*$)
Root N uptake (kg ha^{-1})	$\hat{Y} = 3.6795 + 0.0072x$ ($R^2 = 0.66^{**}$)
Root Si uptake (kg ha^{-1})	$\hat{Y} = 1.6381 + 0.0074x - 0.00002x^2$ ($R^2 = 0.99^{**}$ and $\text{PM} = 185 \text{ kg ha}^{-1}$)
	2017 crop season
Leaf NH_4^+ concentration (mg kg^{-1})	$\hat{Y} = 197.1998 + 0.1380x$ ($R^2 = 0.86^{**}$)
Shoot NH_4^+ uptake (g ha^{-1})	$\hat{Y} = 1031.4235 + 2.5499x$ ($R^2 = 0.95^{**}$)
Shoot N uptake (kg ha^{-1})	$\hat{Y} = 119.2160 + 0.2004x$ ($R^2 = 0.85^{**}$)
Shoot Si uptake (kg ha^{-1})	$\hat{Y} = 8.7547 + 0.0264x$ ($R^2 = 0.72^{**}$)
Root NH_4^+ uptake (g ha^{-1})	$\hat{Y} = 78.1615 + 0.1354x$ ($R^2 = 0.48^*$)

** and * : significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Figure 8. Interaction between N rates and liming sources in leaf chlorophyll index (LCI) in 2017 (A), leaf NO_3^- concentration in 2016 (B), interaction between liming sources and inoculation in leaf NO_3^- concentration in 2016 (C), interaction between N rates and inoculation in leaf N concentration in 2016 (D), and 2017 (E) and interaction between liming sources and inoculation in leaf N concentration in 2016 (F) in wheat crop.



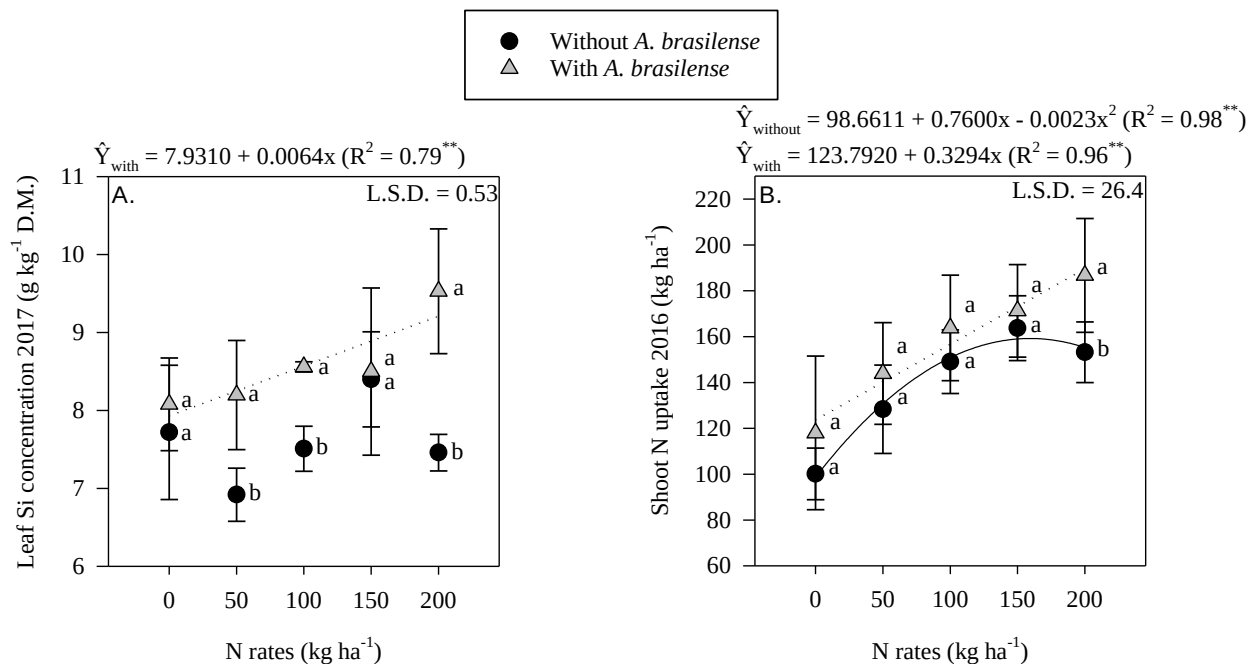
Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference. Error bars indicate the standard deviation ($n = 4$).

Leaf NO_3^- concentration was greater when limestone was applied in 2016 (Table 23). Also in 2016, leaf NO_3^- was greater in non-inoculated plots (Table 23). In 2017, leaf NO_3^- was greater with limestone application when associated with 200 kg N ha^{-1} compared to silicate application (Figure 8B). Leaf NO_3^- concentration was found to respond linearly to increasing N rates when limestone was applied (Figure 8B). In addition, leaf NO_3^- concentration was greater with limestone application in the absence of inoculation when compared to silicate application, however, leaf NO_3^- was greater with silicate application associated with *A. brasilense* compared to limestone application in 2017 (Figure 8C). Limestone provided greater leaf NO_3^- concentration in absence of inoculation compared to inoculated plants (Figure 8C). Leaf NH_4^+ concentration was found to respond linearly to increasing N rates in both years (Table 24). In addition, also in 2016, leaf NH_4^+ was greater when limestone was applied and was greater in non-inoculated plots (Table 23).

Leaf N concentration was greater in inoculated plants when associated with 150 kg N ha^{-1} compared to non-inoculated plants in 2016 (Figure 8D). Leaf N concentration was found to respond linearly to increasing N rates with and without *A. brasilense* inoculation (Figure 8D). In 2017, leaf N was greater in inoculated plants in the absence of N fertilization and associated with 50 and 200 kg N ha^{-1} compared to non-inoculated plants (Figure 8E). Leaf N responded linearly and non-linearly ($P_{\text{min}} = 96$ kg N ha^{-1}) to increasing N rates without inoculation and with *A. brasilense* inoculation, respectively (Figure 8E). In addition, leaf N concentration was greater with limestone application in the absence of inoculation when compared to silicate application, however, leaf N was greater with silicate application associated with *A. brasilense* compared to limestone application in 2016 (Figure 8F). Limestone provided greater leaf N concentration in absence of inoculation compared to inoculated plants (Figure 8F). Differently, silicate provided greater leaf N concentration when the plants were inoculated with *A. brasilense*, compared to non-inoculated plants (Figure 8F).

Leaf Si concentration was greater when silicate was applied in both years (Table 23). In 2017, leaf Si concentration was greater in inoculated plants when associated with 50, 100 and 200 kg N ha^{-1} compared to non-inoculated plants (Figure 9A). Leaf Si responded linearly to increasing N rates in inoculated plants (Figure 9A).

Figure 9. Interaction between N rates and inoculation in leaf Si concentration in 2017 (A) and shoot N uptake in 2016 (B) in wheat crop.



Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference. Error bars indicate the standard deviation ($n = 4$).

Shoot NO_3^- uptake was found to respond non-linearly (up to 161 kg N ha^{-1}) to increasing N rates in 2016 (Table 24). In addition, shoot NO_3^- uptake was greater when silicate was applied in 2017 (Table 25). Shoot NH_4^+ uptake was found to respond linearly to increasing N rates in both years (Table 24). Also, shoot NH_4^+ was greater in inoculated plants in 2016 (Table 25). In 2017, shoot NH_4^+ was greater when silicate was applied (Table 25). Shoot N uptake was greater in inoculated plants when associated with 200 kg N ha^{-1} compared to non-inoculated plants in 2016 (Figure 8B). Shoot N uptake was found to respond linearly and non-linearly (up to 165 kg N ha^{-1}) to increasing N rates with and without *A. brasilense* inoculation, respectively (Figure 8B). In 2017, shoot N uptake responded linearly to increasing N rates (Table 24). Also in 2017, shoot N uptake was greater when silicate was applied (Table 25). Shoot Si uptake was found to respond linearly to increasing N rates in both crops (Table 24). Also in both years, shoot Si was greater when silicate was applied (Table 25).

Root NO_3^- uptake was found to respond linearly to increasing N rates in 2016 (Table 24). Also in 2016, root NO_3^- was greater when limestone was applied (Table 25). Root NH_4^+ uptake was found to respond linearly to increasing N rates in 2017 (Table 24). Similarly, root N uptake responded linearly to N rates in 2016 (Table 24). Root Si uptake was found to respond non-linearly (up to 185 kg N ha^{-1}) to increasing N rates in 2016 (Table 25). In 2017, root Si was greater when silicate was applied (Table 25).

Table 25. Shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

	Shoot NO_3^- uptake		Shoot NH_4^+ uptake		Shoot N uptake		Shoot Si uptake	
	----- (g ha^{-1}) -----		----- (g ha^{-1}) -----		----- (kg ha^{-1}) -----		----- (kg ha^{-1}) -----	
N rate	2016	2017	2016	2017	2016	2017	2016	2017
0	1306	1386	839	990	109	114	35.2	8.9
50	1575	1560	1053	1225	136	138	35.9	10.2
100	1941	1427	1288	1297	156	140	47.4	12.1
150	2026	1474	1322	1360	167	142	46.6	10.6
200	1938	1727	1361	1560	170	162	49.1	15.3
Liming source								
Dolomitic limestone	1669 a†	1288 b	1106 a	1096 b	144 a	125 b	38.6 b	8.0 b
Ca and Mg silicate	1845 a	1742 a	1239 a	1477 a	151 a	154 a	47.1 a	14.8 a
Inoculation								
Without <i>A. brasilense</i>	1744 a	1562 a	1039 b	1270 a	139	133 a	41.5 a	11.5 a
With <i>A. brasilense</i>	1770 a	1468 a	1307 a	1303 a	157	146 a	44.2 a	11.3 a
L.S.D. (5%)	193	217	185	220	11.8	17.1	6.1	2.20
C.V. (5%)	16.6	21.7	23.9	25.9	12.1	18.6	21.3	29.1
Overall Mean	1757	1515	1173	1286	148	139	42.8	11.4
	Root NO_3^- uptake		Root NH_4^+ uptake		Root N uptake		Root Si uptake	
	----- (g ha^{-1}) -----		----- (g ha^{-1}) -----		----- (kg ha^{-1}) -----		----- (kg ha^{-1}) -----	
N rate	2016	2017	2016	2017	2016	2017	2016	2017
0	124	122	93.3	82.2	3.51	6.0	1.65	2.56
50	149	131	86.0	88.8	3.90	6.0	1.93	2.37
100	189	111	96.1	72.8	4.69	5.82	2.17	2.27
150	204	113	93.2	108	5.3	5.7	2.21	2.46
200	193	121	95.1	106	4.62	6.8	2.17	2.94
Liming source								
Dolomitic limestone	190 a	128 a	94 a	93.3 a	4.67 a	5.9 a	1.96 a	2.08 b
Ca and Mg silicate	154 b	111 a	91 a	90.1 a	4.14 a	6.3 a	2.09 a	2.96 a
Inoculation								
Without <i>A. brasilense</i>	159 a	114 a	91 a	89.5 a	4.14 a	5.7 a	2.03 a	2.42 a
With <i>A. brasilense</i>	185 a	125 a	94 a	93.9 a	4.67 a	6.4 a	2.02 a	2.62 a
L.S.D. (5%)	29.4	21.3	13.2	16.1	0.68	0.83	0.16	0.24
C.V. (5%)	25.8	27.0	21.5	26.6	23.3	20.6	11.9	14.1
Overall Mean	172	120	92.7	91.7	4.40	6.1	2.02	2.52

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Similarly as verified in corn crop, applied N was absorbed as verified by the increased leaf N concentration, shoot and root N uptake and LCI and favoured Si uptake, with an increased Si leaf concentration and Si shoot and root accumulation. Nitrogen is the nutrient that is most demanded by wheat plants and directly affects crop development and yield, providing building blocks for the synthesis biomolecules, such as proteins, nucleic acids and chlorophyll (CARLSSON *et al.*, 2017).

Also, silicate was effective in making Si available for wheat plants, as verified by the increased leaf Si concentration and shoot and root Si uptake. Silicon fertilization can benefit the foliar architecture of plants by improving erectness of leaves, leading to a greater light interception, reducing self-shading and lodging, postponing senescence and improving photosynthesis (MA; YAMAKI, 2008; TAMAI; MA, 2008; GONG; CHEN, 2012), as verified by an increase in LCI when the silicate was applied associated with 50 and 100 kg N ha⁻¹ in 2017. Our results showed that most of the Si that was absorbed by the plant accumulated in the leaf tissue. The accumulated Si is deposited within the leaf epidermis, following the plant transpiration flux. Inside plant epidermis, Si becomes condensed into a polymerized silica gel known as a phytolith that is immobile and makes up a protective structural layer in plant cell walls (ISA *et al.*, 2010; MCLARNON *et al.*, 2017; WALSH *et al.*, 2018). Some plant species, such as wheat, can take up and translocate large quantities of Si in the aboveground tissue due to specific Si transporters (MA; YAMAJI, 2015). Shoot Si uptake for wheat may vary between 30 and 133 kg Si ha⁻¹ depending on the Si source applied (HECKMAN, 2013; TUBANA *et al.*, 2016; WALSH *et al.*, 2018; GALINDO *et al.*, 2019c).

The N/Si ratio in the leaf tissue, shoot and root was, on average, in both seasons, 5.5, 5.3 and 2.3, respectively. The leaf N concentration was greater than the leaf Si concentration (39.6 and 38.3 for N and 5.9 and 8.1 g kg⁻¹ D.M. for Si in 2016 and 2017, respectively). However, leaf Si concentration was near the appropriate concentration range for Ca (2.5-10 g kg⁻¹ D.M.) and above the appropriate concentration for Mg (1.5-4.0 g kg⁻¹ of D.M.), which are the third and fourth nutrients, respectively, most absorbed by wheat (CANTARELLA *et al.*, 1997).

Inoculation with *A. brasilense* improved LCI, leaf N concentration and shoot NH₄⁺ uptake. Also, *A. brasilense* inoculation increased leaf N concentration while reduced

leaf NO_3^- concentration when associated with silicate application. When associated with average (50 and 100 kg N ha⁻¹) and high (200 kg N ha⁻¹) N rates, inoculation increased leaf Si concentration in 2017. Also, when associated with 200 kg N ha⁻¹, inoculation increased shoot N uptake in 2016. Recently, Pii *et al.* (2019) reported that *A. brasilense* inoculation can counteracted the nitrate uptake inducing phenomenon without affect the overall N uptake to the plant, most likely through supplementation by bacteria-derived ammonium. Therefore, these specific mechanisms could have benefited the ability of wheat plants to more efficiently explore the soil volume and consequently improve N uptake, and LCI similarly as verified in some studies using *A. brasilense* (MARTINS *et al.*, 2018; FUKAMI *et al.*, 2018a, b, c; LEITE *et al.*, 2019).

4.2 PRODUCTIVE COMPONENTS, NUE, AND GRAIN YIELD

Shoot biomass was significantly affected by the main effect of liming sources in both years, also by the main effects of N rates and inoculation in 2016 (Appendix B). Root biomass was significantly affected by the main effect of liming sources in both years, also by the main effect of N rates in 2016 (Appendix B). Number of spikes per meter was significantly affected by the main effects of N rates and inoculation in 2017 (Appendix B). Spike length was significantly affected by the main effect of N rates in both years (Appendix B). Number of defective grains was significantly affected by the main effect of inoculation in 2016 (Appendix B). Number of spikelets per spike was significantly affected by the main effects of liming sources in 2016 and N rates in 2017 (Appendix B). Number of grains per spikelet was significantly affected by the main effect of inoculation in 2016 (Appendix B).

Number of grains per spike was significantly affected by the main effect of N rates in 2017 (Appendix B). Mass of 1000 grains was significantly affected by the main effect of inoculation in both years (Appendix B). Straw biomass was significantly affected by the main effect of N rates in both years (Appendix B). Harvest index was significantly affected by the main effects of liming sources in 2016 and inoculation in 2017 (Appendix B). Grain yield was significantly affected by the interaction between N rates × inoculation in both years, also by the interaction between N rates × liming

sources in 2017 (Appendix B). Plant height and hectoliter mass were not significantly affected by N rates, liming sources, inoculation and their interactions (Appendix B).

Nitrogen uptake was significantly affected by the main effect of N rates in both years, also by the main effects of liming sources and inoculation in 2017 (Appendix B). Nitrogen utilization was significantly affected by the main effect of N rates in both years (Appendix B). Nitrogen use efficiency was significantly affected by the main effect of N rates in both years, also by the main effect of inoculation in 2017 (Appendix B).

Shoot biomass was greater when silicate was applied in both years (Table 26). In 2016, shoot biomass was found to respond linearly to increasing N rates (Table 27). Also in 2016, shoot biomass was greater in inoculated plants (Table 26). Root biomass was greater when silicate was applied in both years (Table 26). In 2016, root biomass was found to respond non-linearly (up to 138 kg N ha^{-1}) to increasing N rates (Table 27). Number of spikes per meter was found to respond linearly to increasing N rates in 2017 (Table 27). Also in 2017, spikes per meter was greater in inoculated plants (Table 27). Spike length was found to respond linearly to increasing N rates in both years (Table 27). Number of defective grains was greater in non-inoculated plants in 2016 (Table 26). Number of spikelets per spike was greater when limestone was applied in 2016 (Table 27). In 2017, spikelets per spike responded non-linearly (up to 144 kg N ha^{-1}) to increasing N rates (Table 27). Number of grains per spikelet was greater in inoculated plants in 2016 (Table 26).

Table 26. Shoot and root biomass, plant height, number of spikes per meter, spike length, number of defective grains, spikelets per spike and grains per spikelet as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

N rate	Shoot biomass		Root biomass		Plant height		Spikes per meter	
	(kg ha ⁻¹)		(m)					
	2016	2017	2016	2017	2016	2017	2016	2017
0	4796	5343	519	534	88.6	90.6	117	154
50	5529	5690	572	536	88.9	94.1	123	172
100	6076	5202	643	485	88.2	96.5	133	163
150	6441	5190	694	505	89.2	96.9	119	163
200	6181	5801	612	565	89.0	94.0	132	190
Liming source								
Dolomitic limestone	5375 b†	4831 b	566 b	489 b	88.6 a	95.8 a	123 a	171 a
Ca and Mg silicate	6234 a	6059 a	651 a	560 a	89.0 a	93.0 a	127 a	166 a
Inoculation								
Without <i>A. brasilense</i>	5434 b	5364 a	583 a	510 a	88.3 a	94.6 a	123 a	165 b
With <i>A. brasilense</i>	6175 a	5527 a	633 a	539 a	89.2 a	94.3 a	126 a	172 a
L.S.D. (5%)	706	595	70.6	39.1	1.02	4.03	13.3	7.45
C.V. (5%)	18.4	16.5	17.5	11.2	2.19	8.2	20.3	8.47
Overall Mean	5805	5445	608	525	88.8	94.4	125	168
N rate	Spike length		Defective grains		Spikelets per spike		Grains per spikelet	
	(cm)							
	2016	2017	2016	2017	2016	2017	2016	2017
0	8.2	7.7	1.05	1.33	16.7	14.9	2.38	2.65
50	8.1	7.8	0.92	1.42	16.7	15.1	2.37	2.62
100	8.3	8.1	1.12	1.17	17.0	16.1	2.30	2.62
150	8.5	8.3	0.83	1.28	17.0	16.1	2.40	2.68
200	8.5	8.2	1.08	1.53	16.9	15.8	2.43	2.55
Liming source								
Dolomitic limestone	8.4 a	8.0 a	0.94 a	1.35 a	17.1 a	15.6 a	2.41 a	2.56 a
Ca and Mg silicate	8.3 a	8.1 a	1.06 a	1.34 a	16.6 b	15.6 a	2.35 a	2.69 a
Inoculation								
Without <i>A. brasilense</i>	8.3 a	8.0 a	1.11 a	1.38 a	16.9 a	15.7 a	2.34 b	2.64 a
With <i>A. brasilense</i>	8.4 a	8.0 a	0.89 b	1.31 a	16.8 a	15.5 a	2.42 a	2.60 a
L.S.D. (5%)	0.21	0.20	0.15	0.14	0.40	0.39	0.07	0.13
C.V. (5%)	4.90	4.85	28.2	19.9	4.57	4.73	5.6	9.7
Overall Mean	8.3	8.0	1.00	1.35	16.9	15.6	2.38	2.62

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Table 27. Shoot and root biomass, number of spikes per meter, spike length, spikelets per spike, grains per spike and straw biomass as a function of N rates in wheat crop.

Independent variable	2016 crop season
Shoot biomass (kg ha ⁻¹)	$\hat{Y} = 5067.9420 + 7.3673x$ ($R^2 = 0.79^{**}$)
Root biomass (kg ha ⁻¹)	$\hat{Y} = 505.4219 + 2.2670x - 0.0082x^2$ ($R^2 = 0.87^{*}$ and PM = 138 kg ha ⁻¹)
Spike length (cm)	$\hat{Y} = 8.1363 + 0.0020x$ ($R^2 = 0.83^{**}$)
Straw biomass (kg ha ⁻¹)	$\hat{Y} = 5134.6000 + 5.1640x$ ($R^2 = 0.88^{**}$)
	2017 crop season
Spikes per meter	$\hat{Y} = 155.7166 + 0.1275x$ ($R^2 = 0.55^{**}$)
Spike length (cm)	$\hat{Y} = 7.7168 + 0.0030x$ ($R^2 = 0.82^{**}$)
Spikelets per spike	$\hat{Y} = 14.7557 + 0.0173x - 0.00006x^2$ ($R^2 = 0.83^{**}$ and PM = 144 kg ha ⁻¹)
Grains per spike	$\hat{Y} = 37.9733 + 0.0198x$ ($R^2 = 0.64^{**}$)
Straw biomass (kg ha ⁻¹)	$\hat{Y} = 4870.3430 + 5.8070x$ ($R^2 = 0.72^{**}$)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Number of grains per spike was found to respond linearly to increasing N rates in 2017 (Table 27). Mass of 1000 grains was greater in inoculated plants in both years (Table 28). Straw biomass was found to respond linearly to increasing N rates in both years (Table 27). Harvest index (HI) was greater when silicate was applied in 2016 (Table 28). In 2017, HI was greater in inoculated plants (Table 28).

Table 28. Number of grains per spike, hectoliter mass, mass of 1000 grains, straw biomass, harvest index and grain yield as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

Crop.	Grains per spike		Hectoliter mass (kg 100 L ⁻¹)		Mass of 1000 grains (g)		
	N rate	2016	2017	2016	2017	2016	2017
	0	39.9	38.0	75.7	82.7	39.3	33.9
	50	39.7	37.8	77.3	83.0	38.3	33.7
	100	39.0	41.1	74.1	82.3	38.8	34.6
	150	40.8	42.2	75.5	82.1	37.5	34.3
	200	41.1	40.8	76.8	82.2	38.1	34.2
Liming source							
	Dolomitic limestone	40.1 a†	39.2 a	76.0 a	82.5 a	39.0 a	33.9 a
	Ca and Mg silicate	40.1 a	40.8 a	75.8 a	82.5 a	37.8 a	34.4 a
Inoculation							
	Without <i>A. brasilense</i>	39.5 a	39.8 a	75.9 a	82.4 a	37.5 b	33.7 b
	With <i>A. brasilense</i>	40.7 a	40.1 a	75.8 a	82.6 a	39.3 a	34.6 a
	L.S.D. (5%)	1.38	1.77	1.80	0.30	1.50	0.49
	C.V. (5%)	6.6	8.5	5.6	0.81	9.0	2.74
	Overall Mean	40.1	40.0	75.9	82.4	38.4	34.1
		Straw biomass (kg ha ⁻¹)		Harvest index (%)		Grain yield (kg ha ⁻¹)	
	N rate	2016	2017	2016	2017	2016	2017
	0	5208	4938	42.5	50.0	3516	3559
	50	5381	4943	47.5	46.0	3906	3905
	100	5633	5815	45.3	48.1	3979	4507
	150	5687	5395	44.4	48.2	4003	4493
	200	6346	6164	41.8	48.7	3909	4210
Liming source							
	Dolomitic limestone	5349 a	5254 a	42.2 b	47.5 a	3844 a	4095
	Ca and Mg silicate	5952 a	5648 a	46.4 a	49.0 a	3881 a	4174
Inoculation							
	Without <i>A. brasilense</i>	5626	5369 a	43.9 a	44.7 b	3750	3985
	With <i>A. brasilense</i>	5676	5533 a	44.7 a	51.8 a	3975	4284
	L.S.D. (5%)	773	469	4.17	7.0	215	244
	C.V. (5%)	23.6	13.0	14.2	20.6	12.4	13.2
	Overall Mean	5651	5451	44.3	48.2	3863	4135

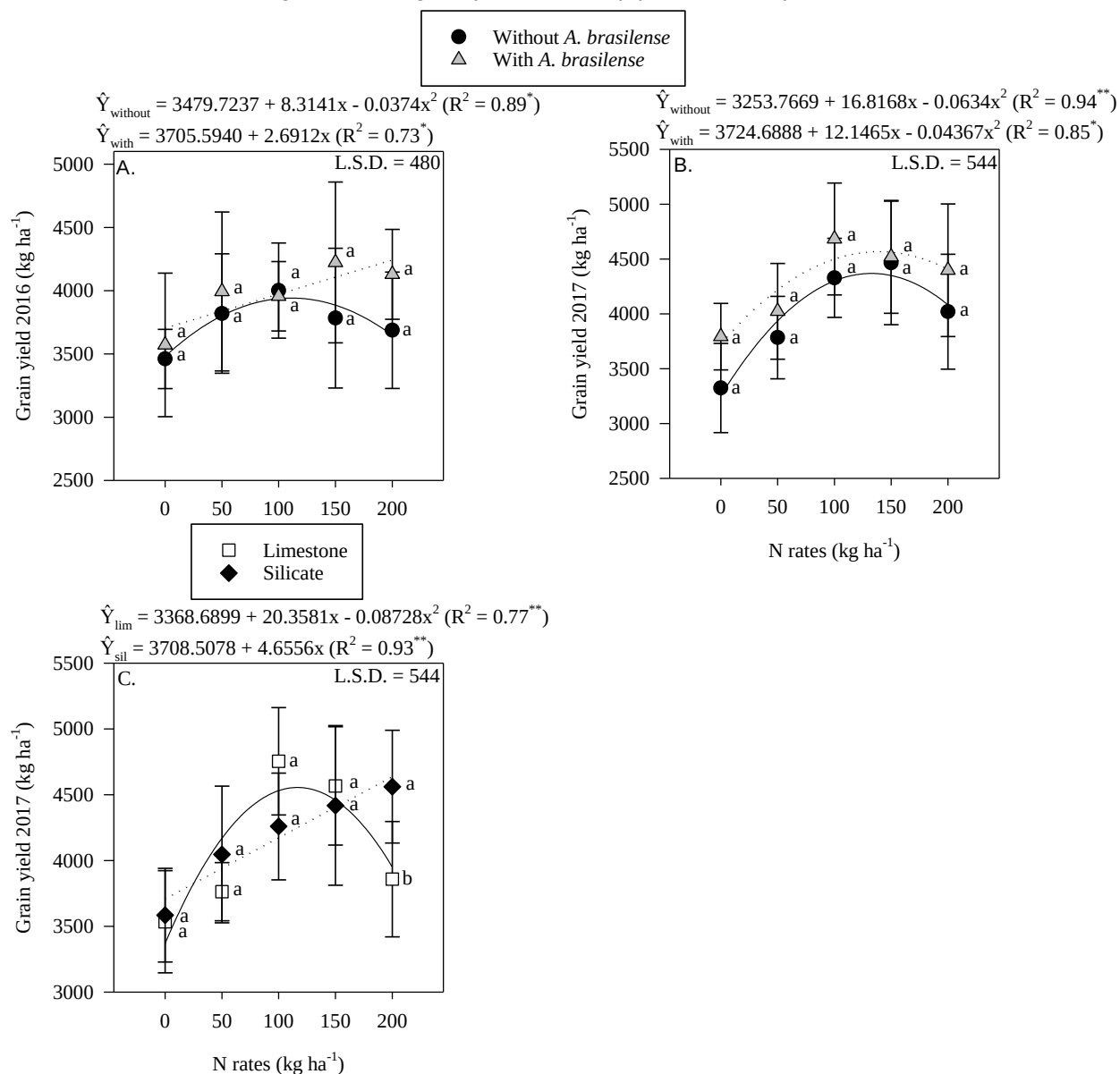
† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

The applied N rates improved wheat development, as verified by the increased shoot and root dry matter, number of spikes per meter, spike length, spikelets per spike and grains per spike. Similarly as verified in corn crop, the higher N availability with N application likely favoured root system development, leading to improved shoot development and wheat grain yield. The positive Pearson's correlations obtained in the present study between leaf N concentration and shoot and root N uptake with the productive components, NUE and grain yield support this hypothesis (Appendix D).

As mentioned before, the Si application can also affect the amount of structural plant compounds such as cellulose and lignin for different species (SCHOELYNCK *et al.*, 2010). According to Schaller *et al.* (2012), Si effect on phenol content, cellulose and lignin depending on the function of the tissue in the plant (stabilization tissue or photosynthetic tissue). Higher Si availability results in higher cellulose content in photosynthetic tissues, such as leaves and less cellulose content in tissues with stabilization function, such as culm. In this way, Si could benefit corn foliar architecture, promote erectness, improving the leaves angle and light interception, avoiding the excessive self-shading, reducing lodging, delaying senescence and increasing the structural rigidity of the tissues (MA; YAMAJI, 2008; GONG; CHEN, 2012; SARTO *et al.*, 2015). Therefore, Si application could provide greater tissue biomass, such as shoot and root biomass, as verified in this study.

Grain yield was found to respond linearly and non-linearly (up to 111 kg N ha⁻¹) to increasing N rates with *A. brasilense* and without inoculation, respectively, in 2016 (Figure 10A). In 2017, grain yield responded non-linearly (up to 133 and 139 kg N ha⁻¹) to increasing N rates without inoculation and with *A. brasilense* inoculation, respectively (Figure 10B). In addition, grain yield was greater with silicate application when associated with 200 kg N ha⁻¹ compared to limestone application (Figure 10C). Grain yield responded linearly and non-linearly (up to 117 kg N ha⁻¹) to increasing N rates when silicate and limestone were applied, respectively (Figure 10C).

Figure 10. Interaction between N rates and inoculation in grain yield in 2016 (A) 2017 (B), interaction between N rates and liming sources in grain yield in 2017 (C) in wheat crop.



Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference. Error bars indicate the standard deviation ($n = 4$).

Different responses to N application rates in side-dressing in wheat have been reported in Brazil. According to De Bona *et al.* (2016), the amount of N to be applied in side-dressing in wheat crop generally varies from 60 to 120 kg N ha⁻¹. Under irrigated conditions in low altitude Cerrado region, Teixeira Filho *et al.* (2007) verified greater wheat grain yield, mass of 100 grains, N leaf concentration, number of spikes per meter, spike length and wheat grain yield with application of 70 kg N ha⁻¹. Similarly, under the

same conditions, Alves *et al.* (2017), Melero *et al.* (2013) and Galindo *et al.* (2017b) verified greater wheat grain yield with application of 120, 122 and 140 kg N ha⁻¹, respectively.

The greater Si uptake associated with increased N absorption and LCI due to the silicate application positively influenced the shoot and root biomass and harvest index. In comparison to the limestone application, the silicate application associated with 200 kg N ha⁻¹ also resulted in a greater grain yield (an average increase of 18.2%). In addition, the silicate application did not negatively affect the inoculation with *A. brasilense* and showed greater leaf N concentration in 2016 when inoculation was performed.

Recently, the interaction of Si application and PGPB inoculation has been determined to be a sustainable strategy to enhance plant growth under sub-optimal conditions (ETESAMI, 2018). Mahmood *et al.* (2016) verified that the combined inoculation of *Bacillus drentensis* with 2 kg Si ha⁻¹ led to substantial intensification of mung bean (*Vigna radiata* (L.) Wilczek) growth, physiology, and yield under salinity-affected conditions. Galindo *et al.* (2019c) studied inoculation methods associated with Si application and reported an average wheat grain yield increase of 6.7% when seed inoculation and Si application were performed. Additionally, Ca and Mg silicate as a liming source can correct soil acidity; increase soil pH, extractable levels of Si, Ca, Mg and P; and decrease the harmful effects of toxic heavy metals such as Al, Fe and Cd (ADREES *et al.*, 2015; CASTRO *et al.*, 2016; CRUSCIOL *et al.*, 2016; ANTONANGELO *et al.*, 2017). In addition, the positive Pearson's correlation between leaf Si concentration and shoot and root Si uptake with the productive components showed that silicate application, in part, benefited wheat development (Appendix D).

In the first season, our data showed that grain yield in the inoculated plots at the higher applied N rates (150 and 200 kg N ha⁻¹) tended to be greater than that in the non-inoculated plots (11.6 and 12.0%, respectively). The plots that were inoculated showed a slower rate of response to applied N compared with plots that were not inoculated. In the second season, our data showed that grain yield in the inoculated plots in the absence and at the average and higher applied N rates (0, 100 and 200 kg N ha⁻¹) tended to be greater than that in the non-inoculated plots (14.1, 8.2 and 9.4%,

respectively). Considering the isolated effect of inoculation, the increase on wheat grain yield was approximately 225 and 299 kg ha⁻¹ in both crops, equivalent to an increase in yield of 6 and 7.5%, respectively. Therefore, our results showed an additive effect of N rates on *A. brasilense* inoculation. In the presence and absence of N application, *A. brasilense* benefited wheat development and yield.

Positive results in wheat crop with the use of *A. brasilense* were reported by Pereira *et al.* (2017), working with 60 kg N ha⁻¹ and seed inoculation with *A. brasilense*. According to the authors, inoculation resulted in an increase of 11.1% on wheat grain yield. Similar results were obtained by Nunes *et al.* (2015), where wheat yields were 7.6% higher with inoculation of *A. brasilense* when compared to control, even in an area with high N availability (the crops that preceded wheat were soybeans or carrots - soybean is a legume with a symbiotic association that allows the N₂ fixation, and the carrot, due to the high N amounts applied, leave high N residue in the soil and tend not to have the effect of N immobilization of the soil in the initial phases of succession crop). Alves *et al.* (2017) also verified increased wheat yield with seed inoculation with *A. brasilense* equivalent to 4.1%. Fukami *et al.* (2016) concluded that inoculation with *A. brasilense* can reduce by 25% the N-fertilizer application in wheat, without affecting grain yield. Galindo *et al.* (2017b), studying N rates in side-dressing (0, 50, 100, 150, and 200 kg N ha⁻¹) with and without inoculation with *A. brasilense* verified increased wheat grain yield as a function of inoculation equivalent to 3.3%, regardless the N rate applied. Additionally, Galindo *et al.* (2017b) reported an average NUE increase of 51.2% when the inoculation with *A. brasilense* was associated with N application rates varying between 50 and 200 kg ha⁻¹. Mumbach *et al.* (2017) reported an increase of wheat grain yield by 64.9% when associated with 70 kg N ha⁻¹ in side-dressing application. Munareto *et al.* (2019) verified the positive influence of side-dressing N application associated with *A. brasilense* seed inoculation in wheat and reported an average yield increase of 36.8% and 13.5% when the inoculation was associated with N application rates of 70 and 140 kg ha⁻¹, respectively.

Nitrogen uptake was found to decrease linearly to increasing N rates in both years (Table 29). In addition, NUP was greater when silicate was applied and greater in inoculated plants in 2017 (Table 30). Similarly, NUT and NUE was found to decrease

linearly to increasing N rates in both years (Table 29). Also, NUE was greater in inoculated plants in 2017 (Table 30).

Table 29. NUP, NUT and NUE as a function of N rates in wheat crop.

Independent variable	2016 crop season
NUP (kg N accumulated kg N applied ⁻¹)	$\hat{Y} = 0.5409 - 0.0020x$ ($R^2 = 0.94^{**}$)
NUT (kg grain kg N accumulated ⁻¹)	$\hat{Y} = 48.8428 - 0.1072x$ ($R^2 = 0.98^{**}$)
NUE (kg grain kg N applied ⁻¹)	$\hat{Y} = 23.9034 - 0.1052x$ ($R^2 = 0.91^{**}$)
	2017 crop season
NUP (kg N accumulated kg N applied ⁻¹)	$\hat{Y} = 0.6043 - 0.0015x$ ($R^2 = 0.78^{**}$)
NUT (kg grain kg N accumulated ⁻¹)	$\hat{Y} = 52.9393 - 0.1451x$ ($R^2 = 0.99^{**}$)
NUE (kg grain kg N applied ⁻¹)	$\hat{Y} = 25.8953 - 0.0950x$ ($R^2 = 0.93^{**}$)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates. NUP = N uptake, NUT = N utilization and NUE = N use efficiency.

Table 30. NUP, NUT and NUE as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

	NUP		NUT		NUE	
	(kg N accumulated kg N applied ⁻¹)		(kg grain kg N accumulated ⁻¹)		(kg grain kg N applied ⁻¹)	
N rate	2016	2017	2016	2017	2016	2017
0	-	-	-	-	-	-
50	0.46	0.47	44.5	46.5	20.7	19.7
100	0.29	0.52	36.7	37.0	10.6	18.8
150	0.20	0.37	32.6	31.3	7.4	11.3
200	0.15	0.26	28.0	24.2	4.23	6.3
Liming source						
Dolomitic limestone	0.28 a	0.36 b	34.1 a	36.0 a	10.6 a	13.3 a
Ca and Mg silicate	0.27 a	0.45 a	36.8 a	33.6 a	10.9 a	14.8 a
Inoculation						
Without <i>A. brasilense</i>	0.27 a	0.33 b	32.5 a	34.5 a	10.0 a	10.7 b
With <i>A. brasilense</i>	0.29 a	0.48 a	38.4 a	35.1 a	11.5 a	17.3 a
L.S.D. (5%)	0.04	0.05	6.21	3.82	2.63	2.49
C.V. (5%)	34.0	27.0	32.9	21.9	20.2#	32.4
Overall Mean	0.28	0.40	35.4	34.8	10.7	14.0

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference, C.V. = coefficient of variation, NUP = N uptake, NUT = N utilization and NUE = N use efficiency.

Nitrogen rates benefited wheat grain yield, however, the side-dressing N application must follow best management practices developed for each region. The application of excessive N rates may result in increased N losses due to low utilization by cereal crops (GALINDO *et al.*, 2016), as verified by decreased NUE with increasing N rates.

The use of Si had little effect on NUE and grain yield associated with low and average N rates (0 to 150 kg N ha⁻¹). In contrast, Detmann *et al.* (2012) and Neu *et al.* (2017) verified an increase of 2.3 and 11.5% in NUE, respectively, when Si was applied.

Some studies have reported that Si can be beneficial under stress conditions (ARTYSZAK *et al.*, 2018). For example, Guével *et al.* (2007) observed that with foliar applications of three different Si products (Kasil - a standard potassium silicate solution, with 26.5% SiO₂, Silamol - a potassium silicate solution formulated for foliar applications, with 2% of SiO₂ and 46% of K₂O and MRD-250 - a volcanic rock dust, high in Si content, with 43.2% of SiO₂) did not promote wheat growth compared to the control treatment under greenhouse conditions, except when the plants were infected by powdery mildew. The environment under greenhouse conditions is usually controlled, and plants are usually exposed to minimal or no adverse conditions (SOUSA; KORNDÖRFER, 2010). As verified in corn, the fact that adequate amounts of Si were present in the soil used for this study and the lack of a positive response to added Si suggest that little to no biotic or abiotic stress was present during the growing seasons studied. Si increased benefits and yield was associated with proline and total sugars accumulation under stress condition (CRUSCIOL *et al.*, 2009). As this research was carried out under supplementar irrigation, the stress caused by the drought was not so evident, moreover sandy soils are more responsive than clay soils to silicate application (DEMATTE *et al.*, 2011; MARAFON; ENDRES, 2013), which may explain the lack of response in the wheat grain yield.

Si application was reported to increase rice yield (SOUSA; KORNDÖRFER, 2010), corn (CASTRO; CRUSCIOL, 2013), sugarcane (REIS *et al.*, 2013), wheat (PROVANCE-BOWLEY *et al.*, 2010; SARTO *et al.*, 2015), sorghum (FLORES *et al.*, 2018), sunflower (FLORES *et al.*, 2019), peanut, soybean and common bean (CRUSCIOL *et al.*, 2013). Tropical and subtropical soils generally have low levels of Si because of desilication due to leaching and weathering processes (EPSTEIN, 2009). Silicon levels in Rhodic Haplustox (Oxisols) soils in tropical areas can be less than 1% due to the presence of extremely active desilification processes (FUJII *et al.*, 2018). Additionally, some crops (for example, sugarcane, rice, wheat, and corn) can remove large amounts of Si from soil (TUBANA *et al.*, 2016), which can significantly reduce cereal yields if not properly restored (GUNTZER *et al.*, 2012; BAKHAT *et al.*, 2018) it is estimated that annually 190–204 million Mg of Si is taken out from the world's arable soils (MEENA *et al.*, 2014). Therefore, studies conducted under tropical conditions with

Ca and Mg silicate application at soil amendment are needed to better understand the role of Si, applied alone or in combination with growth-promoting bacteria such as *A. brasilense*. In addition, it is important to point out that although it did not influence development and corn and wheat grain yield, the use of Ca and Mg silicate also did not affect crop development nor the nitrogen fertilization, even associated with N doses and inoculation with *A. brasilense* and besides being a source of Si, the silicate can neutralize the acidity and toxic Al in tropical soils.

Although many mechanisms have been described to explain the plant growth promotion by *A. brasilense*, in this context, a single mechanism, most of the times, is not responsible for the total effect (CASSÁN; DÍAZ-ZORITA, 2016). Thus, the action mode of the *A. brasilense* could be better explained by the additive hypothesis, which considers multiple mechanisms in the successful association of *A. brasilense* with plants (ZEFFA *et al.*, 2018). The productive components that were most influenced by inoculation with *A. brasilense* were shoot biomass, number of spikes per meter, grains per spikelet, mass of 1000 grains and harvest index. Additionally, inoculation increased NUE in 2017 (an increase of 61.7%) and favoured NUE in 2016 (an increase of 14.8%), reflecting greater grain filling and wheat grain yield, as verified by the increased number of grains per spikelet in 2016, the mass of 1000 grains in 2016 and the reduction in the number of defective grains in 2016. The positive Pearson's correlation between leaf N and Si concentration, shoot and root N and Si uptake with the productive components when treatments were inoculated with *A. brasilense* support this hypothesis. Therefore, our results show that *A. brasilense* seed inoculation may be a potential strategy to help improve NUE by a multiple mechanisms provided by inoculation.

According to Hungria (2011), the effects of seed inoculation affecting on grain yield depend on the genetic characteristics of plants and strains (bacteria), in addition to the environmental conditions. In this sense, the interaction between crop genotypes with efficient bacteria strains is the key fact for the success of BNF on grasses (LANA *et al.*, 2012) and can explain the variation in inoculation effects increasing corn and wheat grain yield verified in the literature.

Evidently, there is still great divergence in the use of *A. brasilense* in corn and wheat crop and even in other grasses, due to the very variable results with inoculation.

However, it is important to highlight the importance of research on the subject and the potential of using this technology, mainly because it is easy to apply and inexpensive, and with great potential to increase the efficiency of N fertilizers, since the N rates can be reduced and or better used when inoculation with *A. brasilense* is associated. In this way, this technology can allow corn and wheat cropping systems in a more sustainable way.

4.3 NUTRIENT UPTAKE IN WHEAT STRAW AND GRAIN (UPTAKE AND REMOVAL)

Straw N and P uptake were significantly affected by the main effect of N rates in 2017 (Appendix B). Straw K uptake was significantly affected by the main effects of N rates and inoculation in 2017 (Appendix B). Straw Ca and Mg uptake were significantly affected by the main effect of N rates in 2017 (Appendix B). Straw S uptake was significantly affected by the main effects of N rates and inoculation in 2017 (Appendix B).

Straw B uptake was significantly affected by the main effect of N rates in both years (Appendix B). Straw Mn and Zn uptake were significantly affected by the main effect of N rates in 2017 (Appendix B). Straw Si uptake was significantly affected by the main effect of liming sources in both years, also by the main effect of N rates in 2017 (Appendix B). Straw Cu and Fe uptake were not significantly affected by N rates, liming sources, inoculation and their interactions (Appendix B).

Grain N uptake was significantly affected by the main effect of N rates in both years, also by the main effect of liming sources in 2016 and inoculation in 2017 (Appendix B). Grain P uptake was significantly affected by the main effect of liming sources in 2016, also by the main effects of N rates and inoculation in 2017 (Appendix B). Grain K and Mg uptake were significantly affected by the main effects of N rates and inoculation in 2017 (Appendix B). Also, grain K was significantly affected by liming sources in 2017 (Appendix B). Grain S uptake was significantly affected by the main effect of N rates in both years, also by the main effect of inoculation in 2017 (Appendix B).

Grain B uptake was significantly affected by the main effect of liming sources in 2016 and N rates and inoculation in 2017 (Appendix B). Grain Cu uptake was

significantly affected by the main effect of N rates and liming sources in 2016, also by the main effect of inoculation in 2017 (Appendix B). Grain Fe uptake was significantly affected by the main effects of liming sources in 2016 and N rates in 2017 (Appendix B). Grain Mn uptake was significantly affected by the main effects of liming sources in 2016 and inoculation in 2017 (Appendix B). Grain Zn uptake was significantly affected by the main effects of N rates and inoculation in 2017 (Appendix B). Grain Si uptake was significantly affected by the main effect of liming sources in both years, also by the main effects of N rates in 2016 and inoculation in 2017 (Appendix B). Grain Ca uptake was not significantly affected by N rates, liming sources, inoculation and their interactions (Appendix B).

Straw N and P were found to respond linearly to increasing N rates in 2017 (Table 31). Straw K was found to respond non-linearly (up to 143 kg N ha⁻¹) to increasing N rates in 2017 (Table 31). Also in 2017, straw K was greater in inoculated plants (Table 32). Straw Ca and Mg uptake were found to respond linearly to increasing N rates (Table 31). Similarly, straw S uptake responded linearly to N rates in 2017. Also in 2017, straw S uptake was greater in inoculated plants (Table 32).

Straw B uptake was found to respond linearly to increasing N rates in both years (Table 31). Similarly, straw Mn and Zn uptake responded linearly to N rates (Table 31). Straw Si uptake was greater when silicate was applied in both years (Table 32). In addition, straw Si was found to respond linearly to increasing N rates in 2017 (Table 31).

Table 31. Straw N, P, K, Ca, Mg, S, B, Mn, Zn and Si uptake as a function of N rates in wheat crop.

Independent variable	2016 crop season
Straw B uptake (g ha ⁻¹)	$\hat{Y} = 47.4946 + 0.0889x$ ($R^2 = 0.79^*$)
	2017 crop season
Straw N uptake (kg ha ⁻¹)	$\hat{Y} = 22.6107 + 0.0527x$ ($R^2 = 0.79^{**}$)
Straw P uptake (kg ha ⁻¹)	$\hat{Y} = 3.1657 + 0.0036x$ ($R^2 = 0.51^{**}$)
Straw K uptake (kg ha ⁻¹)	$\hat{Y} = 28.6596 + 0.1710x - 0.0006x^2$ ($R^2 = 0.85^{**}$ and PM = 143 kg ha ⁻¹)
Straw Ca uptake (kg ha ⁻¹)	$\hat{Y} = 5.8765 + 0.0163x$ ($R^2 = 0.84^{**}$)
Straw Mg uptake (kg ha ⁻¹)	$\hat{Y} = 3.5150 + 0.0102x$ ($R^2 = 0.91^{**}$)
Straw S uptake (kg ha ⁻¹)	$\hat{Y} = 6.4255 + 0.0131x$ ($R^2 = 0.83^{**}$)
Straw B uptake (g ha ⁻¹)	$\hat{Y} = 35.7827 + 0.0418x$ ($R^2 = 0.54^*$)
Straw Mn uptake (g ha ⁻¹)	$\hat{Y} = 298.9418 + 0.5611x$ ($R^2 = 0.58^{**}$)
Straw Zn uptake (g ha ⁻¹)	$\hat{Y} = 67.7075 + 0.0791x$ ($R^2 = 0.65^{**}$)
Straw Si uptake (kg ha ⁻¹)	$\hat{Y} = 7.4075 + 0.0218x$ ($R^2 = 0.70^{**}$)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Table 32. Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

	Straw N uptake		Straw P uptake		Straw K uptake		Straw Ca uptake		Straw Mg uptake		Straw S uptake	
	----- (kg ha ⁻¹) -----											
N rate	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	33.9	22.1	3.6	3.09	35.2	29.9	14.7	6.1	5.2	3.63	4.42	6.5
50	35.3	23.8	3.76	3.31	32.8	32.6	14.0	6.1	4.65	3.73	4.27	6.7
100	38.1	31.7	4.64	3.90	35.8	41.1	14.7	8.3	5.0	4.86	4.34	8.4
150	37.2	29.6	4.39	3.32	32.9	40.7	15.2	7.8	4.9	4.80	4.41	7.9
200	38.0	32.6	4.83	3.99	39.1	36.7	18.6	9.3	5.6	5.7	5.6	9.2
Liming source												
Dolomitic limestone	34.1 a†	27.0 a	4.22 a	3.41 a	33.2 a	35.6 a	15.3 a	7.5 a	5.1 a	4.44 a	4.40 a	7.8 a
Ca and Mg silicate	39.0 a	28.7 a	4.29 a	3.63 a	37.2 a	36.8 a	15.6 a	7.6 a	5.1 a	4.63 a	4.83 a	7.8 a
Inoculation												
Without <i>A. brasilense</i>	35.4 a	27.2 a	4.15 a	3.46 a	33.6 a	32.9 b	15.3 a	7.4 a	5.0 a	4.53 a	4.51 a	7.2 b
With <i>A. brasilense</i>	37.6 a	28.6 a	4.36 a	3.58 a	36.8 a	39.5 a	15.6 a	7.6 a	5.1 a	4.54 a	4.72 a	8.2 a
L.S.D. (5%)	6.6	3.31	0.93	0.34	5.6	2.82	2.79	0.79	0.91	0.63	0.78	0.94
C.V. (5%)	27.2	17.9	30.3	14.6	24.2	11.8	27.3	16.0	27.3	21.1	25.8	18.5
Overall Mean	36.5	27.9	4.25	3.52	35.2	36.2	15.5	7.5	5.1	4.53	4.61	7.7
	Straw B uptake		Straw Cu uptake		Straw Fe uptake		Straw Mn uptake		Straw Zn uptake		Straw Si uptake	
	----- (g ha ⁻¹) -----											
N rate	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	51.3	36.8	29.5	34.3	779	718	387	296	101	67.6	38.8	7.5
50	50.2	36.1	32.3	34.6	739	722	360	302	91.3	69.1	35.4	7.6
100	51.8	42.8	38.2	33.8	796	1024	352	416	106	82.4	36.8	11.4
150	59.7	37.6	33.3	33.6	844	843	329	346	101	74.0	43.9	9.6
200	68.8	46.5	40.1	41.8	924	965	391	414	120	85.0	45.1	11.9
Liming source												
Dolomitic limestone	54.7 a	38.9 a	34.4 a	35.6 a	790 a	846 a	348 a	345 a	96.6 a	73.9 a	33.5 b	8.0 b
Ca and Mg silicate	58.1 a	41.0 a	35.0 a	35.6 a	843 a	863 a	380 a	365 a	111 a	77.3 a	46.5 a	11.2 a
Inoculation												
Without <i>A. brasilense</i>	56.0 a	39.6 a	34.4 a	34.1 a	785 a	829 a	351 a	348 a	100 a	74.9 a	37.8 a	8.9 a
With <i>A. brasilense</i>	56.7 a	40.3 a	35.0 a	37.2 a	848 a	880 a	376 a	362 a	108 a	76.3 a	42.2 a	10.3 a
L.S.D. (5%)	9.6	4.52	7.9	7.1	199	119	79.7	46.6	21.5	7.4	9.6	2.13
C.V. (5%)	25.7	17.1	29.6	29.4	32.9	21.1	31.1	19.8	29.2	14.9	29.2	29.6
Overall Mean	56.4	40.0	34.7	35.6	817	854	364	355	104	75.6	40.0	9.6

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference, C.V. = coefficient of variation.

Grain N uptake was found to respond non-linearly (up to 157 kg N ha⁻¹ and 165 kg N ha⁻¹, respectively) in 2016 and 2017 (Table 33). In 2016, grain N was greater when silicate was applied (Table 34). In 2017, grain N was greater in inoculated plants (Table 34). Grain P uptake was greater when silicate was applied in 2016 (Table 34). In 2017, grain P uptake was found to respond non-linearly (up to 152 kg N ha⁻¹) to increasing N rates (Table 33). Also in 2017, grain P uptake was greater in inoculated plants (Table 34). Grain K uptake was found to respond linearly to increasing N rates in 2017 (Table 33). Also in 2017, grain K uptake was greater when silicate was applied and greater in inoculated plants (Table 34). Similarly, grain Mg uptake responded linearly to N rates (Table 33) and was greater in inoculated plots, both in 2017 (Table 34). Grain S uptake was found to respond linearly to increasing N rates in both years (Table 33). In addition, grain S uptake was greater in inoculated plants in 2017 (Table 34).

Grain B uptake was greater when silicate was applied in 2016 (Table 34). In 2017, grain B uptake was found to respond non-linearly (up to 179 kg N ha⁻¹) to increasing N rates (Table 33). Also in 2017, grain B uptake was greater in inoculated plots (Table 34). Grain Cu uptake was found to respond linearly to increasing N rates in 2016 (Table 33). Also in 2016, grain Cu uptake was greater when silicate was applied (Table 34). In 2017, grain Cu was greater in inoculated plants (Table 34). Grain Fe was greater when silicate was applied in 2016 (Table 34). In 2017, grain Fe uptake was found to respond linearly to increasing N rates (Table 33). Grain Mn was greater when silicate was applied in 2016 (Table 34). In 2017, grain Mn uptake was greater in inoculated plants (Table 34). Grain Zn uptake was found to respond non-linearly (up to 139 kg N ha⁻¹) to increasing N rates in 2017 (Table 33). Grain Zn uptake was greater with silicate application in 2016 and in inoculated plants in 2017 (Table 34). Grain Si uptake was greater when silicate was applied in both years (Table 34). In 2016, grain Si uptake was found to respond linearly to increasing N rates (Table 33). In 2017, grain Si was greater in inoculated plots (Table 34).

Table 33. Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Zn and Si uptake as a function of N rates in wheat crop.

Independent variable	2016 crop season
Grain N uptake (kg ha ⁻¹)	$\hat{Y} = 103.2773 + 0.2834x - 0.0009x^2$ ($R^2 = 0.82^{**}$ and PM = 157 kg ha ⁻¹)
Grain S uptake (kg ha ⁻¹)	$\hat{Y} = 9.3161 + 0.0112x$ ($R^2 = 0.81^*$)
Grain Cu uptake (g ha ⁻¹)	$\hat{Y} = 24.6095 + 0.0558x$ ($R^2 = 0.90^{**}$)
Grain Si uptake (kg ha ⁻¹)	$\hat{Y} = 4.0440 + 0.0040x$ ($R^2 = 0.56^*$)
	2017 crop season
Grain N uptake (kg ha ⁻¹)	$\hat{Y} = 86.3432 + 0.5926x - 0.0018x^2$ ($R^2 = 0.93^{**}$ and PM = 165 kg ha ⁻¹)
Grain P uptake (kg ha ⁻¹)	$\hat{Y} = 18.2198 + 0.0913x - 0.0003x^2$ ($R^2 = 0.89^{**}$ and PM = 152 kg ha ⁻¹)
Grain K uptake (kg ha ⁻¹)	$\hat{Y} = 83.3444 + 0.1622x$ ($R^2 = 0.77^{**}$)
Grain Mg uptake (kg ha ⁻¹)	$\hat{Y} = 5.3417 + 0.0071x$ ($R^2 = 0.62^{**}$)
Grain S uptake (kg ha ⁻¹)	$\hat{Y} = 5.7370 + 0.0113x$ ($R^2 = 0.69^{**}$)
Grain B uptake (g ha ⁻¹)	$\hat{Y} = 36.5207 + 0.1787x - 0.0005x^2$ ($R^2 = 0.86^{**}$ and PM = 179 kg ha ⁻¹)
Grain Fe uptake (g ha ⁻¹)	$\hat{Y} = 300.3145 + 0.7574x$ ($R^2 = 0.65^*$)
Grain Zn uptake (g ha ⁻¹)	$\hat{Y} = 68.6469 + 0.3623x - 0.0013x^2$ ($R^2 = 0.74^{**}$ and PM = 139 kg ha ⁻¹)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. PM = point of maximum response to N increasing rates.

Table 34. Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

Heat crop:	Grain N uptake		Grain P uptake		Grain K uptake		Grain Ca uptake		Grain Mg uptake		Grain S uptake	
	----- (kg ha ⁻¹) -----											
	N rate	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016
0	101	90.0	23.6	18.8	100	84.1	2.16	1.30	7.65	5.16	8.82	5.52
50	121	103	29.0	20.5	111	85.1	2.47	1.26	9.46	5.45	10.54	5.88
100	119	130	25.7	25.2	111	110	2.00	1.42	8.52	6.57	10.50	7.73
150	121	138	24.2	24.8	105	102	2.35	1.59	8.37	6.82	10.83	7.80
200	123	127	25.9	23.4	101	116	2.17	1.34	8.31	6.26	11.48	7.40
Liming source												
Dolomitic limestone	113 b†	113 a	24.4 b	22.3 a	104 a	91.6 b	2.18 a	1.41 a	8.5 a	6.2 a	10.0 a	6.6 a
Ca and Mg silicate	121 a	122 a	27.0 a	22.8 a	105 a	108 a	2.28 a	1.36 a	8.4 a	6.0 a	10.8 a	7.1 a
Inoculation												
Without <i>A. brasilense</i>	115 a	108 b	25.4 a	20.1 b	103 a	94.1 b	2.22 a	1.29 a	8.5 a	5.5 b	9.9 a	6.4 b
With <i>A. brasilense</i>	119 a	127 a	26.0 a	24.9 a	106 a	105 a	2.24 a	1.47 a	8.5 a	6.6 a	11.0 a	7.3 a
L.S.D. (5%)	7.1	13.7	1.81	2.19	9.4	10.6	0.51	0.34	0.94	0.68	1.17	0.87
C.V. (5%)	9.2	17.6	10.7	14.7	13.6	16.1	30.7	32.5	16.9	17.0	17.0	19.2
Overall Mean	117	118	25.7	22.5	105	99.6	2.23	1.38	8.5	6.1	10.4	6.9
	Grain B uptake		Grain Cu uptake		Grain Fe uptake		Grain Mn uptake		Grain Zn uptake		Grain Si uptake	
	----- (g ha ⁻¹) -----											
N rate	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	24.4	37.9	62.4	92.5	363	273	319	231	54.2	72.3	3.88	2.33
50	35.0	40.4	76.8	94.7	379	331	360	241	67.6	74.6	4.61	1.95
100	27.7	51.2	73.9	120	406	417	370	292	59.8	95.3	4.36	3.01
150	38.4	50.4	84.8	106	393	461	340	304	60.1	95.1	4.31	3.02
200	32.3	48.7	94.7	107	420	398	386	263	60.6	83.7	5.04	2.55
Liming source												
Dolomitic limestone	27.3 b	44.2 a	66.4 b	101 a	341 b	393 a	316 b	260 a	52.4 b	82.3 a	3.84 b	1.95 b
Ca and Mg silicate	35.8 a	47.3 a	90.6 a	107 a	443 a	359 a	394 a	272 a	68.5 a	86.1 a	5.04 a	3.19 a
Inoculation												
Without <i>A. brasilense</i>	30.4 a	41.9 b	76.5 a	92.0 b	371 a	367 a	352 a	240 b	60.3 a	74.8 b	4.22 a	2.30 b
With <i>A. brasilense</i>	32.7 a	49.6 a	80.5 a	116 a	414 a	385 a	358 a	293 a	60.6 a	93.6 a	4.65 a	2.84 a
L.S.D. (5%)	4.43	4.61	15.16	19.0	63.2	80.8	44.4	38.7	7.60	7.7	0.55	0.48
C.V. (5%)	21.3	15.3	29.2	27.5	24.4	30.5	18.9	22.0	19.0	13.7	19.0	28.7
Overall Mean	31.5	45.7	78.5	104	392	376	355	266	60.5	84.2	4.44	2.57

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference, C.V. = coefficient of variation

Similarly as verified in corn crop, N application rates positive influenced nutrient uptake as verified by increased straw N, P, K, Ca, Mg, S, B, Fe, Mn, Zn and Si uptake and grain N, P, K, Mg, S, B, Cu, Fe, Zn and Si uptake. As mentioned before, N is the nutrient that is most demanded by wheat plants and directly affects crop development and yield. The higher N availability as a function of the N application likely favoured the development of a well-established root system leading to improved uptake of water and nutrients. Increased root biomass can positively influence root scavenging, which is important for the interception of nutrients in crop systems. Based on two years of cultivation, the average nutrient uptake in the wheat straw and grain in descending order was $K > N > Si > Ca > S > Mg > P > Fe > Mn > Zn > B > Cu$ for straw and $N > K > P > S > Mg > Si > Ca > Fe > Mn > Cu > Zn > B$ for grains.

The increased uptake of macronutrients as a function of the increasing N rates is very interesting for wheat nutrition since P plays an important role in the composition of ATP. It is responsible for the storage and transport of energy for endergonic processes, such as the synthesis of organic compounds and the active uptake of nutrients (MARSHNER, 2012). Also, P levels in the Brazilian Savannah soil solution are generally low, making it necessary to apply large amounts of phosphate fertilizer to meet the crop demands. Potassium can participate in osmoregulation (control of salt concentrations in tissues or cells) and resistance of the wheat plant to the dry matter. Potassium also acts in important functions such as grain filling and final product quality (BARKER; PILBEAM, 2015). Calcium can contribute to the formation and growth of the plant root system, since this nutrient is essential in the synthesis of new cells of the root apical region (meristems) when acting on the composition of the cell wall structure (MARSHNER, 2012). Magnesium is essential for conformational stabilization of macromolecules such as nucleic acids, proteins, cell membranes, and walls, maintenance of enzyme activities such as of H_p-ATPase, kinases, and polymerases and homeostasis of reactive oxygen species (ROS) under Al stress. Also, Mg is involved in protein synthesis and associated with chlorophyll pigments, acting mainly as a cofactor of a series of enzymes involved in photosynthetic carbon fixation and metabolism (GUO *et al.*, 2016). Adequate S contents in plant are very important for the success of the wheat crop because this nutrient increases the N use efficiency (protein and amino acids synthesis such as cystine, cysteine, and methionine) and grain quality due to increased gliadin and

glutenin proteins, which give gluten protein the viscosity and elasticity necessary for the development and formation of a dough with a good capacity to withstand fermentation (retention of carbon dioxide) and kneading (WIESER, 2007).

Also, the increased uptake of micronutrients as a function of N rates is very interesting for wheat nutrition since B is related to several plant physiological processes such as sugar transport, cell wall synthesis, lignification, cell wall structuring, carbohydrate metabolism, RNA metabolism, respiration, indole acetic acid (IAA) metabolism, phenol metabolism, ascorbate metabolism, and plasma membrane integrity (BELL; DELL, 2008; SONGKHUM *et al.*, 2018), as well as pollen grain germination and tube elongation. Also, this element is indirectly responsible for activation of dehydrogenase enzymes, sugar translocation, nucleic acids, and plant hormones (EL-SHEIKH *et al.*, 2007; MARSCHNER, 2012). Copper in the plant is fundamental for the formation of pollen and for the biosynthesis of chlorophyll and the cell wall (lignification) of the plant. The deficiency of this micronutrient causes sterility of pollen in the ear, which leads to poor grain formation and loss of productivity (BARKER; PILBEAM, 2015). The Fe essentiality to plants is proven by action on the metabolism of proteins of heme and non-heme structure or in the coordination of protein structures, and these compounds act as enzymes (for example, catalases, peroxidases) or as electron carriers in the process of photosynthesis (ferredoxin, cytochrome) and in the metabolic routes of assimilation of other nutrients (N and S) in the plant (BARKER; PILBEAM, 2015). Manganese is an essential element in plants and participates in the structure of phosphorylating proteins and enzymes. Its deficiency causes damage to chloroplasts, affecting the photolysis of water in photosystem II (SANTOS *et al.*, 2017b), which provides the electrons necessary for photosynthesis (FERNANDO; LYNCH, 2015). In cereals, such as wheat, Mn deficiency affects grain quality and yield, because the deficiency of this nutrient causes a reduction in the number and mass of the grains produced, possibly associated to the combination of low fertility pollen grains and decreased carbohydrate supply for the grain filling process (MARSCHNER, 2012). Zinc is one of the most important micronutrients for the growth and development of higher plants. It is involved in completing many vital physiological functions such as protein synthesis, energy production and maintenance of membrane integrity (DRISSI *et al.*, 2017; HANSCH; MENDEL, 2009). One of the most important functions of Zn in the plant is to participate as a component of a large number of enzymes such as

dehydrogenases, proteinases, peptidases, and phosphohydrolases, and the basic functions in the plant are related to the metabolism of carbohydrates, proteins, and phosphates and in the formation of the structure of auxins, RNA, and ribosomes (MARSCHNER, 2012; OJEDA-BARRIOS *et al.*, 2014). Zn is also related to the metabolism of phenols, formation of starch, increase in cell size and cell multiplication, and fertility of the pollen grains (DAVARPANA *et al.*, 2016; MARSCHNER, 2012).

The silicate application improved nutrient uptake as verified by greater shoot and grain Si uptake in both years, grain N, P, B, Cu, Fe, Mn and Zn uptake in 2015/16, also, greater grain K uptake in 2016/17. The positive Pearson's correlations verified between the shoot and grain Si uptake and the other nutrients uptake (Appendix D) support this hypothesis. Also, Si was the third and sixth element most absorbed in wheat straw and grain respectively, reinforcing the importance of this element in wheat nutrition. As mentioned before, with greater plant architecture provided by Si application, leading to a greater shoot and root biomass and LCI may positive influenced grain nutrient uptake, even without an increase in wheat grain yield as a function of silicate application.

Inoculation with *A. brasilense* also positive influenced nutrient uptake, mainly in 2017 as verified by increased straw K and S uptake in 2017 and Grain N, P, K, Mg, S, B, Cu, Mn, Zn and Si uptake, also in 2017. The greater N uptake provided by *A. brasilense* inoculation (an increase of 5.8 and 8.9% on straw and grain uptake, in both years, respectively) positively influenced wheat shoot development, leading to a greater grain filling and grain yield, as mentioned before. With an increased shoot development, wheat plant may also improve nutrient uptake to promote greater shoot development and grain filling. To be a free-living bacteria, with endophytic characteristics, it is possible that *A. brasilense* performed some of the metabolic and vital process use of nutrients in the plant (GALINDO *et al.*, 2017b), which would then be made available to reflect in increased uptake in grains. *Azospirillum brasilense* inoculation can improve the uptake of several macro- and micronutrients, which in turn increases a plant's efficiency at using available nutrients (GALINDO *et al.*, 2019d, 2020).

4.4 SOIL CHEMICAL ATTRIBUTES AFTER WHEAT CULTIVATION (0-0.20M DEPTH)

Soil P-resin and OM content was significantly affected by the main effect of N rates in 2016 and 2017, respectively (Appendix B). Soil pH was significantly affected by the main effects of liming sources and inoculation in 2017 (Appendix B). Soil K content was significantly affected by the main effect of N rates in 2016 (Appendix B). Soil S content was significantly affected by the main effect of inoculation in 2017 (Appendix B). Soil V was significantly affected by the main effects of liming sources and inoculation in 2017 (Appendix B).

Soil H+Al and Al content were significantly affected by the main effects of liming source, initially applied in 2015, and by inoculation in 2017 (Appendix B). Soil Fe content was significantly affected by the main effect of inoculation in both years (Appendix B). Soil Mn content was significantly affected by the main effect of inoculation in 2017 (Appendix B). Soil Si content was significantly affected by the main effect of liming sources in both years (Appendix B). Soil Ca, Mg, SB, CEC, B, Cu and Zn content were not significantly affected by N rates, liming sources, inoculation and their interactions (Appendix B).

Soil P-resin content was found to respond non-linearly (up to 123 kg N ha⁻¹) to increasing N rates in 2016 (Table 35). Similarly, OM was found to respond non-linearly (up to 136 kg N ha⁻¹) to increasing N rates in 2017 (Table 35). Soil pH was greater when silicate was applied in 2017 (Table 36). Also in 2017, pH was greater in inoculated plants (Table 36). Soil K content was found to respond non-linearly (up to 70 kg N ha⁻¹) to increasing N rates in 2016 (Table 35). Soil S content was greater in non-inoculated plots in 2017 (Table 36). Soil V was greater when silicate was applied in 2017 (Table 35). Also in 2017, V was greater in inoculated plants (Table 36).

Table 35. Soil P-resin, K and OM in 0-0.20m depth as a function of N rates in wheat crop.

Independent variable	2016 crop season
P-resin (mg dm ⁻³)	$\hat{Y} = 20.9392 + 0.1969x - 0.0008x^2$ ($R^2 = 0.83^*$ and PM = 123 kg ha ⁻¹)
K (mmol _c dm ⁻³)	$\hat{Y} = 2.2742 + 0.0042x - 0.00003x^2$ ($R^2 = 0.62^*$ and PM = 70 kg ha ⁻¹)
	2017 crop season
OM (g dm ⁻³)	$\hat{Y} = 22.5392 + 0.0271x - 0.0001x^2$ ($R^2 = 0.98^*$ and PM = 136 kg ha ⁻¹)

** and *: significant at $p < 0.01$ and $p < 0.05$, respectively. OM = organic matter, PM = point of maximum response to N increasing rates.

Table 36. Soil P-resin, organic matter (OM), pH, K, Ca, Mg, sum of bases (SB), S-SO₄, cation exchange capacity (CEC), base saturation (V) in 0-0.20m depth as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

N rate	P-resin (mg dm ⁻³)		OM (g dm ⁻³)		pH (CaCl ₂)		K ----- (mmol _c dm ⁻³) -----		Ca -----	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	21.3	26.6	22.3	22.5	5.1	4.89	2.36	1.87	26.9	22.9
50	27.1	25.5	22.8	23.6	5.2	5.2	2.26	1.98	30.0	26.1
100	35.6	25.7	23.0	24.0	4.95	4.80	2.27	1.73	26.9	24.3
150	29.9	26.2	21.3	23.5	5.1	4.92	2.46	1.98	26.5	23.7
200	28.8	24.4	21.4	22.8	4.97	4.77	1.71	1.77	26.6	22.1
Liming source										
Dolomitic limestone	26.7 a†	27.4 a	21.8 a	23.0 a	5.0 a	4.8 b	2.22 a	1.85 a	26.4 a	22.5 a
Ca and Mg silicate	30.4 a	24.0 a	22.5 a	23.6 a	5.0 a	5.1 a	2.20 a	1.89 a	28.4 a	25.2 a
Inoculation										
Without <i>A. brasilense</i>	27.9 a	26.8 a	22.1 a	23.0 a	5.0 a	4.81 b	2.16 a	1.90 a	27.3 a	22.2 a
With <i>A. brasilense</i>	29.2 a	24.6 a	22.2 a	23.6 a	5.0 a	5.0 a	2.27 a	1.84 a	27.5 a	25.5 a
L.S.D. (5%)	5.3	3.71	1.14	1.03	0.11	0.19	0.28	0.22	2.70	3.95
C.V. (5%)	28.2	21.8	7.8	6.7	3.35	6.1	19.7	18.4	14.9	25.1
Overall Mean	28.5	25.7	22.1	23.3	5.0	4.91	2.21	1.87	27.4	23.8
N rate	Mg ----- (mmol _c dm ⁻³) -----		SB -----		S (mg dm ⁻³)		CEC (mmol _c dm ⁻³)		V (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
0	16.3	15.1	45.5	39.9	3.75	4.20	78.9	75.7	57.5	46.2
50	19.0	17.0	51.3	45.1	4.50	4.20	83.3	75.8	60.6	52.3
100	15.5	14.7	44.7	40.7	4.12	3.50	80.9	77.2	54.9	46.2
150	16.4	14.7	45.3	40.4	3.50	3.58	78.0	75.6	58.0	46.5
200	17.0	14.0	45.3	37.9	4.00	3.85	80.5	75.4	56.1	43.9
Liming source										
Dolomitic limestone	17.6 a	16.1 a	46.2 a	40.5 a	4.00 a	3.85 a	80.27 a	78.8 a	57.3 a	44.7 b
Ca and Mg silicate	16.1 a	14.1 a	46.6 a	41.1 a	3.95 a	3.88 a	80.30 a	73.1 a	57.5 a	49.4 a
Inoculation										
Without <i>A. brasilense</i>	16.7 a	14.1 a	46.2 a	38.2 a	4.05 a	4.16 a	80.8 a	74.9 a	56.5 a	44.7 b
With <i>A. brasilense</i>	17.0 a	16.1 a	46.7 a	43.4 a	3.90 a	3.57 b	79.8 a	77.0 a	58.3 a	49.4 a
L.S.D. (5%)	2.44	2.48	4.88	6.3	0.85	0.51	4.02	6.8	3.60	3.92
C.V. (5%)	21.9	24.9	15.9	23.2	29.4	20.3	7.6	13.5	9.5	12.6
Overall Mean	16.8	15.1	46.4	40.8	3.97	3.86	80.3	75.9	57.4	47.0

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Soil H+Al and Al content were greater when limestone was applied in 2017 (Table 37). Also in 2017, H+Al content was greater in non-inoculated plants (Table 37). Soil Fe content was greater in inoculated plants in both years (Table 37). Similarly, soil Mn content was greater in inoculated plants in 2017 (Table 37). Soil Si content was greater when silicate was applied in both years (Table 37).

Table 37. Soil H+Al, Al, B, Cu, Fe, Mn, Zn and Si content in 0-0.20m depth as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

N rate	H + Al		Al		B		Cu	
	(mmol _c dm ⁻³)				(mg dm ⁻³)			
	2016	2017	2016	2017	2016	2017	2016	2017
0	33.4	36.0	0.75	1.25	0.20	0.18	4.18	3.47
50	32.0	29.5	0.37	0.87	0.21	0.18	4.10	3.13
100	36.3	36.7	0.87	1.62	0.22	0.17	4.17	3.61
150	32.6	35.2	0.62	1.12	0.21	0.18	4.10	3.31
200	35.1	38.3	1.00	1.37	0.19	0.18	4.22	3.57
Liming source								
Dolomitic limestone	34.1 a	38.8 a	0.70 a	1.85 a	0.20 a	0.18 a	4.18 a	3.48 a
Ca and Mg silicate	33.7 a	31.5 b	0.75 a	0.65 b	0.22 a	0.18 a	4.13 a	3.36 a
Inoculation								
Without <i>A. brasilense</i>	34.7 a	37.3 a	0.85 a	1.60 a	0.21 a	0.17 a	4.14 a	3.45 a
With <i>A. brasilense</i>	33.1 a	33.0 b	0.60 a	0.90 b	0.21 a	0.19 a	4.17 a	3.39 a
L.S.D. (5%)	2.29	3.32	0.47	0.64	0.02	0.01	0.13	0.17
C.V. (5%)	10.3	14.3	28.7	29.2	20.9	13.9	4.90	7.8
Overall Mean	33.9	35.1	0.72	1.25	0.21	0.18	4.15	3.42
N rate	Fe		Mn		Zn		Si	
			(mg dm ⁻³)					
	2016	2017	2016	2017	2016	2017	2016	2017
0	26.8	26.2	49.7	36.9	1.31	1.35	12.5	10.3
50	23.0	22.4	46.1	35.1	1.13	1.26	12.9	11.3
100	28.2	27.1	52.1	37.1	1.27	1.40	13.3	10.0
150	24.2	25.0	47.4	36.2	1.16	1.32	12.6	10.2
200	26.8	25.1	49.1	39.0	1.30	1.20	12.9	9.4
Liming source								
Dolomitic limestone	26.1 a	25.2 a	49.2 a	35.9 a	1.21 a	1.22 a	12.3 b	8.8 b
Ca and Mg silicate	25.5 a	25.2 a	48.5 a	37.8 a	1.26 a	1.39 a	13.4 a	11.6 a
Inoculation								
Without <i>A. brasilense</i>	24.1 b	22.4 b	48.3 a	34.9 b	1.26 a	1.23 a	13.2 a	9.9 a
With <i>A. brasilense</i>	27.5 a	27.9 a	49.5 a	38.8 a	1.21 a	1.38 a	12.5 a	10.6 a
L.S.D. (5%)	3.23	3.57	3.62	3.13	0.19	0.23	1.07	1.07
C.V. (5%)	18.9	21.4	11.2	12.8	24.2	27.6	12.7	15.9
Overall Mean	25.8	25.1	48.9	36.9	1.23	1.30	12.8	10.2

† Means within the column followed by different letters are significantly different by Tukey Test ($p \leq 0.05$). L.S.D. = Least significant difference and C.V. = coefficient of variation.

Increasing N rates provided greater soil P and K content in 2016 and OM in 2017. Probably, the nutrient cycling that occurs as a function of straw decomposition (corn 2015/16 – straw biomass = 12738 kg ha⁻¹, C/N ratio = 38.2, wheat 2016 – straw biomass = 5781 kg ha⁻¹, C/N ratio = 34.6 and corn 2016/17 – straw biomass = 12423 kg ha⁻¹, C/N ratio = 37.9) and the fact that the study was conducted under no-tillage for the last 15 years may improved soil P, K and OM.

After four cropping seasons (2015/16, 2016, 2016/17 and 2017, with corn and wheat in succession), silicate application was more efficient as a liming source to reduce soil acidity, as verified by decreased soil H+Al and Al content, and increased pH and V. Similarly, it has been reported that Ca and Mg silicate as a liming source can correct soil acidity; increase soil pH, extractable levels of Si, Ca, Mg and P; and

decrease the harmful effects of toxic heavy metals such as Al, Fe and Cd (ADREES *et al.*, 2015; SARTO *et al.*, 2015; CASTRO *et al.*, 2016; CRUSCIOL *et al.*, 2016; ANTONANGELO *et al.*, 2017). Silicon content in soil decreased from 9.4 to 8.8 in limestone applied plots, while Si content increased from 9.4 to 11.6 (31.8% greater Si content compared to limestone applied plots), even after these four cropping season. Given that the Si content in Typic Haplustox soils (Oxisols) under tropical conditions, such as the Brazilian Savannah, can be less than 1% due to the presence of extremely active desilication processes (FUJII *et al.*, 2018) and that some crops (mostly grasses, e.g., sugarcane, rice, wheat, and corn) can remove Si quickly and in great quantity (TUBANA *et al.*, 2016), studies with Si application aiming to increase NUE and N uptake in tropical conditions should be performed. Also, intensive cultivation removes Si from the soil, as it is estimated that annually 190–204 million Mg of Si is taken out from the world's arable soils (MEENA *et al.*, 2014). In cultivated areas, the removal of crops does not allow the recycling of Si by plants, and the biogeochemical cycle of Si is disturbed (BAKHAT *et al.*, 2018). In this way, the decrease of bioavailable Si might have significant impacts on cereal yields if not properly restored (GUNTZER *et al.*, 2012).

Similarly results verified in soil chemical attributes after corn cultivation were verified after wheat cropping, where the inoculation with *A. brasilense* reduced soil acidity in 2017 as observed by reduced soil H+Al, Al and increased pH, also in 2017. This increase on pH may positively affect S content and V. Galindo *et al.* (2017c) studying N rates (0, 50, 100, 150 and 200 kg N ha⁻¹ applied in side-dressing) with and without inoculation with *A. brasilense* also reported that inoculation can reduce the negative effect of soil acidity on irrigated wheat crop. Also, these authors concluded that cultivation of wheat inoculated with *A. brasilense* is not harmful to soil fertility because it does not reduce the base saturation, OM and nutrient content (P, K, Ca, Mg, and S). Inoculation with *A. brasilense* was reported to increase nitrogenase activity in soil, microbial biomass N, enzyme activities and rice biomass (ZHANG *et al.*, 2017), which would benefit straw biomass decomposition. With an increased straw decomposition, some nutrients, such as Fe and Mn would be more available as a function of inoculation, partially explaining the obtained results.

4.5 ECONOMIC ANALYSIS

Table 38 shows the total operating cost (TOC) structure for wheat crop in Selvíria, state of Mato Grosso do Sul, describing the treatment with dolomitic limestone application, in the absence of N fertilization (0 kg N ha^{-1}) and without inoculation with *A. brasilense*. This TOC structure model was used in all treatments. On average, the expenses with mechanized operations, followed by fertilizers, represented the highest costs, corresponding to 44.8 and 30.6% of the TOC, respectively.

Table 38. Total operating cost (TOC) structure model for wheat crop in the control treatment (0 kg ha^{-1} N in side-dressing), with limestone application and without inoculation with *A. brasilense* per hectare.

Description	Specification ¹	Times	Coefficient	Unitary Value (R\$)	Total Value (R\$)
A. OPERATIONS					
Liming sources application	HM	1.00	0.30	85.0	25.5
Weed control	HM	1.00	0.50	85.0	42.5
Hoeing (triton)	HM	1.00	0.50	85.0	42.5
Planting	HM	1.00	0.60	150	90.0
Pulverization	HM	1.00	0.60	85.0	51.0
Side-dressing	HM	1.00	0.40	150	60.0
Harvest	HM	1.00	1.00	118	118
Irrigation (pivot)	mm	1.00	150	2.50	375
Subtotal A					805
B. AGRICULTURAL INPUTS					
Dolomitic limestone	Mg	1.00	0.49	85.0	41.2
Fertilizer 08-28-16	Mg	1.00	0.28	1998	549
Urea	Mg	1.00	0.00	1780	0.00
Inoculant (<i>A. brasilense</i>)	L	1.00	0.00	10.0	0.00
Wheat seed CD 1104	50-kg sc	1.00	2.90	60.0	174
Glyphosate	L	1.00	4.00	14.5	58.0
2,4-D	L	1.00	1.00	13.2	13.2
Metsulfuron methyl	g ha^{-1}	1.00	3.00	1.02	3.05
Seed treatment pyraclostrobin + thiophanate-methyl + fipronil	L	1.00	0.04	350	14.0
Subtotal B					853
Effective operating costs (EOC)					1658
Other expenses					83.9
Interest cost					53.9
Total operating cost (TOC)					1794

HM = hour machine, sc = sack. 2016 and 2017 average exchange rate: R\$3.32 = U\$1.00.

Similar results have been reported by Galindo *et al.* (2018a) working with N rates and sources (0, 50, 100, 150, and 200 kg ha^{-1} , as urea or urea with NBPT), with and without inoculation with *A. brasilense* in wheat crop under Brazilian Cerrado conditions. These authors found higher expenditures with mechanized operations and fertilizers, corresponding to 43.6 and 35.7% of TOC. It should be pointed out that with the elevation of N doses and change of analyzed acidity corrective source, the

percentage of expenses in relation to the TOC of the fertilizers tends to increase.

The costs with side-dressing N application, as a function of increasing N rates, ranged from 12.2 to 30.9% of the TOC. Galindo *et al.* (2018a), verified costs with fertilization at planting that ranged from 9.8 to 30.4% of the TOC as a function of the increasing N rates. Gitti *et al.* (2012), studying the wheat crop economic viability under similar conditions with cover crops and N rates (0, 25, 50, 75, and 125 kg ha⁻¹), reported that the expenditure with fertilization was 23.52% of the TOC, for yields ranging from 34.5 and 66.5 of 60-kg sc ha⁻¹, and cost with N fertilization in side-dressing, varied from 4.9 to 20.3% of TOC. For the costs with liming sources, the values ranged (in both crops) between 2.5 and 3.7% (limestone) and 2.9 and 4.2% (silicate) (Table 38).

Regarding TOC and yield (Table 39), the highest cost for the liming sources was found in the treatment with silicate application associated with 200 kg N ha⁻¹ and inoculation with *A. brasilense* (Table 39). The lowest cost in TOC corresponded to the treatments with limestone application, in the absence of N fertilization in side-dressing (0 kg N ha⁻¹) and without inoculation with *A. brasilense* (Table 39). For grain yield, the highest values were verified in the treatment with limestone application associated with 150 kg N ha⁻¹ and with inoculation with *A. brasilense* in 2016, and with dolomitic limestone associated with 100 kg N ha⁻¹ also with inoculation with *A. brasilense* in 2017, with average yields of 72.2 and 82.8 of 60-kg sc ha⁻¹, respectively (Table 39).

Concerning gross revenue (GR) (Table 39), being the price of wheat constant, the GR of the treatments followed the same trend as the yields (Table 39); that is, the increases in revenue are due to the increases in grain yield. Thus, investment in management practices, such as balanced N fertilization, elevates the grain yield and the gross margin with wheat cultivation.

Table 39. Total operating cost (TOC), grain yield and gross revenue (GR) as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

Without <i>Azospirillum brasilense</i>										
----- Dolomitic limestone -----					----- Ca and Mg silicate -----					
	2016	2017	2016	2017		2016	2017	2016	2017	
N rates	TOC	YIELD	GR		TOC	YIELD	GR			
	R\$	60-kg sc ha ⁻¹	----- (R\$) -----			60-kg sc ha ⁻¹	R\$			
0	1794	55.7	54.1	2265	2198	1804	59.7	56.8	2426	2309
50	2008	66.7	61.1	2711	2485	2019	60.64	65.1	2466	2646
100	2222	64.3	75.7	2614	3080	2233	69.1	68.6	2806	2789
150	2437	63.3	77.9	2574	3169	2447	62.8	71.0	2555	2885
200	2651	62.5	61.5	2543	2502	2661	60.4	72.5	2457	2949
Mean	2222	62.5	66.1	2541	2687	2233	62.5	66.8	2542	2716
With <i>Azospirillum brasilense</i>										
----- Dolomitic limestone -----					----- Ca and Mg silicate -----					
	2016	2017	2016	2017		2016	2017	2016	2017	
N rates	TOC	YIELD	GR		TOC	YIELD	GR			
	R\$	60-kg sc ha ⁻¹	----- (R\$) -----			60-kg sc ha ⁻¹	R\$			
0	1888	53.3	63.8	2169	2593	1899	65.7	62.7	2672	2550
50	2103	72.1	64.3	2934	2616	2113	61.0	69.8	2480	2840
100	2317	67.9	82.8	2760	3366	2327	64.0	73.4	2604	2985
150	2531	72.2	74.3	2934	3023	2541	68.6	76.3	2791	3105
200	2745	68.9	67.1	2802	2728	2755	68.8	79.6	2796	3235
Mean	2317	66.9	70.5	2720	2865	2327	65.6	72.4	2669	2943

*Average wheat trading price R\$ 40.67 per 60-kg sc according to IEA (2017).

The operating profit (OP) was positive for the majority of evaluated treatments, regardless of N rate, liming sources, and inoculation (Table 40), with the exception of the following treatments: i) 200 kg N ha⁻¹ with dolomitic limestone application and without inoculation, in 2016 and 2017, ii) 200 kg N ha⁻¹ with silicate application and without inoculation in 2016, and iii) 200 kg N ha⁻¹ with limestone application and with inoculation in 2017, respectively (Table 40).

In 2016, the greater OP was obtained with limestone application associated with 50 kg N ha⁻¹ and inoculation with *A. brasilense* (R\$ 831) (Table 40). For 2017, the greater OP (R\$1042) was obtained by using limestone associated with 100 kg N ha⁻¹ and inoculation with *A. brasilense* (Table 40). Even with N fertilization in side-dressing in high N doses up to 150 kg ha⁻¹ (which would increase costs, possibly reducing the OP), wheat growing would be viable because of the high yield obtained; this reinforces the importance of N fertilization management to achieve high yields and consequently high financial return.

Regarding the profitability index (PI), in 2016 the highest value was obtained with Ca and Mg silicate application in the absence of N fertilization and with *A. brasilense* providing a PI of 29.0 % (Table 40). In 2017 crop, the highest value was obtained with application of limestone associated with 100 kg N ha⁻¹ and inoculation

with *A. brasilense* provided a PI of 31.2 % (Table 40). Teixeira Filho *et al.* (2010), studying N rates (0, 50, 100, 150, and 200 kg ha⁻¹), sources (ammonium sulfonitrate with nitrification inhibitor – dimethylpyrazolophosphate (ENTEC), ammonium sulfate, and urea), and wheat cultivars (E21 and IAC 370) in the low altitude Cerrado in irrigated cultivation, obtained higher gross margin of gain with 50 kg N ha⁻¹ in side-dressing, presenting values close to R\$ 500.00 with urea source. Gitti *et al.* (2012) obtained a higher cost-benefit ratio; in doses between 50 and 75 kg ha⁻¹, values that were in agreement with the rates that provided higher PI in the two crops evaluated in the present study (50 and 100 kg ha⁻¹).

Table 40. Operating profit (OP) and profitability index (PI) as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

Without <i>Azospirillum brasilense</i>								
----- Dolomitic limestone -----				----- Ca and Mg silicate -----				
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
N rates	OP R\$		PI ----- (%)-----		OP R\$		PI ----- (%)-----	
0	471	404	20.8	18.4	622	505	25.6	21.9
50	703	477	25.9	19.2	448	627	18.2	23.7
100	392	857	15.0	27.8	577	557	20.6	20.0
150	137	732	5.3	23.1	108	439	4.25	15.2
200	-108	-149	-4.25	-6.0	-204	288	-8.3	9.8
Mean	319	464	12.6	16.5	310	483	12.1	18.1
With <i>Azospirillum brasilense</i>								
----- Dolomitic limestone -----				----- Ca and Mg silicate -----				
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
N rates	OP R\$		PI ----- (%)-----		OP R\$		PI ----- (%)-----	
0	281	705	13.0	27.2	774	651	29.0	25.5
50	831	513	28.3	19.6	368	727	14.8	25.6
100	443	1042	16.1	31.2	277	658	10.6	22.0
150	403	492	13.8	16.3	250	564	9.0	18.2
200	57.2	-16.5	2.04	-0.61	41.1	480	1.47	14.9
Mean	403	547	14.6	18.7	342	616	13.0	21.2

Evaluating the accumulated operating profit (AOP), in the absence of inoculation, the greater AOP was obtained with application of 100 kg N ha⁻¹ associated with limestone, providing R\$ 1249 (Table 41). When the treatments were inoculated with *A. brasilense*, the highest AOP was also obtained with application of 100 kg N ha⁻¹ associated with dolomitic limestone, providing R\$ 1485 (Table 41). Analyzing the liming sources application, the greater AOP was obtained with limestone application associated with 100 kg N ha⁻¹ and with inoculation with *A. brasilense*, providing R\$ 1485 (Table 41). Concerning the silicate application, the highest AOP was obtained in the absence of N fertilization and with inoculation with

A. brasilense, providing R\$ 1424 (Table 41). An expressive increase in AOP was observed when inoculation with *A. brasilense* was performed, with an increase of 18.9% from the highest obtained AOP without inoculation (R\$ 1249 – 100 kg N ha⁻¹ with limestone application and without inoculation) to the treatment that provided the highest AOP with inoculation (R\$ 1485 – 100 kg N ha⁻¹ with limestone and *A. brasilense* inoculation) (Table 41).

Table 41. Accumulated operating profit (AOP), equilibrium price (EP) and equilibrium yield (EY) as a function of N rates, liming sources and inoculation with *A. brasilense* in wheat crop.

Without <i>Azospirillum brasilense</i>									
----- Dolomitic limestone -----					----- Ca and Mg silicate -----				
		2015/16 2016/17				2015/16 2016/17			
N rates	AOP	EP		EY	AOP	EP		EY	
	R\$	R\$ sc ⁻¹		60 kg ha ⁻¹ sc	R\$	R\$ sc ⁻¹		60 kg ha ⁻¹ sc	
0	875	32.2	33.2	44.1	1127	30.3	31.8	44.4	
50	1180	30.1	32.9	49.4	1075	33.3	31.0	49.6	
100	1249	34.6	29.4	54.7	1134	32.3	32.6	54.9	
150	870	38.5	31.3	59.9	547	38.9	34.5	60.2	
200	-257	42.4	43.1	65.2	84.3	44.1	36.7	65.4	
Mean	783	35.6	34.0	54.7	793	35.8	33.3	54.9	
With <i>Azospirillum brasilense</i>									
----- Dolomitic limestone -----					----- Ca and Mg silicate -----				
		2015/16 2016/17				2015/16 2016/17			
N rates	AOP	EP		EY	AOP	EP		EY	
	R\$	R\$ sc ⁻¹		60 kg ha ⁻¹ sc	R\$	R\$ sc ⁻¹		60 kg ha ⁻¹ sc	
0	986	35.4	29.6	46.4	1424	28.9	30.3	46.7	
50	1345	29.2	32.7	51.7	1095	34.6	30.3	52.0	
100	1485	34.1	28.0	57.0	935	36.4	31.7	57.2	
150	896	35.1	34.1	62.2	814	37.0	33.3	62.5	
200	40.6	40.0	40.9	67.5	521	40.1	34.6	67.7	
Mean	950	34.7	33.1	57.0	958	35.4	32.0	57.2	

The values (in R\$) of the 60-kg wheat sack for the equilibrium price (EP) (minimum price to cover the TOC) are shown in Table 41. Analyzing the liming sources, the application of limestone provided lower EP compared to silicate application in 2016 (average of R\$ 35.2 for limestone and R\$ 35.6 for silicate, respectively), however, in 2017 silicate application provided lower EP (average of R\$ 32.7 for silicate and R\$ 33.6 for limestone, respectively) (Table 41). Concerning the N rates, in 2016 the lower EP was verified in the absence of N fertilization, when inoculation with *A. brasilense* was performed, associated with silicate application (R\$ 28.9), and in 2017, the lower EP was obtained with 100 kg N ha⁻¹ associated with limestone and inoculation with *A. brasilense* (R\$ 28.0) (Table 41). In 2016, the greater EP was verified with application of 200 kg N ha⁻¹ associated with silicate and without inoculation (R\$ 44.1) (Table 41). In 2017, the greater EP was verified with

application of 200 kg N ha⁻¹ associated with limestone and without inoculation (R\$ 43.1) (Table 41).

The greater equilibrium yield (EY) was verified with application of 200 kg N ha⁻¹ associated with silicate application and *A. brasilense* inoculation (Table 41) (67.4 of 60-kg sc ha⁻¹), while the lowest EY were observed in the absence of N fertilization (44.12 and 46.43 of 60-kg sc ha⁻¹ without and with *A. brasilense* inoculation, respectively), to cover the production costs. Similarly as verified to corn crop, although the lowest EY was obtained, in general, in the absence of N fertilization, it is noteworthy that if N is not properly replenished, a lack of N supply in agricultural soils will negatively affect the success of agricultural activity over time. Also, a little variation was observed between non-inoculated and inoculated treatments in EY, regardless of N rates and liming sources applied, which is due to the low cost of the inoculation (R\$ 10.00 per rate), which is one of the main advantages of this technique, for both corn and wheat crops.

5 CONCLUSIONS

Increasing N rates positively influenced corn nutrition with increased nutrient and Si uptake in shoot, straw and grain, plant development and grain filling, leading to a greater corn grain yield. Based on two years of cultivation, the average nutrient uptake in the corn straw and grain in descending order was $K > N > Ca > Si > Mg > S > P > Fe > Mn > Zn > Cu > B$ for straw, and $N > K > P > S > Mg > Ca > Si > Fe > Zn > Mn > B > Cu$ for grains.

Increasing N rates also positively influenced wheat nutrition with increased nutrient and Si uptake in shoot, straw and grain, plant development and grain filling, leading to a greater wheat grain yield. Based on two years of cultivation, the average nutrient uptake in the wheat straw and grain in descending order was $K > N > Si > Ca > S > Mg > P > Fe > Mn > Zn > B > Cu$ for straw and $N > K > P > S > Mg > Si > Ca > Fe > Mn > Cu > Zn > B$ for grains.

The utilization of Ca and Mg silicate as a Si source had small effects on corn and wheat development and grain yield and mostly increased Si uptake by the plant. However, Si was the fourth and seventh chemical element most absorbed in corn straw and grain. In addition, Si was the third and sixth chemical element most absorbed in wheat straw and grain, respectively.

Although silicate application as Si source did not greatly favor nitrogen use efficiency (NUE), Si also did not negatively affect the inoculation and N fertilization, and correlates positively with nutritional, productive components, soil chemical attributes and corn and wheat grain yield. After four cropping seasons (2015/16, 2016, 2016/17 and 2017, with corn and wheat in succession), silicate application was more efficient as a liming source to reduce soil acidity, with decreased soil H+Al and Al content, and increased pH and base saturation (V).

Inoculation with *A. brasilense* increased NUE in corn crop by 19.1% (average of both years) leading to a greater leaf chlorophyll index (LCI), nutrient uptake, corn development, grain filling, grain yield and operating profit. Also, inoculation could increase leaf N concentration when associated with silicate application. Inoculation provided greater grain yield by 5.6% (average of both years). Under high N application rates (200 kg N ha^{-1}), inoculation provided an increase of 11.4% in grain yield, in 2015/16. In the absence of N fertilization, inoculation provided an increase of 20.2% in grain yield, in 2016/17.

Inoculation also increased NUE in wheat crop by 38.6% (average of both years) leading to a greater LCI, nutrient uptake, wheat development, grain filling, grain yield and operating profit. Under high N application rates (200 kg N ha⁻¹), inoculation provided an increase of 22.2% in shoot N uptake, in 2016. In addition, inoculation could increase leaf N concentration when associated with silicate application. Inoculation provided greater grain yield by 6.8% (average of both years).

The application of 200 kg N ha⁻¹ and 140 kg N ha⁻¹ in side-dressing with dolomitic limestone, associated with *A. brasilense* inoculation provided greater corn grain and wheat yield, respectively. However, the highest economic return was obtained with application of 100 kg N ha⁻¹, with limestone and *A. brasilense* inoculation, ensuring profitability from production of irrigated corn and wheat under tropical condition (Brazilian Savannah - Cerrado).

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APPENDIX A – Corn F-test values

1. F-test values for corn leaf chlorophyll index (LCI), leaf NO_3^- , NH_4^+ , N and Si concentration as a function of N rate, liming source and inoculation with *A. brasilense*.

	LCI		Leaf NO_3^- concentration		Leaf NH_4^+ concentration	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test						
N rate (R)	0.631 ^{ns}	15.403 ^{**}	1.210 ^{ns}	0.522 ^{ns}	0.291 ^{ns}	0.448 ^{ns}
Liming source (S)	10.489 ^{**}	4.497 [*]	1.190 ^{ns}	0.438 ^{ns}	2.661 ^{ns}	6.214 [*]
Inoculation (I)	7.535 ^{**}	5.767 [*]	2.968 ^{ns}	2.962 ^{ns}	2.903 ^{ns}	14.970 ^{**}
R × S	10.232 ^{**}	11.119 ^{**}	1.734 ^{ns}	0.634 ^{ns}	5.367 [*]	4.707 ^{**}
R × I	0.164 ^{ns}	0.084 ^{ns}	1.561 ^{ns}	0.813 ^{ns}	1.660 ^{ns}	2.279 ^{ns}
S × I	1.623 ^{ns}	0.026 ^{ns}	0.008 ^{ns}	0.127 ^{ns}	0.390 ^{ns}	2.593 ^{ns}
R × S × I	1.238 ^{ns}	0.385 ^{ns}	1.184 ^{ns}	0.478 ^{ns}	0.421 ^{ns}	0.647 ^{ns}
	Leaf N concentration		Leaf Si concentration			
	2015/16	2016/17	2015/16	2016/17		
F-test						
N rate (R)	7.921 ^{**}	14.783 ^{**}	0.175 ^{ns}		5.399 [*]	
Liming source (S)	0.249 ^{ns}	0.288 ^{ns}	15.922 ^{**}		7.875 ^{**}	
Inoculation (I)	0.017 ^{ns}	2.887 ^{ns}	2.692 ^{ns}		0.212 ^{ns}	
R × S	1.276 ^{ns}	0.190 ^{ns}	1.873 ^{ns}		1.261 ^{ns}	
R × I	1.181 ^{ns}	4.997 [*]	0.189 ^{ns}		0.183 ^{ns}	
S × I	0.000 ^{ns}	12.767 ^{**}	1.936 ^{ns}		1.721 ^{ns}	
R × S × I	1.403 ^{ns}	1.871 ^{ns}	0.689 ^{ns}		0.443 ^{ns}	

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

2. F-test values for corn shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rate, liming source and inoculation with *A. brasilense*.

	Shoot NO_3^- uptake		Shoot NH_4^+ uptake		Shoot N uptake		Shoot Si uptake	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test								
N rate (R)	2.416 ^{ns}	4.463 [*]	1.475 ^{ns}	0.611 ^{ns}	5.793 [*]	7.922 ^{**}	2.433 ^{ns}	8.923 ^{**}
Liming source (S)	0.156 ^{ns}	0.602 ^{ns}	1.007 ^{ns}	1.945 ^{ns}	1.135 ^{ns}	1.687 ^{ns}	4.607 [*]	6.081 ^{**}
Inoculation (I)	0.013 ^{ns}	0.071 ^{ns}	0.012 ^{ns}	0.100 ^{ns}	0.556 ^{ns}	0.274 ^{ns}	0.321 ^{ns}	0.364 ^{ns}
R × S	0.132 ^{ns}	0.135 ^{ns}	0.135 ^{ns}	0.402 ^{ns}	0.458 ^{ns}	0.102 ^{ns}	0.033 ^{ns}	0.481 ^{ns}
R × I	0.208 ^{ns}	0.531 ^{ns}	0.499 ^{ns}	0.767 ^{ns}	4.687 [*]	0.417 ^{ns}	0.767 ^{ns}	1.115 ^{ns}
S × I	1.827 ^{ns}	2.135 ^{ns}	1.339 ^{ns}	2.719 ^{ns}	0.064 ^{ns}	1.905 ^{ns}	0.792 ^{ns}	0.364 ^{ns}
R × S × I	0.488 ^{ns}	0.188 ^{ns}	0.726 ^{ns}	0.458 ^{ns}	1.000 ^{ns}	0.306 ^{ns}	0.920 ^{ns}	0.395 ^{ns}
	Root NO_3^- uptake		Root NH_4^+ uptake		Root N uptake		Root Si uptake	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test								
N rate (R)	20.478 ^{**}	10.096 ^{**}	12.357 ^{**}	11.568 ^{**}	11.127 ^{**}	31.510 ^{**}	19.526 ^{**}	7.140 [*]
Liming source (S)	0.982 ^{ns}	0.296 ^{ns}	9.375 ^{**}	1.172 ^{ns}	0.912 ^{ns}	1.600 ^{ns}	24.088 ^{**}	20.433 ^{**}
Inoculation (I)	2.979 ^{ns}	1.978 ^{ns}	1.424 ^{ns}	0.292 ^{ns}	0.891 ^{ns}	1.857 ^{ns}	0.004 ^{ns}	0.393 ^{ns}
R × S	0.812 ^{ns}	0.375 ^{ns}	2.367 ^{ns}	0.523 ^{ns}	4.225 [*]	4.109 [*]	1.972 ^{ns}	1.329 ^{ns}
R × I	0.131 ^{ns}	0.417 ^{ns}	0.883 ^{ns}	0.377 ^{ns}	3.998 [*]	16.403 ^{**}	0.542 ^{ns}	0.450 ^{ns}
S × I	1.076 ^{ns}	1.044 ^{ns}	1.031 ^{ns}	0.316 ^{ns}	0.162 ^{ns}	0.426 ^{ns}	2.015 ^{ns}	0.524 ^{ns}
R × S × I	1.504 ^{ns}	1.823 ^{ns}	0.420 ^{ns}	0.866 ^{ns}	0.628 ^{ns}	2.242 ^{ns}	1.289 ^{ns}	1.576 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

3. F-test values for corn shoot biomass, root biomass, plant height, stem diameter, ear length, ear diameter and rows per ear as a function of N rate, liming source and inoculation with *A. brasilense*.

	Shoot biomass		Root biomass		Plant height		Stem diameter	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test								
N rate (R)	2.712 ^{ns}	4.631 [*]	21.195 ^{**}	4.636 [*]	0.952 ^{ns}	12.293 ^{**}	0.605 ^{ns}	7.626 ^{**}
Liming source (S)	0.093 ^{ns}	1.137 ^{ns}	4.786 [*]	0.085 ^{ns}	1.423 ^{ns}	0.415 ^{ns}	12.001 ^{**}	20.224 ^{**}
Inoculation (I)	0.460 ^{ns}	0.065 ^{ns}	1.138 ^{ns}	0.297 ^{ns}	3.144 ^{ns}	0.139 ^{ns}	3.655 [*]	0.018 ^{ns}
R × S	0.083 ^{ns}	0.308 ^{ns}	9.183 ^{**}	0.832 ^{ns}	0.311 ^{ns}	0.674 ^{ns}	0.782 ^{ns}	0.089 ^{ns}
R × I	0.602 ^{ns}	0.808 ^{ns}	0.717 ^{ns}	0.430 ^{ns}	0.317 ^{ns}	0.152 ^{ns}	0.484 ^{ns}	0.272 ^{ns}
S × I	1.455 ^{ns}	2.295 ^{ns}	1.024 ^{ns}	0.394 ^{ns}	0.300 ^{ns}	1.182 ^{ns}	0.445 ^{ns}	0.097 ^{ns}
R × S × I	1.086 ^{ns}	0.255 ^{ns}	1.161 ^{ns}	0.705 ^{ns}	0.285 ^{ns}	0.736 ^{ns}	2.197 ^{ns}	0.355 ^{ns}
	Ear length		Ear diameter		Rows per ear			
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test								
N rate (R)	4.872 ^{**}	14.386 ^{**}	1.056 ^{ns}	15.017 ^{**}	0.503 ^{ns}		0.230 ^{ns}	
Liming source (S)	2.675 ^{ns}	0.962 ^{ns}	0.221 ^{ns}	6.659 [*]	1.521 ^{ns}		1.073 ^{ns}	
Inoculation (I)	2.213 ^{ns}	1.458 ^{ns}	0.007 ^{ns}	1.465 ^{ns}	0.057 ^{ns}		7.152 [*]	
R × S	0.742 ^{ns}	1.320 ^{ns}	1.044 ^{ns}	0.829 ^{ns}	2.146 ^{ns}		0.502 ^{ns}	
R × I	1.637 ^{ns}	0.735 ^{ns}	1.890 ^{ns}	1.572 ^{ns}	0.266 ^{ns}		1.576 ^{ns}	
S × I	0.766 ^{ns}	1.249 ^{ns}	0.370 ^{ns}	0.018 ^{ns}	0.452 ^{ns}		1.060 ^{ns}	
R × S × I	1.453 ^{ns}	0.580 ^{ns}	0.645 ^{ns}	0.737 ^{ns}	0.262 ^{ns}		1.193 ^{ns}	

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

4. F-test values for corn number of grains per row, grains per ear, mass of 100 grains, straw biomass, harvest index and grain yield as a function of N rate, liming source and inoculation with *A. brasilense*.

	Grains per row		Grains per ear		Mass of 100 grains	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test						
N rate (R)	1.464 ^{ns}	8.419 ^{**}	6.908 [*]	15.219 ^{**}	4.587 ^{**}	9.153 ^{**}
Liming source (S)	0.930 ^{ns}	0.187 ^{ns}	0.106 ^{ns}	0.614 ^{ns}	7.081 [*]	2.862 ^{ns}
Inoculation (I)	0.069 ^{ns}	1.776 ^{ns}	5.428 [*]	6.512 [*]	3.856 [*]	0.829 ^{ns}
R × S	1.745 ^{ns}	0.739 ^{ns}	1.911 ^{ns}	0.211 ^{ns}	0.193 ^{ns}	2.480 ^{ns}
R × I	1.145 ^{ns}	2.023 ^{ns}	3.865 [*]	1.987 ^{ns}	0.680 ^{ns}	1.112 ^{ns}
S × I	0.268 ^{ns}	1.203 ^{ns}	1.097 ^{ns}	0.930 ^{ns}	0.591 ^{ns}	3.286 ^{ns}
R × S × I	1.661 ^{ns}	0.958 ^{ns}	1.156 ^{ns}	1.585 ^{ns}	0.710 ^{ns}	1.427 ^{ns}
	Straw biomass		Harvest index		Grain yield	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test						
N rate (R)	8.008 [*]	10.832 ^{**}	4.583 [*]	8.117 ^{**}	39.091 ^{**}	37.134 ^{**}
Liming source (S)	0.041 ^{ns}	0.743 ^{ns}	5.398 [*]	1.355 ^{ns}	0.041 ^{ns}	0.000 ^{ns}
Inoculation (I)	1.658 ^{ns}	0.948 ^{ns}	5.667 [*]	10.272 ^{**}	8.104 ^{**}	4.762 [*]
R × S	0.318 ^{ns}	0.387 ^{ns}	0.226 ^{ns}	0.599 ^{ns}	9.050 ^{**}	8.474 ^{**}
R × I	0.567 ^{ns}	0.460 ^{ns}	0.539 ^{ns}	0.762 ^{ns}	9.189 ^{**}	8.157 ^{**}
S × I	2.129 ^{ns}	0.003 ^{ns}	0.106 ^{ns}	0.514 ^{ns}	2.512 ^{ns}	0.987 ^{ns}
R × S × I	1.171 ^{ns}	0.216 ^{ns}	0.676 ^{ns}	0.718 ^{ns}	1.944 ^{ns}	0.437 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

5. F-test values for corn nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use efficiency (NUE) as a function of N rate, liming source and inoculation with *A. brasilense*.

	NUP		NUT		NUE	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test						
N rate (R)	14.146 ^{**}	6.113 ^{**}	2.492 ^{ns}	9.879 ^{**}	16.288 ^{**}	9.218 ^{**}
Liming source (S)	16.421 ^{**}	6.796 ^{**}	5.503 [*]	0.969 ^{ns}	0.011 ^{ns}	0.538 ^{ns}
Inoculation (I)	2.667 ^{ns}	4.944 [*]	2.615 ^{ns}	0.095 ^{ns}	7.764 ^{**}	1.743 ^{ns}
R × S	1.463 ^{ns}	2.347 ^{ns}	2.286 ^{ns}	0.496 ^{ns}	1.055 ^{ns}	1.782 ^{ns}
R × I	1.463 ^{ns}	0.397 ^{ns}	1.245 ^{ns}	0.301 ^{ns}	0.955 ^{ns}	0.821 ^{ns}
S × I	0.223 ^{ns}	1.367 ^{ns}	2.358 ^{ns}	0.172 ^{ns}	0.784 ^{ns}	0.101 ^{ns}
R × S × I	1.968 ^{ns}	1.555 ^{ns}	2.337 ^{ns}	0.928 ^{ns}	2.601 ^{ns}	2.035 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

6. F-test values for corn straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rate, liming source and inoculation with *A. brasilense*.

	Straw N uptake		Straw P uptake		Straw K uptake		Straw Ca uptake		Straw Mg uptake		Straw S uptake	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test												
N rate (R)	16.395 ^{**}	9.045 ^{**}	1.076 ^{ns}	13.210 ^{**}	2.416 ^{ns}	1.165 ^{ns}	6.548 ^{**}	9.726 ^{**}	10.042 ^{**}	6.154 [*]	6.806 [*]	10.714 ^{**}
Liming source (S)	0.712 ^{ns}	0.259 ^{ns}	8.422 ^{**}	0.768 ^{ns}	6.987 [*]	0.202 ^{ns}	10.951 ^{**}	1.802 ^{ns}	0.495 ^{ns}	0.332 ^{ns}	0.002 ^{ns}	4.722 [*]
Inoculation (I)	1.632 ^{ns}	5.797 [*]	1.053 ^{ns}	0.750 ^{ns}	9.927 ^{**}	2.694 ^{ns}	4.629 [*]	0.625 ^{ns}	2.512 ^{ns}	1.194 ^{ns}	2.154 ^{ns}	1.619 ^{ns}
R × S	0.201 ^{ns}	0.615 ^{ns}	0.477 ^{ns}	0.630 ^{ns}	1.091 ^{ns}	0.237 ^{ns}	0.647 ^{ns}	1.066 ^{ns}	0.721 ^{ns}	0.756 ^{ns}	1.308 ^{ns}	0.446 ^{ns}
R × I	0.317 ^{ns}	1.448 ^{ns}	0.696 ^{ns}	0.271 ^{ns}	0.182 ^{ns}	0.341 ^{ns}	0.316 ^{ns}	1.047 ^{ns}	0.423 ^{ns}	0.454 ^{ns}	0.334 ^{ns}	0.362 ^{ns}
S × I	0.849 ^{ns}	0.057 ^{ns}	2.211 ^{ns}	1.429 ^{ns}	0.956 ^{ns}	0.673 ^{ns}	0.057 ^{ns}	0.715 ^{ns}	1.109 ^{ns}	0.748 ^{ns}	2.149 ^{ns}	2.060 ^{ns}
R × S × I	1.810 ^{ns}	0.200 ^{ns}	1.213 ^{ns}	0.284 ^{ns}	0.462 ^{ns}	0.233 ^{ns}	1.471 ^{ns}	0.437 ^{ns}	0.352 ^{ns}	0.261 ^{ns}	1.423 ^{ns}	0.596 ^{ns}
	Straw B uptake		Straw Cu uptake		Straw Fe uptake		Straw Mn uptake		Straw Zn uptake		Straw Si uptake	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test												
N rate (R)	14.102 ^{**}	6.905 ^{**}	1.073 ^{ns}	1.339 ^{ns}	7.614 [*]	12.041 ^{**}	13.901 ^{**}	20.268 ^{**}	1.469 ^{ns}	14.034 ^{**}	1.048 ^{ns}	5.528 [*]
Liming source (S)	5.107 [*]	1.479 ^{ns}	1.130 ^{ns}	0.065 ^{ns}	6.544 [*]	0.466 ^{ns}	1.708 ^{ns}	0.878 ^{ns}	0.728 ^{ns}	1.354 ^{ns}	5.855 [*]	9.805 ^{**}
Inoculation (I)	2.563 ^{ns}	16.627 ^{**}	0.093 ^{ns}	2.640 ^{ns}	2.623 ^{ns}	0.002 ^{ns}	1.703 ^{ns}	0.043 ^{ns}	0.058 ^{ns}	1.001 ^{ns}	1.276 ^{ns}	5.077 [*]
R × S	0.875 ^{ns}	2.443 ^{ns}	0.337 ^{ns}	0.433 ^{ns}	1.392 ^{ns}	0.690 ^{ns}	0.494 ^{ns}	1.179 ^{ns}	1.076 ^{ns}	0.429 ^{ns}	0.553 ^{ns}	1.471 ^{ns}
R × I	0.652 ^{ns}	0.729 ^{ns}	0.345 ^{ns}	1.170 ^{ns}	0.881 ^{ns}	0.418 ^{ns}	0.831 ^{ns}	0.777 ^{ns}	0.748 ^{ns}	0.355 ^{ns}	1.192 ^{ns}	0.399 ^{ns}
S × I	1.648 ^{ns}	1.594 ^{ns}	2.317 ^{ns}	0.000 ^{ns}	2.274 ^{ns}	1.336 ^{ns}	2.449 ^{ns}	2.202 ^{ns}	0.465 ^{ns}	0.012 ^{ns}	2.248 ^{ns}	0.119 ^{ns}
R × S × I	1.911 ^{ns}	0.702 ^{ns}	2.400 ^{ns}	0.264 ^{ns}	1.535 ^{ns}	0.244 ^{ns}	1.478 ^{ns}	1.701 ^{ns}	0.873 ^{ns}	1.143 ^{ns}	0.994 ^{ns}	0.541 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

7. F-test values for corn grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rate, liming source and inoculation with *A. brasilense*.

	Grain N uptake		Grain P uptake		Grain K uptake		Grain Ca uptake		Grain Mg uptake		Grain S uptake	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test												
N rate (R)	8.077 ^{**}	8.284 ^{**}	18.981 ^{**}	14.600 ^{**}	1.297 ^{ns}	7.233 ^{**}	6.063 [*]	12.808 ^{**}	7.810 ^{**}	12.913 ^{**}	7.189 ^{**}	7.023 ^{**}
Liming source (S)	0.276 ^{ns}	0.242 ^{ns}	1.723 ^{ns}	0.083 ^{ns}	0.985 ^{ns}	0.222 ^{ns}	19.311 ^{**}	1.519 ^{ns}	0.306 ^{ns}	0.204 ^{ns}	0.911 ^{ns}	0.487 ^{ns}
Inoculation (I)	2.703 ^{ns}	0.004 ^{ns}	2.521 ^{ns}	0.043 ^{ns}	0.573 ^{ns}	0.119 ^{ns}	1.792 ^{ns}	2.666 ^{ns}	2.491 ^{ns}	0.076 ^{ns}	10.972 ^{**}	0.170 ^{ns}
R × S	2.116 ^{ns}	0.762 ^{ns}	0.945 ^{ns}	0.330 ^{ns}	0.532 ^{ns}	0.173 ^{ns}	0.855 ^{ns}	1.180 ^{ns}	1.078 ^{ns}	0.136 ^{ns}	2.109 ^{ns}	0.267 ^{ns}
R × I	3.763 [*]	1.923 ^{ns}	1.645 ^{ns}	0.395 ^{ns}	0.909 ^{ns}	0.707 ^{ns}	1.725 ^{ns}	0.454 ^{ns}	2.603 ^{ns}	0.269 ^{ns}	4.616 ^{**}	0.091 ^{ns}
S × I	0.046 ^{ns}	2.425 ^{ns}	0.073 ^{ns}	0.370 ^{ns}	0.347 ^{ns}	0.001 ^{ns}	0.101 ^{ns}	1.516 ^{ns}	0.728 ^{ns}	0.013 ^{ns}	1.236 ^{ns}	2.228 ^{ns}
R × S × I	0.721 ^{ns}	0.298 ^{ns}	0.956 ^{ns}	0.419 ^{ns}	0.704 ^{ns}	0.302 ^{ns}	1.918 ^{ns}	0.230 ^{ns}	2.241 ^{ns}	0.089 ^{ns}	0.293 ^{ns}	0.400 ^{ns}
	Grain B uptake		Grain Cu uptake		Grain Fe uptake		Grain Mn uptake		Grain Zn uptake		Grain Si uptake	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test												
N rate (R)	1.373 ^{ns}	10.559 ^{**}	0.387 ^{ns}	1.075 ^{ns}	1.240 ^{ns}	8.576 ^{**}	11.048 ^{**}	7.208 ^{**}	12.060 ^{**}	2.075 ^{ns}	10.306 ^{**}	13.581 ^{**}
Liming source (S)	0.026 ^{ns}	2.657 ^{ns}	0.050 ^{ns}	0.043 ^{ns}	0.303 ^{ns}	13.773 ^{**}	0.010 ^{ns}	1.957 ^{ns}	0.609 ^{ns}	0.881 ^{ns}	1.784 ^{ns}	14.451 ^{**}
Inoculation (I)	1.454 ^{ns}	0.029 ^{ns}	7.680 [*]	0.043 ^{ns}	2.630 ^{ns}	0.019 ^{ns}	0.607 ^{ns}	0.389 ^{ns}	51.997 ^{**}	0.016 ^{ns}	0.028 ^{ns}	2.044 ^{ns}
R × S	0.569 ^{ns}	0.959 ^{ns}	1.099 ^{ns}	0.602 ^{ns}	0.767 ^{ns}	1.484 ^{ns}	0.894 ^{ns}	0.833 ^{ns}	0.443 ^{ns}	0.425 ^{ns}	0.759 ^{ns}	0.665 ^{ns}
R × I	0.211 ^{ns}	0.833 ^{ns}	0.858 ^{ns}	0.649 ^{ns}	0.418 ^{ns}	0.540 ^{ns}	1.755 ^{ns}	0.913 ^{ns}	6.101 ^{**}	0.356 ^{ns}	0.395 ^{ns}	0.414 ^{ns}
S × I	1.426 ^{ns}	2.672 ^{ns}	1.966 ^{ns}	0.636 ^{ns}	0.122 ^{ns}	1.212 ^{ns}	0.974 ^{ns}	1.521 ^{ns}	1.071 ^{ns}	0.374 ^{ns}	0.191 ^{ns}	1.087 ^{ns}
R × S × I	0.956 ^{ns}	0.444 ^{ns}	1.821 ^{ns}	0.282 ^{ns}	0.538 ^{ns}	1.137 ^{ns}	0.379 ^{ns}	1.536 ^{ns}	1.167 ^{ns}	0.273 ^{ns}	2.183 ^{ns}	0.704 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

8. F-test values for soil P, organic matter (OM), pH, K, Ca, Mg, sum of bases (SB), S, cation exchange capacity (CEC) and base saturation (V) content in 0-0.20m depth after corn cultivation as a function of N rate, liming source and inoculation with *A. brasilense*.

	P		OM		pH		K		Ca	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test										
N rate (R)	4.679 [*]	2.109 ^{ns}	6.044 [*]	0.314 ^{ns}	0.971 ^{ns}	0.467 ^{ns}	2.837 ^{ns}	1.558 ^{ns}	8.132 [*]	0.270 ^{ns}
Liming source (S)	0.080 ^{ns}	5.344 [*]	2.238 ^{ns}	0.332 ^{ns}	1.277 ^{ns}	0.051 ^{ns}	16.912 ^{**}	1.388 ^{ns}	0.085 ^{ns}	0.260 ^{ns}
Inoculation (I)	9.321 ^{**}	0.466 ^{ns}	2.482 ^{ns}	0.201 ^{ns}	0.026 ^{ns}	1.287 ^{ns}	9.021 ^{**}	7.085 [*]	0.340 ^{ns}	0.077 ^{ns}
R × S	1.268 ^{ns}	0.313 ^{ns}	0.825 ^{ns}	0.301 ^{ns}	0.652 ^{ns}	0.550 ^{ns}	1.292 ^{ns}	0.783 ^{ns}	2.296 ^{ns}	0.379 ^{ns}
R × I	1.210 ^{ns}	2.181 ^{ns}	0.915 ^{ns}	0.334 ^{ns}	2.144 ^{ns}	0.402 ^{ns}	1.511 ^{ns}	2.092 ^{ns}	1.793 ^{ns}	1.338 ^{ns}
S × I	0.025 ^{ns}	0.012 ^{ns}	2.223 ^{ns}	2.169 ^{ns}	0.691 ^{ns}	2.171 ^{ns}	0.942 ^{ns}	0.746 ^{ns}	0.133 ^{ns}	2.199 ^{ns}
R × S × I	0.965 ^{ns}	2.111 ^{ns}	0.020 ^{ns}	0.765 ^{ns}	1.499 ^{ns}	1.516 ^{ns}	1.086 ^{ns}	0.305 ^{ns}	2.266 ^{ns}	1.806 ^{ns}
	Mg		SB		S		CEC		V	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test										
N rate (R)	1.111 ^{ns}	0.783 ^{ns}	4.698 [*]	0.578 ^{ns}	0.055 ^{ns}	1.074 ^{ns}	5.904 [*]	0.113 ^{ns}	0.885 ^{ns}	0.346 ^{ns}
Liming source (S)	2.423 ^{ns}	8.127 ^{**}	0.424 ^{ns}	1.383 ^{ns}	2.338 ^{ns}	22.376 ^{**}	0.176 ^{ns}	2.527 ^{ns}	0.257 ^{ns}	1.167 ^{ns}
Inoculation (I)	5.222 [*]	0.753 ^{ns}	1.770 ^{ns}	0.026 ^{ns}	2.338 ^{ns}	0.767 ^{ns}	1.344 ^{ns}	1.655 ^{ns}	0.776 ^{ns}	0.492 ^{ns}
R × S	1.753 ^{ns}	0.966 ^{ns}	2.163 ^{ns}	1.255 ^{ns}	0.237 ^{ns}	1.044 ^{ns}	2.510 ^{ns}	0.409 ^{ns}	1.359 ^{ns}	0.529 ^{ns}
R × I	1.120 ^{ns}	0.505 ^{ns}	1.894 ^{ns}	1.335 ^{ns}	0.420 ^{ns}	0.767 ^{ns}	0.883 ^{ns}	1.174 ^{ns}	2.050 ^{ns}	0.872 ^{ns}
S × I	2.499 ^{ns}	0.493 ^{ns}	1.488 ^{ns}	2.210 ^{ns}	1.169 ^{ns}	0.246 ^{ns}	2.449 ^{ns}	0.109 ^{ns}	0.058 ^{ns}	0.984 ^{ns}
R × S × I	1.713 ^{ns}	2.065 ^{ns}	1.962 ^{ns}	1.478 ^{ns}	0.676 ^{ns}	0.123 ^{ns}	1.139 ^{ns}	1.379 ^{ns}	2.474 ^{ns}	1.616 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

9. F-test values for soil H+Al, Al, B, Cu, Fe, Mn, Zn and Si content in 0-0.20m depth after corn cultivation as a function of N rate, liming source and inoculation with *A. brasilense*.

	H + Al		Al		B		Cu	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test								
N rate (R)	0.491 ^{ns}	0.394 ^{ns}	1.407 ^{ns}	0.489 ^{ns}	0.870 ^{ns}	0.604 ^{ns}	0.585 ^{ns}	1.058 ^{ns}
Liming source (S)	0.332 ^{ns}	0.199 ^{ns}	0.000 ^{ns}	0.364 ^{ns}	0.337 ^{ns}	5.623 [*]	2.248 ^{ns}	0.249 ^{ns}
Inoculation (I)	0.420 ^{ns}	5.251 [*]	0.235 ^{ns}	1.455 ^{ns}	0.100 ^{ns}	8.842 ^{**}	0.754 ^{ns}	6.235 [*]
R × S	0.364 ^{ns}	0.616 ^{ns}	1.173 ^{ns}	1.102 ^{ns}	1.050 ^{ns}	0.307 ^{ns}	0.722 ^{ns}	2.013 ^{ns}
R × I	1.908 ^{ns}	0.700 ^{ns}	1.173 ^{ns}	1.057 ^{ns}	1.493 ^{ns}	0.919 ^{ns}	2.491 ^{ns}	1.568 ^{ns}
S × I	0.876 ^{ns}	0.342 ^{ns}	0.235 ^{ns}	2.227 ^{ns}	2.514 ^{ns}	1.283 ^{ns}	1.052 ^{ns}	1.559 ^{ns}
R × S × I	1.395 ^{ns}	0.721 ^{ns}	0.586 ^{ns}	1.170 ^{ns}	1.458 ^{ns}	1.131 ^{ns}	0.710 ^{ns}	1.393 ^{ns}
	Fe		Mn		Zn		Si	
	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17	2015/16	2016/17
F-test								
N rate (R)	0.041 ^{ns}	1.188 ^{ns}	1.819 ^{ns}	0.643 ^{ns}	11.967 ^{**}	6.782 ^{**}	4.553 [*]	6.850 [*]
Liming source (S)	0.532 ^{ns}	1.158 ^{ns}	0.763 ^{ns}	6.522 [*]	16.775 ^{**}	0.735 ^{ns}	8.023 [*]	20.466 ^{**}
Inoculation (I)	0.001 ^{ns}	0.229 ^{ns}	0.006 ^{ns}	2.430 ^{ns}	12.600 ^{**}	32.107 ^{**}	5.940 [*]	8.288 ^{**}
R × S	0.297 ^{ns}	0.756 ^{ns}	0.478 ^{ns}	0.810 ^{ns}	1.804 ^{ns}	1.290 ^{ns}	1.015 ^{ns}	1.720 ^{ns}
R × I	0.811 ^{ns}	0.559 ^{ns}	0.391 ^{ns}	1.025 ^{ns}	0.045 ^{ns}	1.850 ^{ns}	1.449 ^{ns}	1.603 ^{ns}
S × I	2.453 ^{ns}	0.357 ^{ns}	0.438 ^{ns}	0.154 ^{ns}	1.491 ^{ns}	1.652 ^{ns}	0.525 ^{ns}	2.176 ^{ns}
R × S × I	0.878 ^{ns}	0.572 ^{ns}	0.198 ^{ns}	0.320 ^{ns}	1.906 ^{ns}	1.362 ^{ns}	0.460 ^{ns}	1.626 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

APPENDIX B – Wheat F-test values

1. F-test values for wheat leaf chlorophyll index (LCI), leaf NO_3^- , NH_4^+ , N and Si concentration as a function of N rate, liming source and inoculation with *A. brasilense*.

F-test	LCI		Leaf NO_3^- concentration		Leaf NH_4^+ concentration	
	2016	2017	2016	2017	2016	2017
N rate (R)	6.936*	14.301**	1.679 ^{ns}	1.070 ^{ns}	7.016**	7.218**
Liming source (S)	2.743 ^{ns}	2.127 ^{ns}	7.705**	0.117 ^{ns}	18.746**	0.338 ^{ns}
Inoculation (I)	5.362*	4.182*	9.663**	4.538*	7.820**	1.472 ^{ns}
R × S	1.614 ^{ns}	8.628**	0.783 ^{ns}	4.105*	2.640 ^{ns}	2.105 ^{ns}
R × I	0.678 ^{ns}	1.592 ^{ns}	1.655 ^{ns}	1.911 ^{ns}	0.775 ^{ns}	1.197 ^{ns}
S × I	0.592 ^{ns}	0.062 ^{ns}	2.456 ^{ns}	11.334**	2.144 ^{ns}	2.356 ^{ns}
R × S × I	1.143 ^{ns}	0.972 ^{ns}	2.425 ^{ns}	1.306 ^{ns}	2.253 ^{ns}	0.164 ^{ns}
F-test	Leaf N concentration		Leaf Si concentration			
	2016	2017	2016	2017		
N rate (R)	23.220**	26.364**	1.785 ^{ns}		9.723**	
Liming source (S)	2.825 ^{ns}	0.023 ^{ns}	15.884**		11.525**	
Inoculation (I)	0.361 ^{ns}	43.234**	0.455 ^{ns}		73.769**	
R × S	0.208 ^{ns}	1.400 ^{ns}	1.522 ^{ns}		2.506 ^{ns}	
R × I	7.996**	5.001**	0.061 ^{ns}		9.558**	
S × I	25.401**	0.188 ^{ns}	0.238 ^{ns}		0.001 ^{ns}	
R × S × I	0.956 ^{ns}	1.792 ^{ns}	1.545 ^{ns}		2.643 ^{ns}	

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

2. F-test values for wheat shoot and root NO_3^- , NH_4^+ , N and Si uptake as a function of N rate, liming source and inoculation with *A. brasilense*.

F-test	Shoot NO_3^- uptake		Shoot NH_4^+ uptake		Shoot N uptake		Shoot Si uptake	
	2016	2017	2016	2017	2016	2017	2016	2017
N rate (R)	8.829**	1.360 ^{ns}	5.005**	11.758**	16.165**	11.999**	14.227**	12.711**
Liming source (S)	2.663 ^{ns}	19.168**	2.254 ^{ns}	13.100**	1.688 ^{ns}	12.956**	8.524**	42.567**
Inoculation (I)	0.084 ^{ns}	0.823 ^{ns}	9.183**	0.097 ^{ns}	9.958**	2.425 ^{ns}	0.875 ^{ns}	0.038 ^{ns}
R × S	0.463 ^{ns}	0.733 ^{ns}	1.002 ^{ns}	1.202 ^{ns}	2.047 ^{ns}	1.170 ^{ns}	1.484 ^{ns}	2.088 ^{ns}
R × I	1.416 ^{ns}	1.092 ^{ns}	0.471 ^{ns}	0.829 ^{ns}	7.021**	2.100 ^{ns}	1.075 ^{ns}	1.327 ^{ns}
S × I	1.429 ^{ns}	0.129 ^{ns}	0.775 ^{ns}	0.026 ^{ns}	0.018 ^{ns}	0.397 ^{ns}	1.631 ^{ns}	2.230 ^{ns}
R × S × I	1.834 ^{ns}	1.636 ^{ns}	0.667 ^{ns}	1.712 ^{ns}	1.758 ^{ns}	2.127 ^{ns}	1.042 ^{ns}	0.677 ^{ns}
F-test	Root NO_3^- uptake		Root NH_4^+ uptake		Root N uptake		Root Si uptake	
	2016	2017	2016	2017	2016	2017	2016	2017
N rate (R)	4.735**	0.512 ^{ns}	0.320 ^{ns}	6.176*	9.949**	0.895 ^{ns}	6.920*	2.741 ^{ns}
Liming source (S)	6.508*	2.686 ^{ns}	0.197 ^{ns}	0.165 ^{ns}	2.255 ^{ns}	0.839 ^{ns}	2.785 ^{ns}	60.105**
Inoculation (I)	2.344 ^{ns}	1.176 ^{ns}	0.174 ^{ns}	0.325 ^{ns}	2.326 ^{ns}	2.740 ^{ns}	0.010 ^{ns}	2.723 ^{ns}
R × S	2.201 ^{ns}	1.195 ^{ns}	1.226 ^{ns}	1.138 ^{ns}	1.009 ^{ns}	0.720 ^{ns}	0.122 ^{ns}	2.155 ^{ns}
R × I	0.774 ^{ns}	0.818 ^{ns}	2.372 ^{ns}	0.125 ^{ns}	0.078 ^{ns}	1.746 ^{ns}	1.208 ^{ns}	0.845 ^{ns}
S × I	2.293 ^{ns}	2.413 ^{ns}	0.274 ^{ns}	2.478 ^{ns}	0.058 ^{ns}	0.034 ^{ns}	0.476 ^{ns}	0.011 ^{ns}
R × S × I	0.591 ^{ns}	0.814 ^{ns}	0.545 ^{ns}	0.590 ^{ns}	0.512 ^{ns}	1.275 ^{ns}	1.241 ^{ns}	0.589 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

3. F-test values for wheat shoot biomass, root biomass, plant height, spikes per meter, spike length, number of defective grains, spikelets per spike and grains per spikelet as a function of N rate, liming source and inoculation with *A. brasilense*.

	Shoot biomass		Root biomass		Plant height		Spikes per meter	
	2016	2017	2016	2017	2016	2017	2016	2017
F-test								
N rate (R)	9.542 ^{**}	0.795 ^{ns}	5.209 [*]	3.739 ^{ns}	0.486 ^{ns}	1.269 ^{ns}	1.034 ^{ns}	10.834 ^{**}
Liming source (S)	16.221 ^{**}	18.687 ^{**}	18.506 ^{**}	14.451 ^{**}	1.159 ^{ns}	1.980 ^{ns}	0.373 ^{ns}	2.113 ^{ns}
Inoculation (I)	5.646 [*]	0.329 ^{ns}	2.268 ^{ns}	2.226 ^{ns}	2.457 ^{ns}	0.018 ^{ns}	0.166 ^{ns}	4.111 [*]
R × S	1.683 ^{ns}	2.223 ^{ns}	1.753 ^{ns}	0.612 ^{ns}	1.029 ^{ns}	0.545 ^{ns}	0.576 ^{ns}	2.183 ^{ns}
R × I	0.477 ^{ns}	1.355 ^{ns}	0.562 ^{ns}	0.640 ^{ns}	0.765 ^{ns}	0.954 ^{ns}	0.021 ^{ns}	2.287 ^{ns}
S × I	0.008 ^{ns}	0.852 ^{ns}	0.001 ^{ns}	0.147 ^{ns}	0.385 ^{ns}	0.480 ^{ns}	0.201 ^{ns}	0.473 ^{ns}
R × S × I	1.915 ^{ns}	1.891 ^{ns}	1.810 ^{ns}	1.027 ^{ns}	1.609 ^{ns}	0.058 ^{ns}	1.025 ^{ns}	1.918 ^{ns}
	Spike length		Defective grains		Spikelets per spike		Grains per spikelet	
	2016	2017	2016	2017	2016	2017	2016	2017
F-test								
N rate (R)	7.780 ^{**}	18.154 ^{**}	2.183 ^{ns}	1.346 ^{ns}	0.319 ^{ns}	6.898 ^{**}	1.554 ^{ns}	0.433 ^{ns}
Liming source (S)	1.693 ^{ns}	0.559 ^{ns}	1.720 ^{ns}	0.002 ^{ns}	5.496 [*]	0.044 ^{ns}	2.412 ^{ns}	2.243 ^{ns}
Inoculation (I)	0.390 ^{ns}	0.001 ^{ns}	9.706 ^{**}	2.056 ^{ns}	0.072 ^{ns}	1.959 ^{ns}	5.384 [*]	0.376 ^{ns}
R × S	0.594 ^{ns}	1.014 ^{ns}	0.369 ^{ns}	0.747 ^{ns}	1.144 ^{ns}	1.568 ^{ns}	1.062 ^{ns}	0.993 ^{ns}
R × I	0.695 ^{ns}	1.040 ^{ns}	2.275 ^{ns}	0.934 ^{ns}	0.324 ^{ns}	1.515 ^{ns}	0.915 ^{ns}	0.485 ^{ns}
S × I	2.145 ^{ns}	0.706 ^{ns}	0.134 ^{ns}	0.189 ^{ns}	1.149 ^{ns}	0.705 ^{ns}	0.440 ^{ns}	1.099 ^{ns}
R × S × I	1.043 ^{ns}	1.028 ^{ns}	0.428 ^{ns}	1.330 ^{ns}	1.177 ^{ns}	1.265 ^{ns}	1.612 ^{ns}	0.212 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

4. F-test values for wheat number of grains per spike, hectoliter mass, mass of 1000 grains, straw biomass, harvest index and grain yield as a function of N rate, liming source and inoculation with *A. brasilense*.

	Grains per spike		Hectoliter mass		Mass of 1000 grains	
	2016	2017	2016	2017	2016	2017
F-test						
N rate (R)	1.235 ^{ns}	4.021 ^{**}	1.775 ^{ns}	2.124 ^{ns}	0.641 ^{ns}	1.412 ^{ns}
Liming source (S)	1.226 ^{ns}	2.172 ^{ns}	0.078 ^{ns}	0.005 ^{ns}	2.237 ^{ns}	1.607 ^{ns}
Inoculation (I)	2.125 ^{ns}	0.146 ^{ns}	0.007 ^{ns}	1.422 ^{ns}	5.757 [*]	13.584 ^{**}
R × S	2.257 ^{ns}	0.417 ^{ns}	0.948 ^{ns}	1.158 ^{ns}	1.093 ^{ns}	1.161 ^{ns}
R × I	1.344 ^{ns}	2.247 ^{ns}	0.923 ^{ns}	0.771 ^{ns}	1.478 ^{ns}	1.171 ^{ns}
S × I	0.021 ^{ns}	0.431 ^{ns}	1.954 ^{ns}	0.512 ^{ns}	0.540 ^{ns}	2.162 ^{ns}
R × S × I	2.052 ^{ns}	1.424 ^{ns}	1.032 ^{ns}	2.181 ^{ns}	1.045 ^{ns}	0.805 ^{ns}
	Straw biomass		Harvest index		Grain yield	
	2016	2017	2016	2017	2016	2017
F-test						
N rate (R)	10.555 ^{**}	13.447 ^{**}	1.048 ^{ns}	0.126 ^{ns}	5.154 [*]	13.547 ^{**}
Liming source (S)	1.956 ^{ns}	2.084 ^{ns}	4.573 [*]	0.176 ^{ns}	0.117 ^{ns}	0.418 ^{ns}
Inoculation (I)	0.013 ^{ns}	0.535 ^{ns}	0.168 ^{ns}	3.313 [*]	4.379 [*]	6.051 [*]
R × S	0.276 ^{ns}	0.765 ^{ns}	0.327 ^{ns}	0.704 ^{ns}	2.256 ^{ns}	3.932 ^{**}
R × I	0.200 ^{ns}	0.585 ^{ns}	0.206 ^{ns}	0.387 ^{ns}	4.256 [*]	5.588 ^{**}
S × I	0.006 ^{ns}	0.844 ^{ns}	0.016 ^{ns}	1.081 ^{ns}	0.131 ^{ns}	0.086 ^{ns}
R × S × I	0.885 ^{ns}	0.297 ^{ns}	0.479 ^{ns}	0.089 ^{ns}	0.690 ^{ns}	0.297 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

5. F-test values for wheat nitrogen uptake (NUP), nitrogen utilization (NUT) and nitrogen use efficiency (NUE) as a function of N rate, liming source and inoculation with *A. brasilense*.

	NUP		NUT		NUE	
	2016	2017	2016	2017	2016	2017
F-test						
N rate (R)	32.055 ^{**}	17.004 ^{**}	5.145 ^{**}	24.579 ^{**}	29.878 ^{**}	26.277 ^{**}
Liming source (S)	0.110 ^{ns}	11.516 ^{**}	0.793 ^{ns}	1.578 ^{ns}	0.033 ^{ns}	1.491 ^{ns}
Inoculation (I)	0.707 ^{ns}	31.478 ^{**}	2.585 ^{ns}	0.115 ^{ns}	1.211 ^{ns}	28.303 ^{**}
R × S	0.025 ^{ns}	2.456 ^{ns}	0.334 ^{ns}	2.348 ^{ns}	0.397 ^{ns}	0.810 ^{ns}
R × I	0.057 ^{ns}	2.412 ^{ns}	0.019 ^{ns}	2.372 ^{ns}	0.551 ^{ns}	1.791 ^{ns}
S × I	2.647 ^{ns}	0.524 ^{ns}	1.481 ^{ns}	1.817 ^{ns}	0.615 ^{ns}	2.272 ^{ns}
R × S × I	0.263 ^{ns}	1.666 ^{ns}	0.136 ^{ns}	1.795 ^{ns}	0.432 ^{ns}	0.858 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

6. F-test values for wheat straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rate, liming source and inoculation with *A. brasilense*.

	Straw N uptake		Straw P uptake		Straw K uptake		Straw Ca uptake		Straw Mg uptake		Straw S uptake	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
F-test												
N rate (R)	0.267 ^{ns}	7.078 ^{**}	1.101 ^{ns}	4.789 ^{**}	0.728 ^{ns}	6.673 ^{**}	1.484 ^{ns}	10.980 ^{**}	0.584 ^{ns}	6.291 ^{**}	1.817 ^{ns}	5.076 ^{**}
Liming source (S)	2.435 ^{ns}	1.140 ^{ns}	0.027 ^{ns}	1.820 ^{ns}	2.177 ^{ns}	0.885 ^{ns}	0.079 ^{ns}	0.047 ^{ns}	0.006 ^{ns}	0.376 ^{ns}	1.272 ^{ns}	0.049 ^{ns}
Inoculation (I)	0.477 ^{ns}	0.817 ^{ns}	0.220 ^{ns}	0.539 ^{ns}	1.395 ^{ns}	4.691 [*]	0.030 ^{ns}	0.130 ^{ns}	0.083 ^{ns}	0.002 ^{ns}	0.324 ^{ns}	4.731 [*]
R × S	0.560 ^{ns}	2.169 ^{ns}	0.718 ^{ns}	2.202 ^{ns}	1.051 ^{ns}	2.061 ^{ns}	0.048 ^{ns}	2.405 ^{ns}	0.288 ^{ns}	1.964 ^{ns}	0.876 ^{ns}	2.167 ^{ns}
R × I	1.059 ^{ns}	0.536 ^{ns}	0.029 ^{ns}	1.581 ^{ns}	0.103 ^{ns}	0.697 ^{ns}	0.435 ^{ns}	1.772 ^{ns}	0.268 ^{ns}	1.492 ^{ns}	0.256 ^{ns}	0.294 ^{ns}
S × I	0.312 ^{ns}	2.312 ^{ns}	0.151 ^{ns}	0.071 ^{ns}	0.061 ^{ns}	0.531 ^{ns}	0.003 ^{ns}	0.002 ^{ns}	1.415 ^{ns}	0.384 ^{ns}	2.410 ^{ns}	1.633 ^{ns}
R × S × I	1.470 ^{ns}	0.393 ^{ns}	0.593 ^{ns}	1.072 ^{ns}	0.665 ^{ns}	0.726 ^{ns}	0.707 ^{ns}	0.447 ^{ns}	0.946 ^{ns}	0.722 ^{ns}	0.895 ^{ns}	0.282 ^{ns}
	Straw B uptake		Straw Cu uptake		Straw Fe uptake		Straw Mn uptake		Straw Zn uptake		Straw Si uptake	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
F-test												
N rate (R)	5.389 [*]	7.493 [*]	1.042 ^{ns}	0.846 ^{ns}	0.444 ^{ns}	1.076 ^{ns}	0.366 ^{ns}	5.478 ^{**}	0.819 ^{ns}	9.914 ^{**}	0.712 ^{ns}	9.182 ^{**}
Liming source (S)	0.528 ^{ns}	0.995 ^{ns}	0.020 ^{ns}	0.000 ^{ns}	0.306 ^{ns}	0.085 ^{ns}	0.723 ^{ns}	0.799 ^{ns}	2.024 ^{ns}	0.889 ^{ns}	8.156 ^{**}	10.251 ^{**}
Inoculation (I)	0.023 ^{ns}	0.092 ^{ns}	0.031 ^{ns}	0.864 ^{ns}	0.443 ^{ns}	0.792 ^{ns}	0.435 ^{ns}	0.373 ^{ns}	0.607 ^{ns}	0.155 ^{ns}	0.938 ^{ns}	1.830 ^{ns}
R × S	0.304 ^{ns}	1.379 ^{ns}	2.214 ^{ns}	1.139 ^{ns}	0.182 ^{ns}	1.480 ^{ns}	0.562 ^{ns}	1.628 ^{ns}	0.067 ^{ns}	2.496 ^{ns}	0.188 ^{ns}	1.083 ^{ns}
R × I	0.152 ^{ns}	0.574 ^{ns}	1.141 ^{ns}	0.162 ^{ns}	0.153 ^{ns}	1.389 ^{ns}	0.113 ^{ns}	2.172 ^{ns}	0.291 ^{ns}	0.600 ^{ns}	0.148 ^{ns}	0.570 ^{ns}
S × I	0.076 ^{ns}	1.356 ^{ns}	0.402 ^{ns}	0.119 ^{ns}	0.001 ^{ns}	2.473 ^{ns}	0.375 ^{ns}	1.258 ^{ns}	0.157 ^{ns}	0.081 ^{ns}	0.075 ^{ns}	0.122 ^{ns}
R × S × I	0.965 ^{ns}	0.175 ^{ns}	1.887 ^{ns}	1.504 ^{ns}	0.673 ^{ns}	0.514 ^{ns}	0.795 ^{ns}	0.901 ^{ns}	0.453 ^{ns}	0.203 ^{ns}	0.948 ^{ns}	1.084 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

7. F-test values for wheat grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake as a function of N rate, liming source and inoculation with *A. brasilense*.

	Grain N uptake		Grain P uptake		Grain K uptake		Grain Ca uptake		Grain Mg uptake		Grain S uptake	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
F-test												
N rate (R)	5.903 ^{**}	7.573 ^{**}	2.344 ^{ns}	5.610 ^{**}	0.735 ^{ns}	10.689 ^{**}	0.445 ^{ns}	0.517 ^{ns}	1.647 ^{ns}	9.589 ^{**}	8.019 [*]	5.365 ^{**}
Liming source (S)	4.730 [*]	2.054 ^{ns}	9.412 ^{**}	0.194 ^{ns}	0.015 ^{ns}	9.946 ^{**}	0.193 ^{ns}	0.097 ^{ns}	0.036 ^{ns}	0.337 ^{ns}	1.964 ^{ns}	1.379 ^{ns}
Inoculation (I)	1.402 ^{ns}	8.640 ^{**}	0.595 ^{ns}	20.771 ^{**}	0.298 ^{ns}	23.651 ^{**}	0.003 ^{ns}	1.127 ^{ns}	0.000 ^{ns}	11.644 ^{**}	2.669 ^{ns}	4.849 [*]
R × S	0.065 ^{ns}	0.310 ^{ns}	1.295 ^{ns}	1.121 ^{ns}	0.574 ^{ns}	0.806 ^{ns}	0.528 ^{ns}	0.480 ^{ns}	0.826 ^{ns}	1.253 ^{ns}	0.955 ^{ns}	0.680 ^{ns}
R × I	0.880 ^{ns}	0.677 ^{ns}	0.553 ^{ns}	1.003 ^{ns}	0.422 ^{ns}	1.497 ^{ns}	0.801 ^{ns}	0.319 ^{ns}	0.498 ^{ns}	0.759 ^{ns}	0.999 ^{ns}	0.541 ^{ns}
S × I	2.243 ^{ns}	0.087 ^{ns}	2.424 ^{ns}	0.309 ^{ns}	1.209 ^{ns}	0.000 ^{ns}	0.576 ^{ns}	0.809 ^{ns}	2.425 ^{ns}	2.132 ^{ns}	2.241 ^{ns}	0.023 ^{ns}
R × S × I	1.582 ^{ns}	0.332 ^{ns}	0.721 ^{ns}	0.487 ^{ns}	1.938 ^{ns}	0.267 ^{ns}	0.248 ^{ns}	0.158 ^{ns}	0.875 ^{ns}	0.427 ^{ns}	0.222 ^{ns}	0.032 ^{ns}
	Grain B uptake		Grain Cu uptake		Grain Fe uptake		Grain Mn uptake		Grain Zn uptake		Grain Si uptake	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
F-test												
N rate (R)	2.673 ^{ns}	6.206 ^{**}	8.039 ^{**}	1.188 ^{ns}	0.438 ^{ns}	7.703 [*]	1.239 ^{ns}	2.315 ^{ns}	1.393 ^{ns}	7.126 ^{**}	4.523 [*]	1.578 ^{ns}
Liming source (S)	16.156 ^{**}	1.923 ^{ns}	11.176 ^{**}	0.347 ^{ns}	11.366 ^{**}	0.763 ^{ns}	13.451 ^{**}	0.389 ^{ns}	19.469 ^{**}	1.095 ^{ns}	20.438 ^{**}	28.138 ^{**}
Inoculation (I)	1.141 ^{ns}	12.264 ^{**}	0.317 ^{ns}	7.090 [*]	2.053 ^{ns}	0.202 ^{ns}	0.099 ^{ns}	8.142 ^{**}	0.005 ^{ns}	26.219 ^{**}	2.607 ^{ns}	5.302 [*]
R × S	0.986 ^{ns}	1.147 ^{ns}	0.318 ^{ns}	0.414 ^{ns}	1.538 ^{ns}	1.000 ^{ns}	0.642 ^{ns}	0.534 ^{ns}	0.999 ^{ns}	0.532 ^{ns}	0.657 ^{ns}	0.267 ^{ns}
R × I	2.100 ^{ns}	0.673 ^{ns}	1.231 ^{ns}	0.132 ^{ns}	0.230 ^{ns}	0.232 ^{ns}	0.883 ^{ns}	0.203 ^{ns}	1.235 ^{ns}	1.087 ^{ns}	2.478 ^{ns}	0.862 ^{ns}
S × I	1.062 ^{ns}	0.482 ^{ns}	1.843 ^{ns}	0.565 ^{ns}	0.256 ^{ns}	1.507 ^{ns}	1.547 ^{ns}	0.411 ^{ns}	0.640 ^{ns}	2.281 ^{ns}	2.325 ^{ns}	1.735 ^{ns}
R × S × I	1.107 ^{ns}	0.609 ^{ns}	1.092 ^{ns}	0.282 ^{ns}	0.550 ^{ns}	0.347 ^{ns}	1.071 ^{ns}	0.103 ^{ns}	0.082 ^{ns}	0.228 ^{ns}	0.148 ^{ns}	1.052 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

8. F-test values for soil P, organic matter (OM), pH, K, Ca, Mg, sum of bases (SB), S, cation exchange capacity (CEC) and base saturation (V) content in 0-0.20m depth after wheat cultivation as a function of N rate, liming source and inoculation with *A. brasilense*.

	P		OM		pH		K		Ca	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
F-test										
N rate (R)	7.049*	0.166 ^{ns}	1.670 ^{ns}	5.019*	2.080 ^{ns}	2.412 ^{ns}	3.588*	0.858 ^{ns}	1.048 ^{ns}	0.517 ^{ns}
Liming source (S)	2.059 ^{ns}	2.742 ^{ns}	1.411 ^{ns}	1.242 ^{ns}	0.218 ^{ns}	8.276**	0.021 ^{ns}	0.128 ^{ns}	2.284 ^{ns}	2.007 ^{ns}
Inoculation (I)	0.242 ^{ns}	1.551 ^{ns}	0.075 ^{ns}	1.242 ^{ns}	0.078 ^{ns}	5.109*	0.636 ^{ns}	0.289 ^{ns}	0.014 ^{ns}	2.080 ^{ns}
R × S	1.674 ^{ns}	1.500 ^{ns}	0.409 ^{ns}	0.985 ^{ns}	2.071 ^{ns}	0.148 ^{ns}	0.146 ^{ns}	1.277 ^{ns}	0.989 ^{ns}	0.123 ^{ns}
R × I	1.083 ^{ns}	0.426 ^{ns}	0.367 ^{ns}	1.191 ^{ns}	2.136 ^{ns}	2.021 ^{ns}	1.909 ^{ns}	0.479 ^{ns}	1.873 ^{ns}	0.310 ^{ns}
S × I	0.170 ^{ns}	0.018 ^{ns}	2.414 ^{ns}	0.257 ^{ns}	2.148 ^{ns}	1.520 ^{ns}	2.344 ^{ns}	0.004 ^{ns}	0.434 ^{ns}	2.383 ^{ns}
R × S × I	1.155 ^{ns}	0.507 ^{ns}	0.493 ^{ns}	0.513 ^{ns}	1.208 ^{ns}	0.781 ^{ns}	0.796 ^{ns}	1.478 ^{ns}	1.008 ^{ns}	0.695 ^{ns}
	Mg		SB		S		CEC		V	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
F-test										
N rate (R)	1.036 ^{ns}	0.728 ^{ns}	1.096 ^{ns}	0.620 ^{ns}	0.694 ^{ns}	1.413 ^{ns}	0.904 ^{ns}	0.041 ^{ns}	1.229 ^{ns}	2.249 ^{ns}
Liming source (S)	1.766 ^{ns}	2.647 ^{ns}	0.027 ^{ns}	0.052 ^{ns}	0.015 ^{ns}	0.020 ^{ns}	0.000 ^{ns}	2.636 ^{ns}	0.012 ^{ns}	6.192*
Inoculation (I)	0.046 ^{ns}	2.647 ^{ns}	0.048 ^{ns}	2.669 ^{ns}	0.136 ^{ns}	5.750*	0.293 ^{ns}	0.391 ^{ns}	1.174 ^{ns}	6.176*
R × S	0.764 ^{ns}	0.208 ^{ns}	0.819 ^{ns}	0.107 ^{ns}	0.770 ^{ns}	0.517 ^{ns}	0.444 ^{ns}	0.117 ^{ns}	1.308 ^{ns}	0.101 ^{ns}
R × I	1.801 ^{ns}	0.344 ^{ns}	1.542 ^{ns}	0.301 ^{ns}	1.419 ^{ns}	2.487 ^{ns}	2.115 ^{ns}	0.395 ^{ns}	2.472 ^{ns}	0.915 ^{ns}
S × I	0.222 ^{ns}	1.089 ^{ns}	0.256 ^{ns}	1.892 ^{ns}	2.445 ^{ns}	2.547 ^{ns}	0.161 ^{ns}	0.290 ^{ns}	1.524 ^{ns}	2.018 ^{ns}
R × S × I	0.764 ^{ns}	0.474 ^{ns}	0.864 ^{ns}	0.626 ^{ns}	0.166 ^{ns}	0.995 ^{ns}	0.426 ^{ns}	0.540 ^{ns}	1.116 ^{ns}	0.915 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

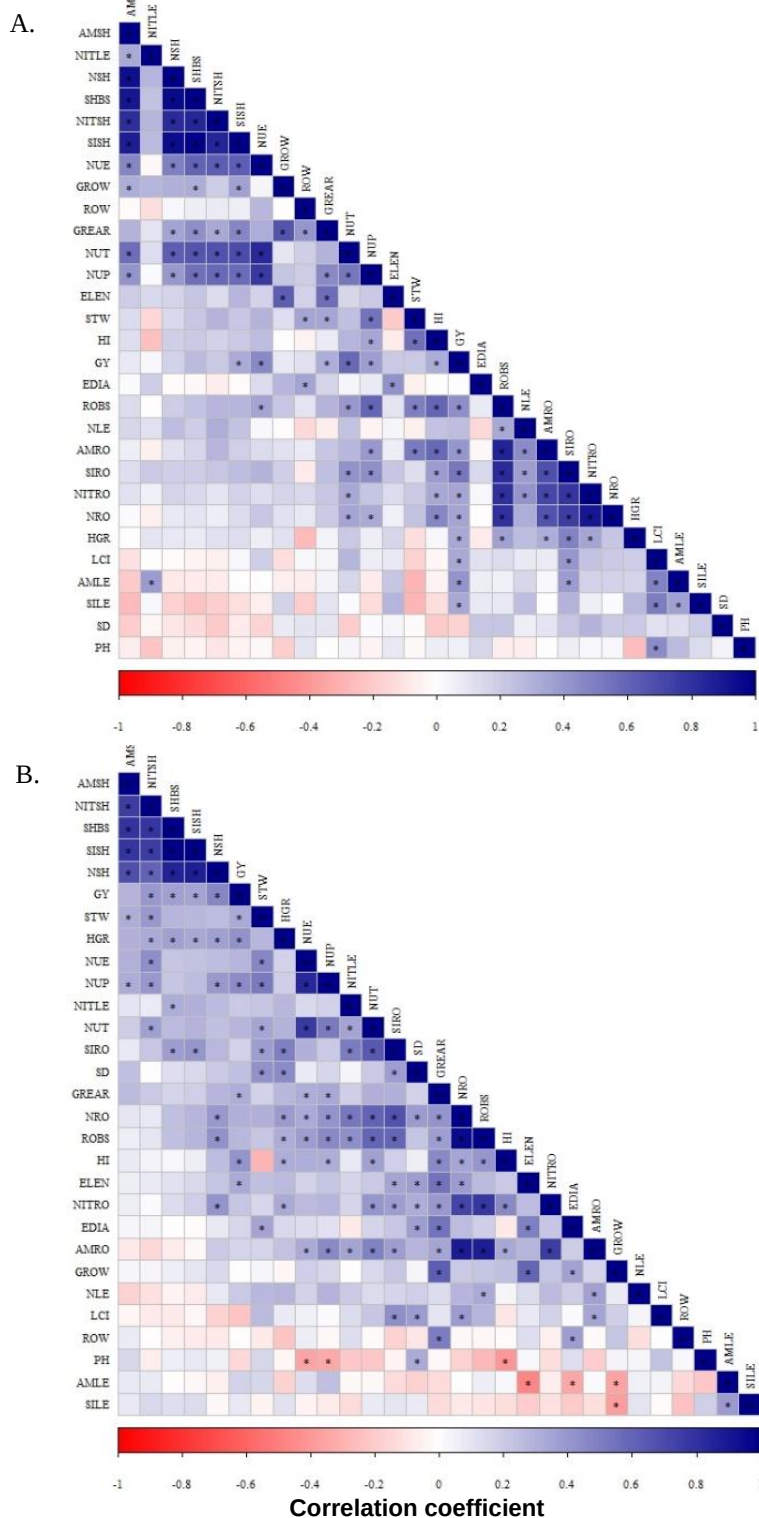
9. F-test values for soil H+Al, Al, B, Cu, Fe, Mn, Zn and Si content in 0-0.20m depth after wheat cultivation as a function of N rate, liming source and inoculation with *A. brasilense*.

	H + Al		Al		B		Cu	
	2016	2017	2016	2017	2016	2017	2016	2017
F-test								
N rate (R)	2.081 ^{ns}	2.371 ^{ns}	0.890 ^{ns}	0.383 ^{ns}	0.378 ^{ns}	0.192 ^{ns}	0.596 ^{ns}	1.562 ^{ns}
Liming source (S)	0.102 ^{ns}	21.566 ^{**}	0.048 ^{ns}	8.826 ^{**}	2.374 ^{ns}	0.000 ^{ns}	0.488 ^{ns}	1.836 ^{ns}
Inoculation (I)	1.994 ^{ns}	7.483 [*]	1.203 ^{ns}	3.603 [*]	0.025 ^{ns}	2.501 ^{ns}	0.151 ^{ns}	0.586 ^{ns}
R × S	1.248 ^{ns}	0.163 ^{ns}	1.130 ^{ns}	0.475 ^{ns}	1.217 ^{ns}	1.297 ^{ns}	0.036 ^{ns}	0.474 ^{ns}
R × I	1.754 ^{ns}	2.090 ^{ns}	2.165 ^{ns}	1.241 ^{ns}	0.237 ^{ns}	0.919 ^{ns}	0.783 ^{ns}	1.463 ^{ns}
S × I	3.157 ^{ns}	2.401 ^{ns}	0.433 ^{ns}	2.206 ^{ns}	2.490 ^{ns}	0.155 ^{ns}	0.054 ^{ns}	0.586 ^{ns}
R × S × I	1.221 ^{ns}	1.235 ^{ns}	0.072 ^{ns}	1.210 ^{ns}	0.567 ^{ns}	1.040 ^{ns}	0.476 ^{ns}	0.855 ^{ns}
	Fe		Mn		Zn		Si	
	2016	2017	2016	2017	2016	2017	2016	2017
F-test								
N rate (R)	1.521 ^{ns}	0.869 ^{ns}	1.389 ^{ns}	0.714 ^{ns}	0.593 ^{ns}	0.373 ^{ns}	0.249 ^{ns}	1.511 ^{ns}
Liming source (S)	0.131 ^{ns}	0.000 ^{ns}	0.177 ^{ns}	1.647 ^{ns}	0.338 ^{ns}	2.353 ^{ns}	4.978 [*]	30.065 ^{**}
Inoculation (I)	4.954 [*]	10.357 ^{**}	0.503 ^{ns}	6.970 [*]	0.338 ^{ns}	1.616 ^{ns}	1.590 ^{ns}	1.647 ^{ns}
R × S	0.379 ^{ns}	1.228 ^{ns}	1.909 ^{ns}	2.016 ^{ns}	1.042 ^{ns}	1.047 ^{ns}	1.143 ^{ns}	2.334 ^{ns}
R × I	0.419 ^{ns}	0.132 ^{ns}	0.352 ^{ns}	0.770 ^{ns}	0.986 ^{ns}	0.857 ^{ns}	1.285 ^{ns}	0.711 ^{ns}
S × I	1.182 ^{ns}	1.113 ^{ns}	2.376 ^{ns}	0.721 ^{ns}	0.003 ^{ns}	0.693 ^{ns}	2.397 ^{ns}	2.633 ^{ns}
R × S × I	0.291 ^{ns}	0.936 ^{ns}	0.800 ^{ns}	0.779 ^{ns}	0.888 ^{ns}	0.156 ^{ns}	0.221 ^{ns}	1.705 ^{ns}

ns, ** and *: not significant, significant at $p < 0.01$ and $p < 0.05$, respectively.

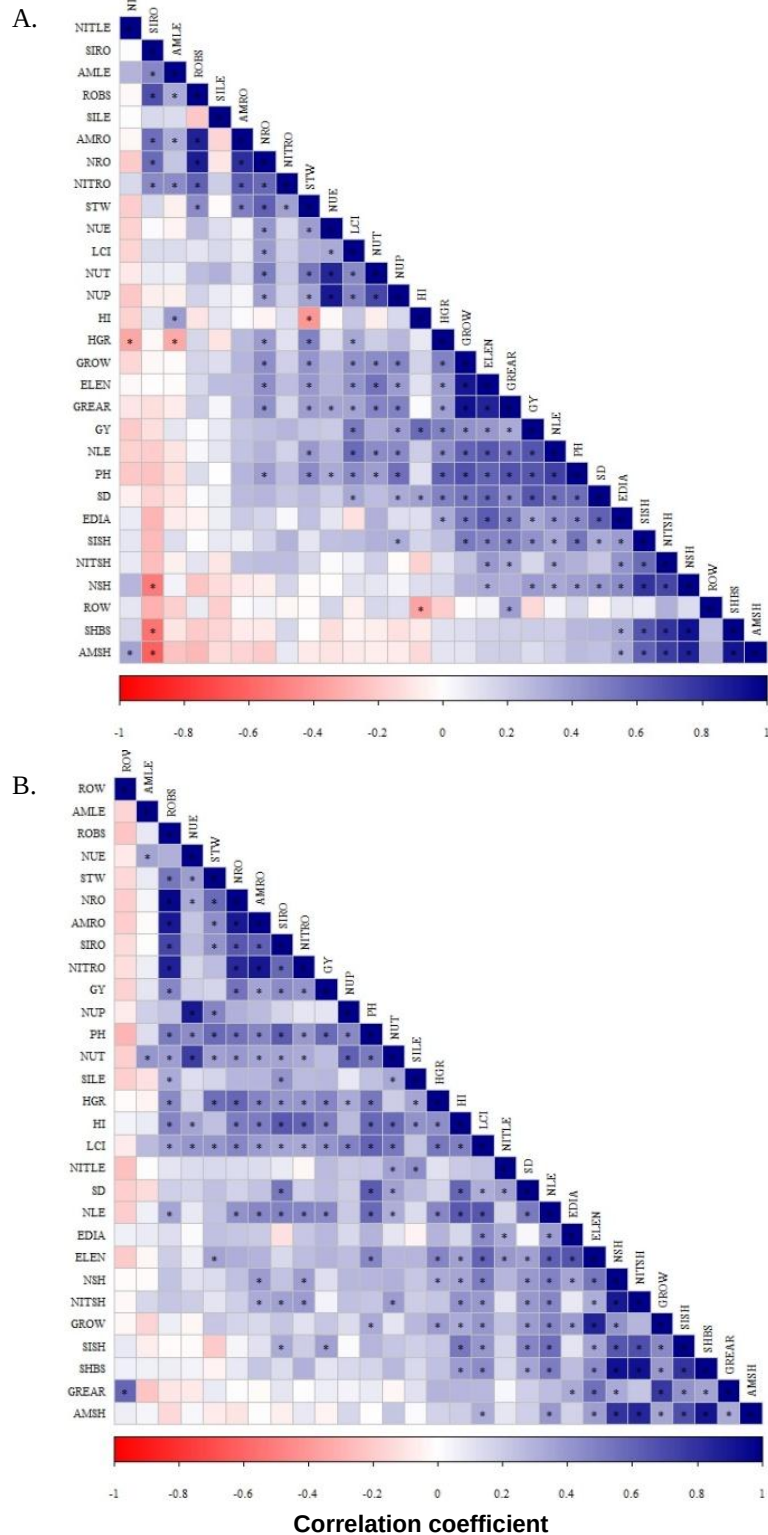
APPENDIX C – Corn heatmaps (Pearson correlation)

1. Heatmap showing the Pearson correlation among in season evaluations, productive components, NUP, NUT and NUE and corn grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2015/16 crop season.



LCI = Leaf chlorophyll index, NITL = Leaf NO_3^- concentration, AMLE = Leaf NH_4^+ concentration, NLE = Leaf N concentration, SILE = Leaf Si concentration, NITSH = Shoot NO_3^- uptake, AMSH = Shoot NH_4^+ uptake, NSH = Shoot N uptake, SISH = Shoot Si uptake, NITRO = Root NO_3^- uptake, AMRO = Root NH_4^+ uptake, NRO = Root N uptake, SIRO = Root Si uptake, SHBS = Shoot biomass, ROBS = Root biomass, PH = Plant height, SD = Stem diameter, ELEN = Ear length, EDIA = Ear diameter, ROW = Number of rows per ear, GROW = Number of grains per row, GREAR = Number of grains per ear, HGR = Mass of 100 grains, STW = Straw biomass, HI = Harvest index, NUP = N uptake, NUT = N utilization, NUE = N use efficiency, GY = Grain yield.

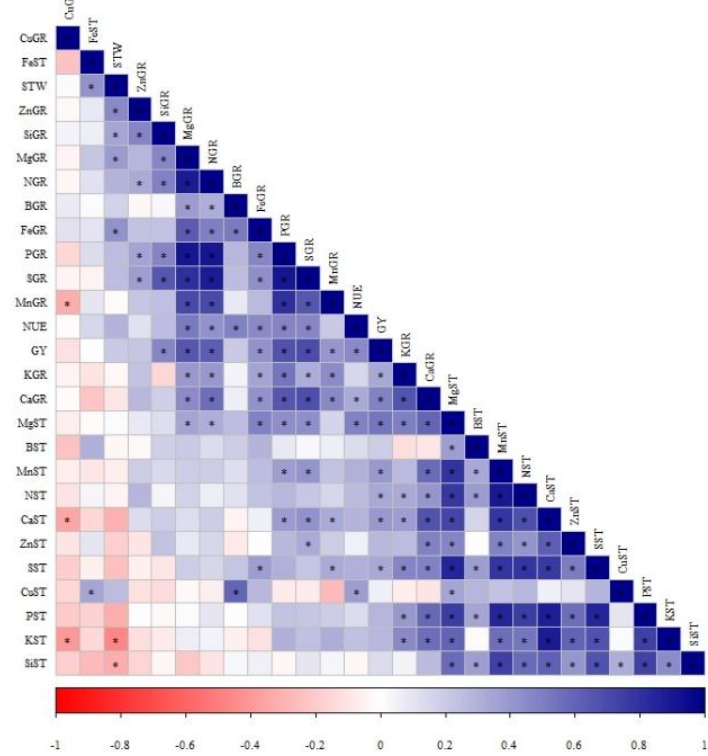
2. Heatmap showing the Pearson correlation among in season evaluations, productive components, NUP, NUT and NUE and corn grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2016/17 crop season.



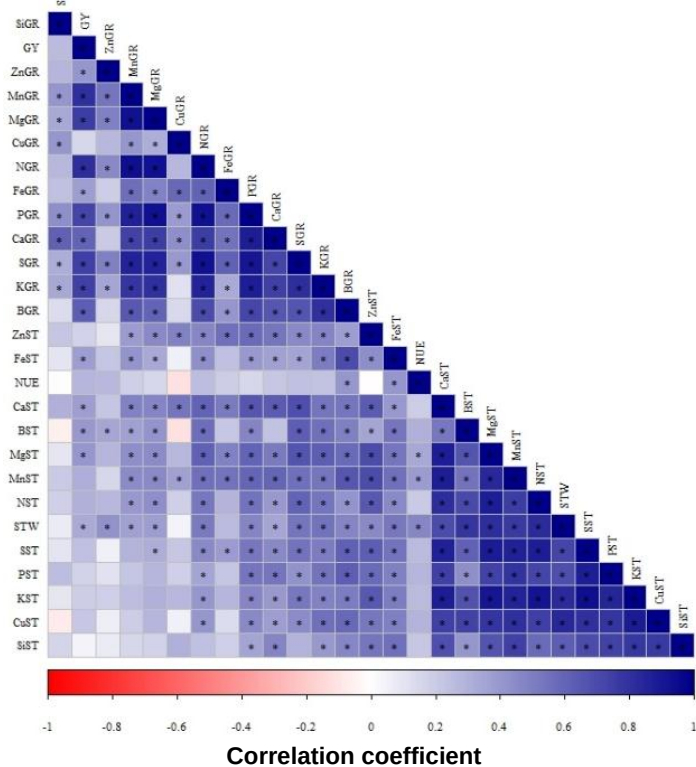
LCI = Leaf chlorophyll index, NITL = Leaf NO_3^- concentration, AMLE = Leaf NH_4^+ concentration, NLE = Leaf N concentration, SILE = Leaf Si concentration, NITSH = Shoot NO_3^- uptake, AMSH = Shoot NH_4^+ uptake, NSH = Shoot N uptake, SISH = Shoot Si uptake, NITRO = Root NO_3^- uptake, AMRO = Root NH_4^+ uptake, NRO = Root N uptake, SIRO = Root Si uptake, SHBS = Shoot biomass, ROBS = Root biomass, PH = Plant height, SD = Stem diameter, ELEN = Ear length, EDIA = Ear diameter, ROW = Number of rows per ear, GROW = Number of grains per row, GREAR = Number of grains per ear, HGR = Mass of 100 grains, STW = Straw biomass, HI = Harvest index, NUP = N uptake, NUT = N utilization, NUE = N use efficiency, GY = Grain yield.

3. Heatmap showing the Pearson correlation among straw and grain nutrient uptake, straw biomass, NUE and corn grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2015/16 crop season.

A.

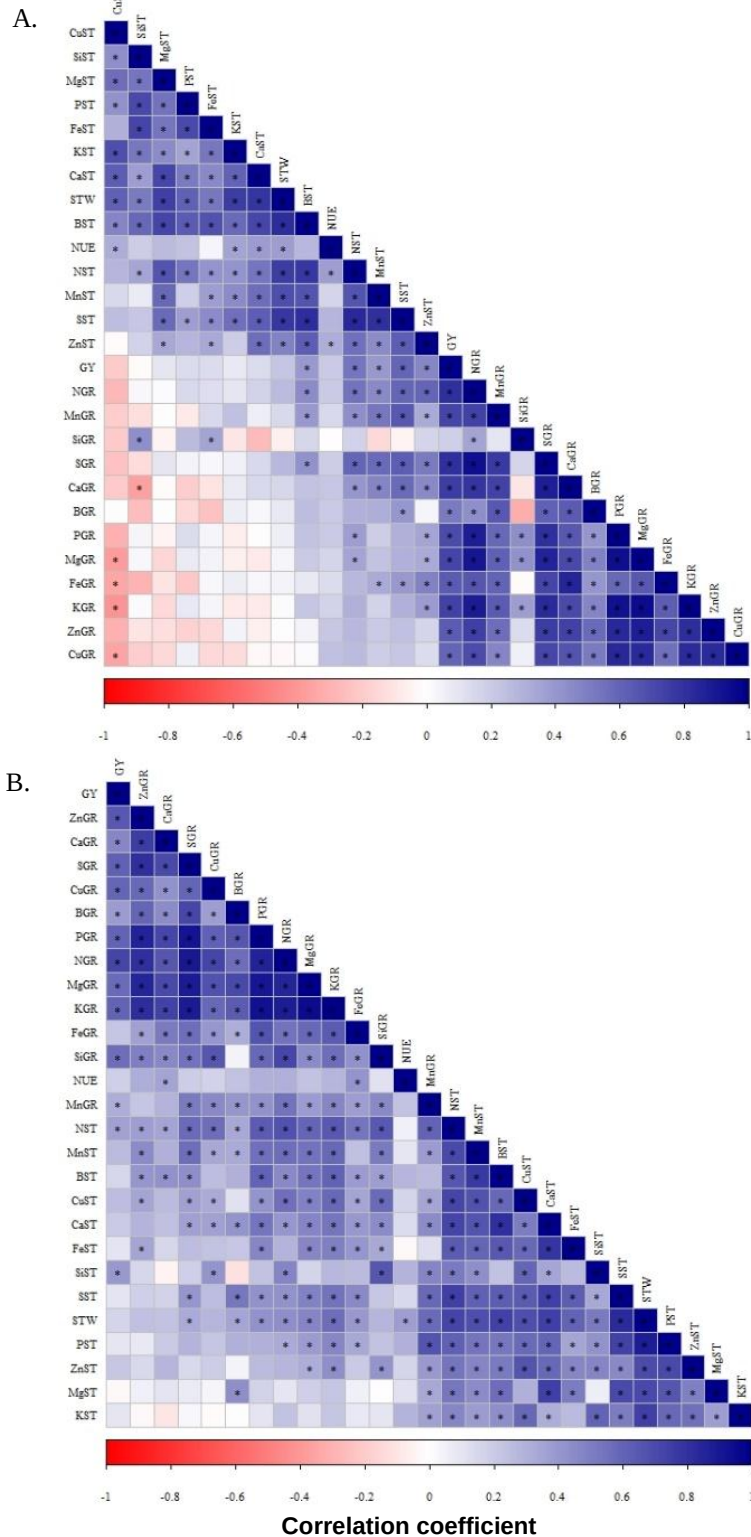


B.



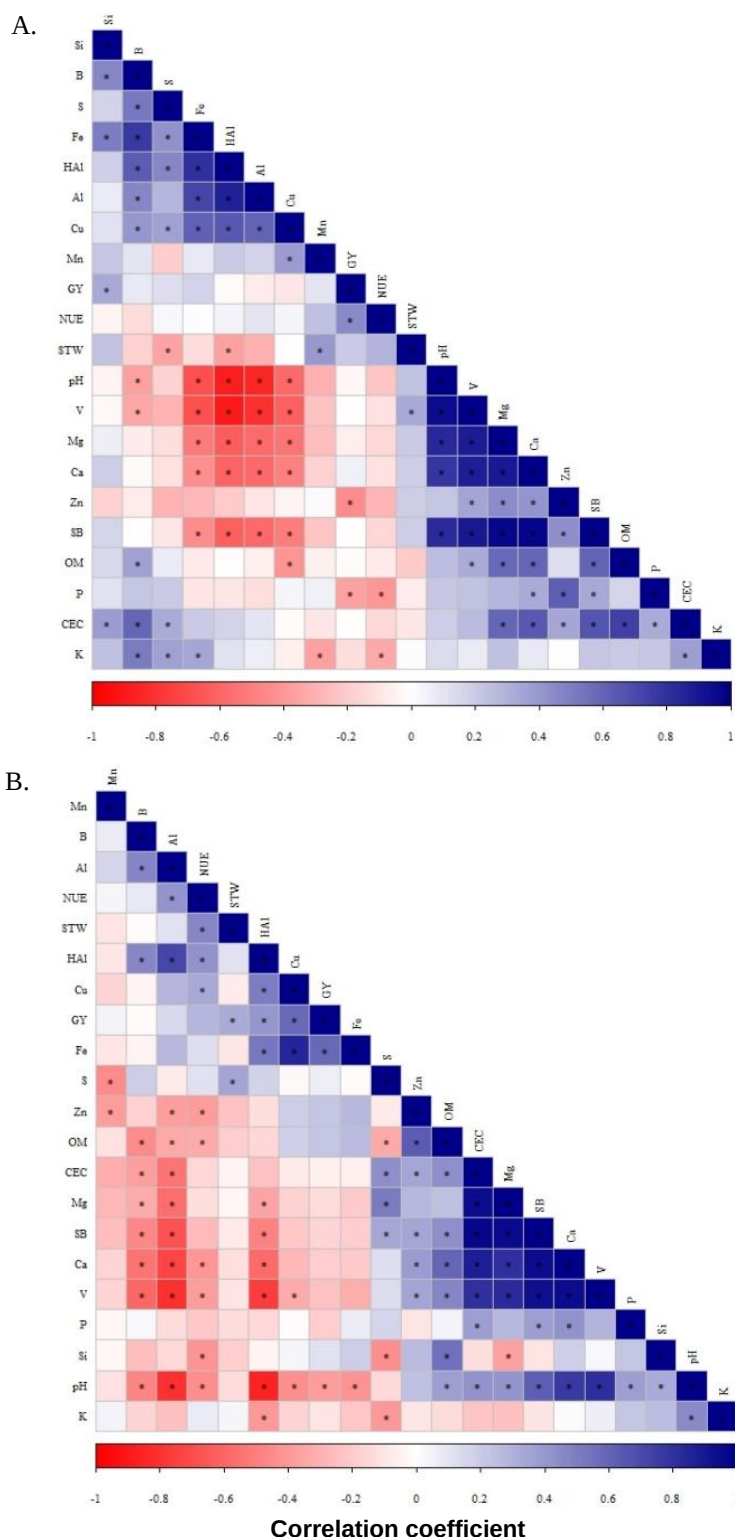
STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, NST, PST, KST, CaST, MgST, SST, BST, CuST, FeST, MnST, ZnST and SiST = Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively, NGR, PGR, KGR, CaGR, MgGR, SGR, BGR, CuGR, FeGR, MnGR, ZnGR and SiGR = Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively.

4. Heatmap showing the Pearson correlation among straw and grain nutrient uptake, straw biomass, NUE and corn grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2016/17 crop season.



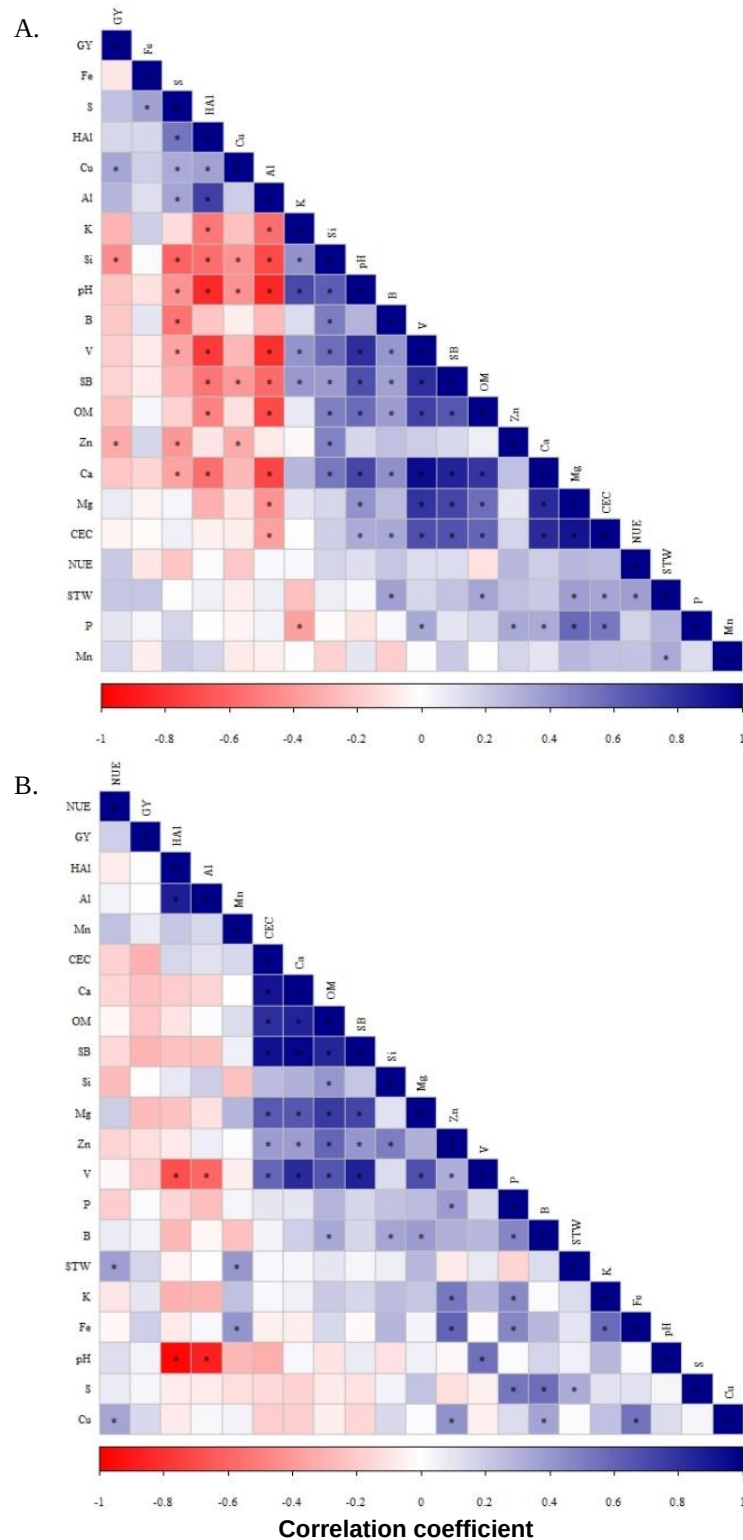
STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, NST, PST, KST, CaST, MgST, SST, BST, CuST, FeST, MnST, ZnST and SiST = Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively, NGR, PGR, KGR, CaGR, MgGR, SGR, BGR, CuGR, FeGR, MnGR, ZnGR and SiGR = Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively.

5. Heatmap showing the Pearson correlation among soil chemical attributes (0-0.20m depth), straw biomass, NUE and corn grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2015/16 crop season.



STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, P, K, Ca, Mg, HAI, Al, S, B, Cu, Fe, Mn, Zn and Si = Soil P-resin, K, Ca, Mg, H+Al, Al, S, B, Cu, Fe, Mn, Zn and Si content, respectively, OM = Soil organic matter, pH = soil pH, SB = soil sum of bases, CEC = soil cation exchange capacity, V = soil base saturation.

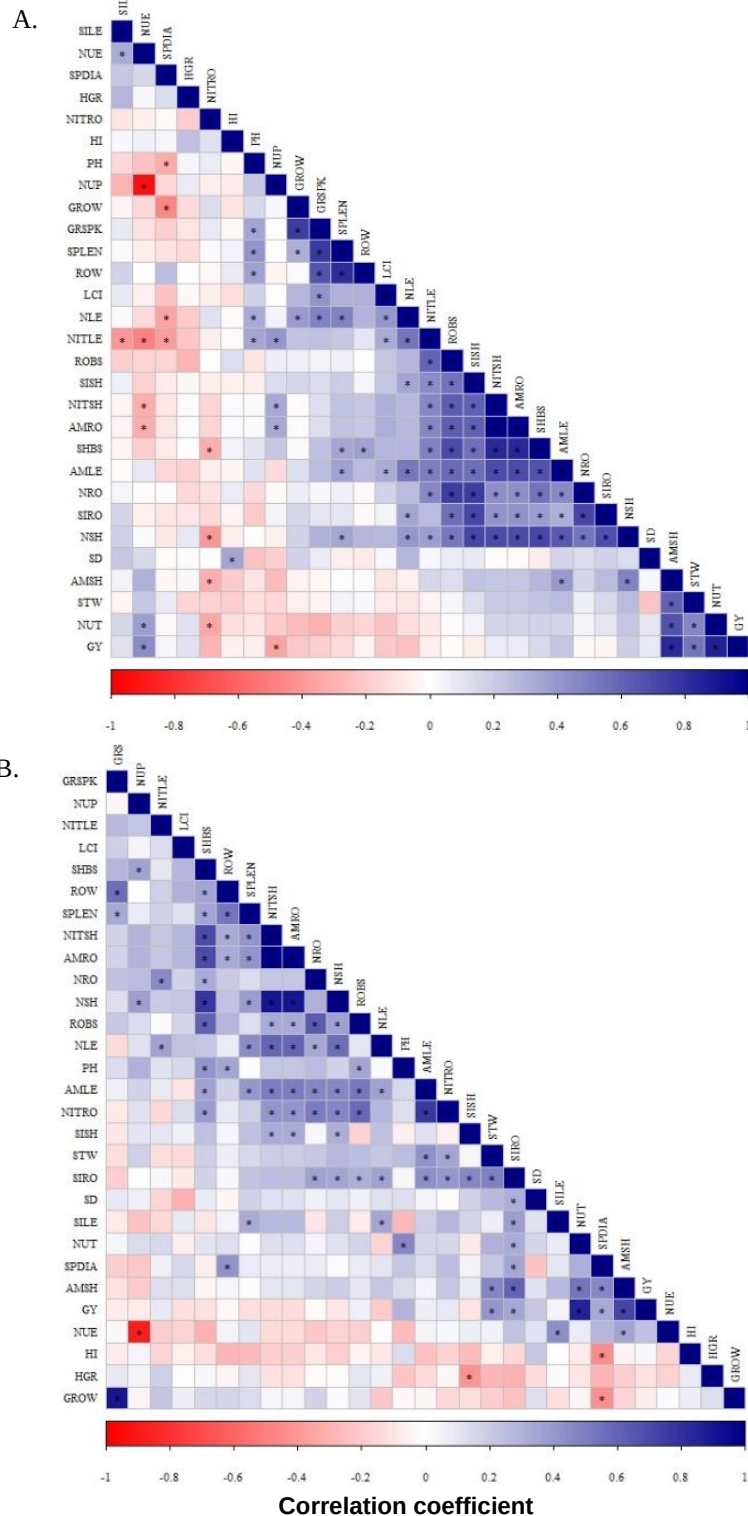
6. Heatmap showing the Pearson correlation among soil chemical attributes (0-0.20m depth), straw biomass, NUE and corn grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2016/17 crop season.



STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, P, K, Ca, Mg, HAI, Al, S, B, Cu, Fe, Mn, Zn and Si = Soil P-resin, K, Ca, Mg, H+Al, Al, S, B, Cu, Fe, Mn, Zn and Si content, respectively, OM = Soil organic matter, pH = soil pH, SB = soil sum of bases, CEC = soil cation exchange capacity, V = soil base saturation.

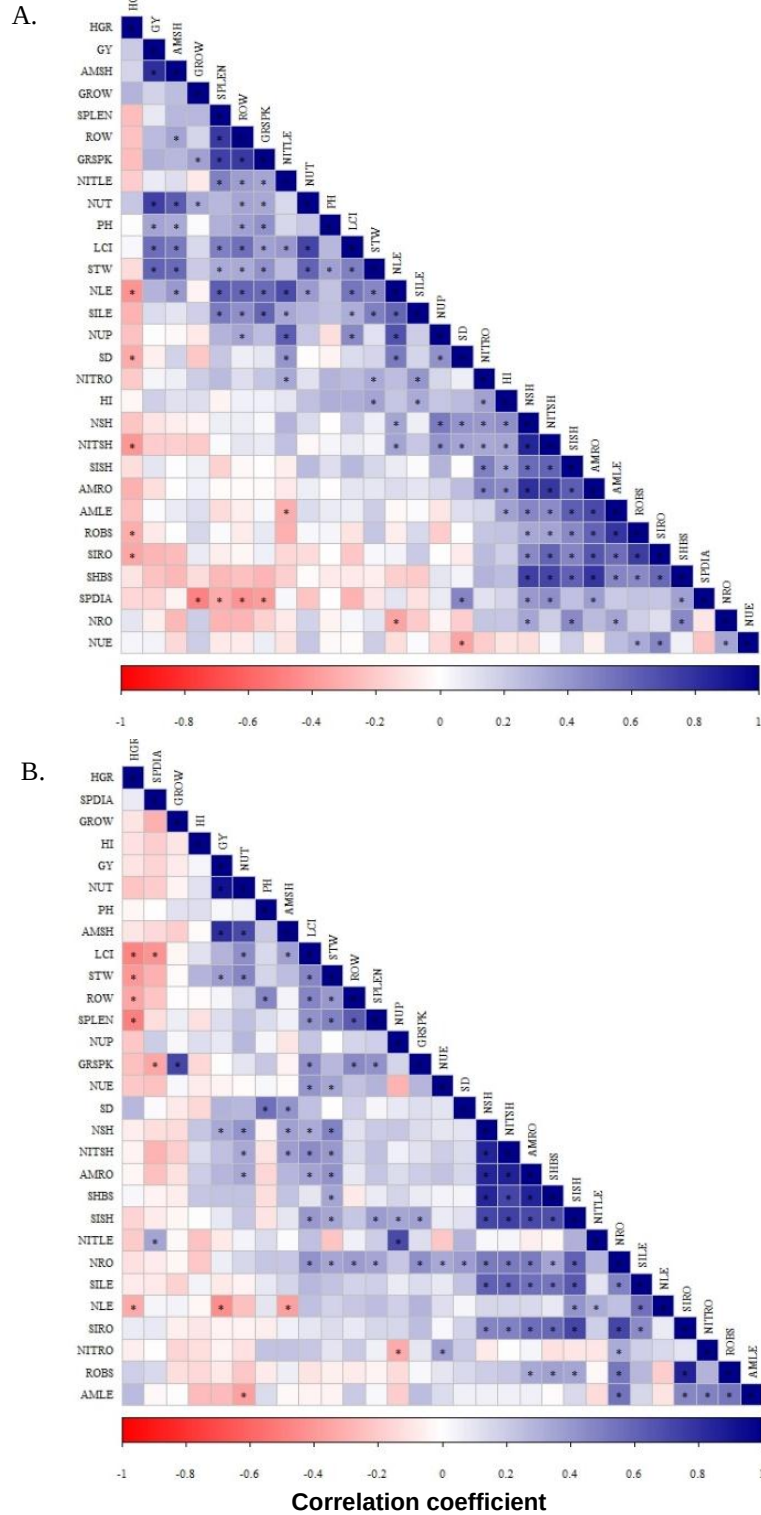
APPENDIX D – Wheat heatmaps (Pearson correlation)

1. Heatmap showing the Pearson correlation among in season evaluations, productive components, NUP, NUT and NUE and wheat grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2016 crop season.



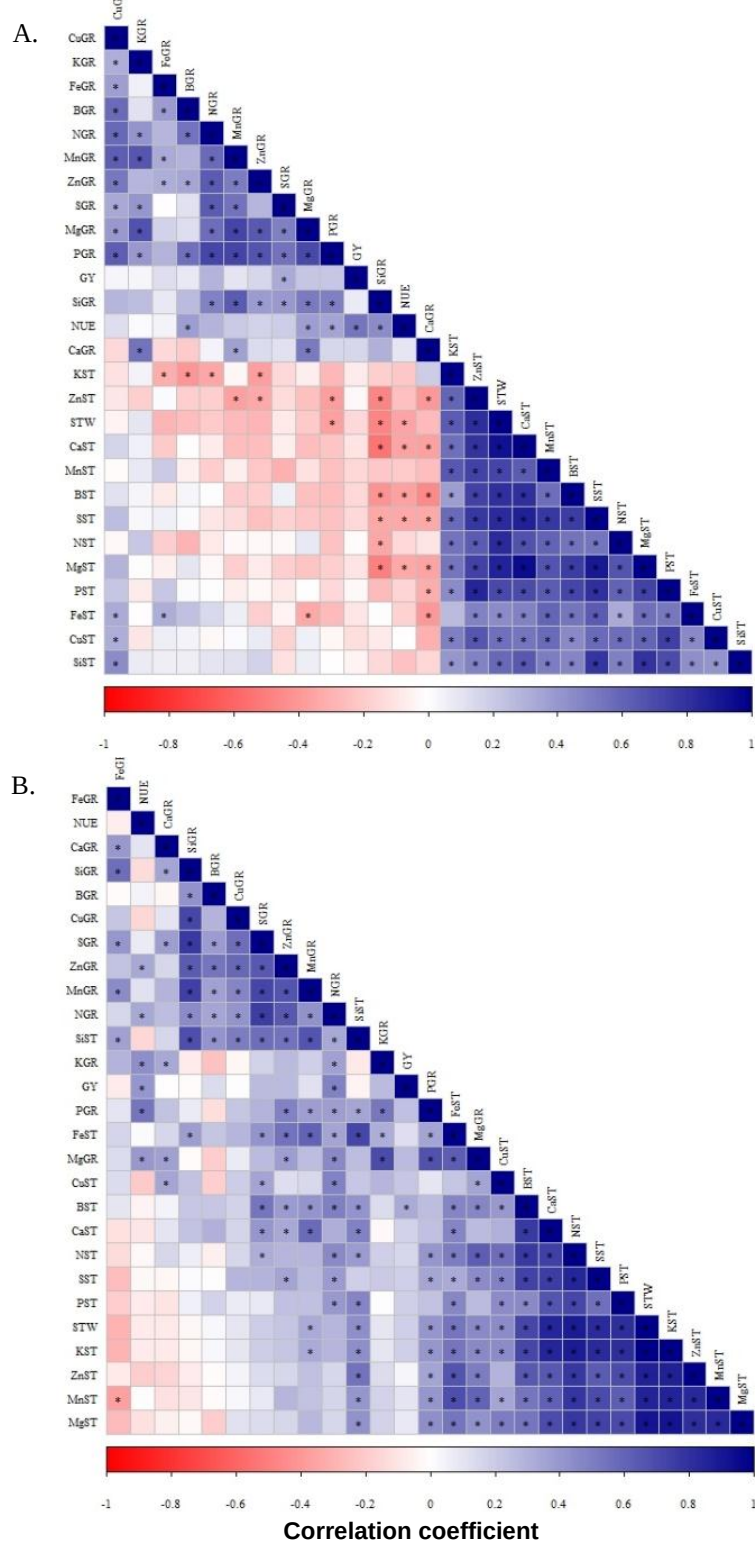
LCI = Leaf chlorophyll index, NITL = Leaf NO_3^- concentration, AMLE = Leaf NH_4^+ concentration, NLE = Leaf N concentration, SILE = Leaf Si concentration, NITSH = Shoot NO_3^- uptake, AMSH = Shoot NH_4^+ uptake, NSH = Shoot N uptake, SISH = Shoot Si uptake, NITRO = Root NO_3^- uptake, AMRO = Root NH_4^+ uptake, NRO = Root Si uptake, SIRO = Root Si uptake, SHBS = Shoot biomass, ROBS = Root biomass, PH = Plant height, SPDLA = Number of spikes per meter, SPLEN = Spike length, DEFGR = Number of defective grains, SPIKLS = Number of spikelets per spike, GRSPK = Number of grains per spikelet, GRSP = Number of grains per spike, HM = hectoliter mass, MTG = Mass of 1000 grains, STW = Straw biomass, HI = Harvest index, NUP = N uptake, NUT = N utilization, NUE = N use efficiency, GY = Grain yield.

2. Heatmap showing the Pearson correlation among in season evaluations, productive components, NUP, NUT and NUE and wheat grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2017 crop season.



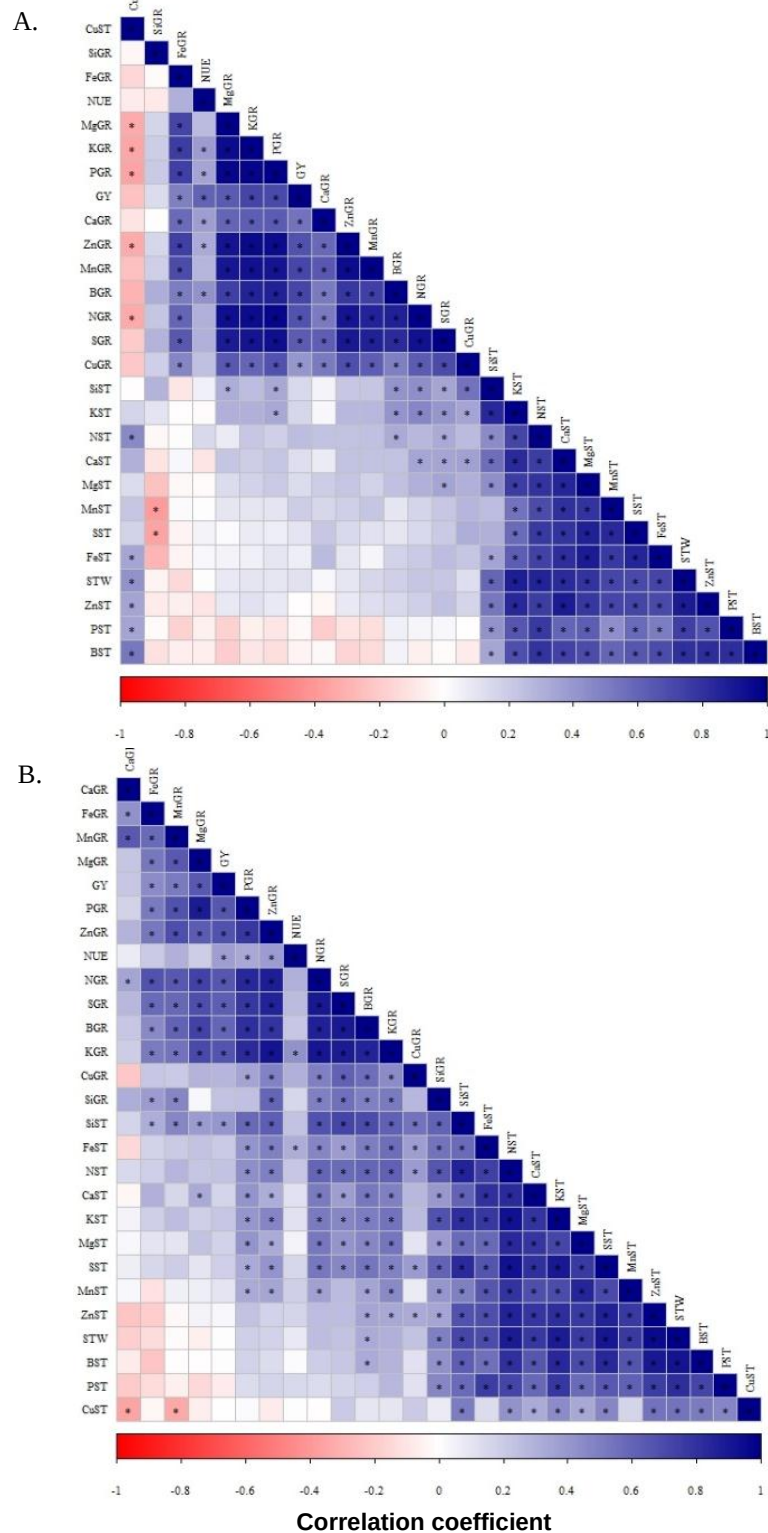
LCI = Leaf chlorophyll index, NITLE = Leaf NO_3^- concentration, AMLE = Leaf NH_4^+ concentration, NLE = Leaf N concentration, SILE = Leaf Si concentration, NITSH = Shoot NO_3^- uptake, AMSH = Shoot NH_4^+ uptake, NSH = Shoot N uptake, SISH = Shoot Si uptake, NITRO = Root NO_3^- uptake, AMRO = Root NH_4^+ uptake, NRO = Root N uptake, SIRO = Root Si uptake, SHBS = Shoot biomass, ROBS = Root biomass, PH = Plant height, SPMET = Number of spikes per meter, SPLEN = Spike length, DEFGR = Number of defective grains, SPIKLSP = Number of spikelets per spike, GRSPKL = Number of grains per spikelet, GRSP = Number of grains per spike, HM = hectoliter mass, MTG = Mass of 1000 grains, STW = Straw biomass, HI = Harvest index, NUP = N uptake, NUT = N utilization, NUE = N use efficiency, GY = Grain yield.

3. Heatmap showing the Pearson correlation among straw and grain nutrient uptake, straw biomass, NUE and wheat grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2016 crop season.



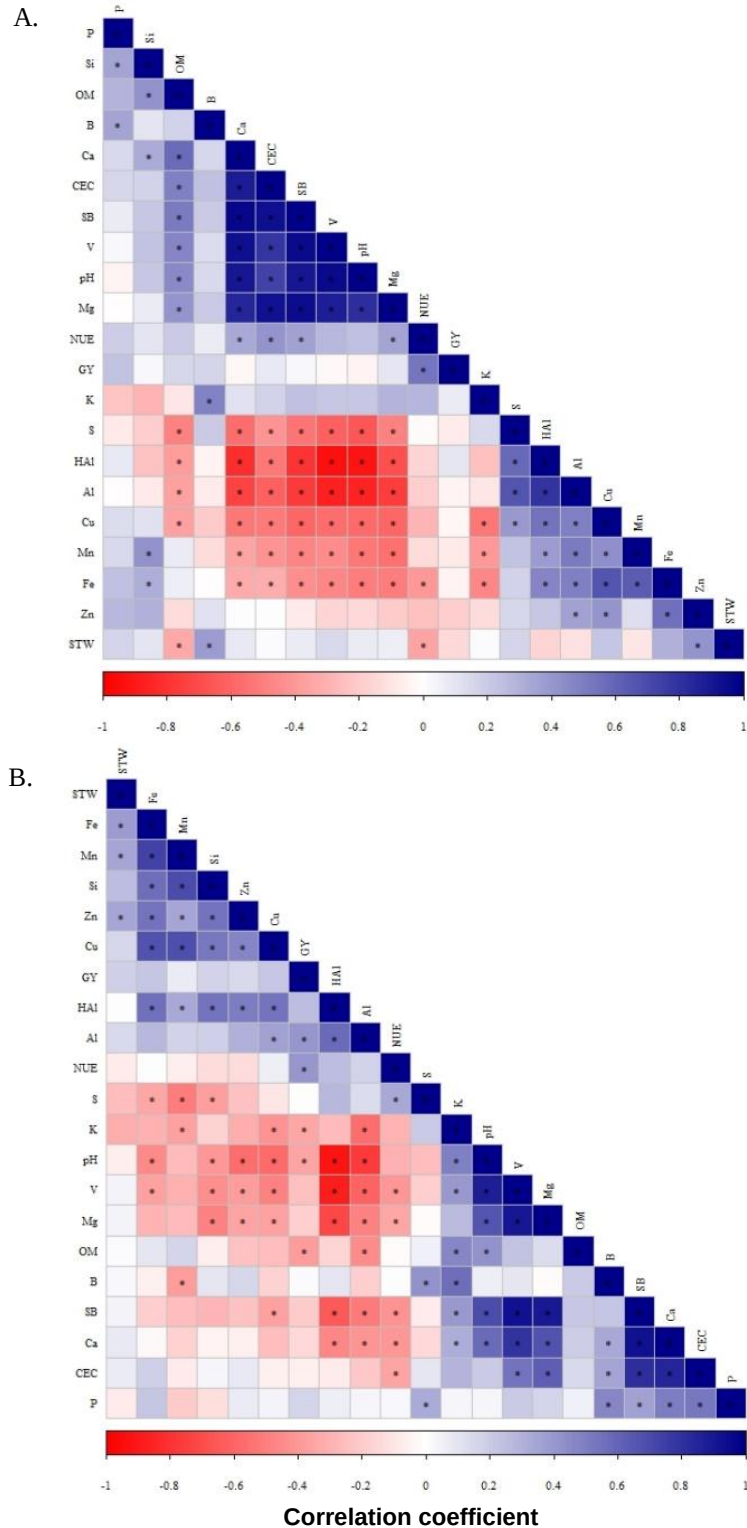
STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, NST, PST, KST, CaST, MgST, SST, BST, CuST, FeST, MnST, ZnST and SiST = Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively, NGR, PGR, KGR, CaGR, MgGR, SGR, BGR, CuGR, FeGR, MnGR, ZnGR and SiGR = Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively.

4. Heatmap showing the Pearson correlation among straw and grain nutrient uptake, straw biomass, NUE and wheat grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2017 crop season.



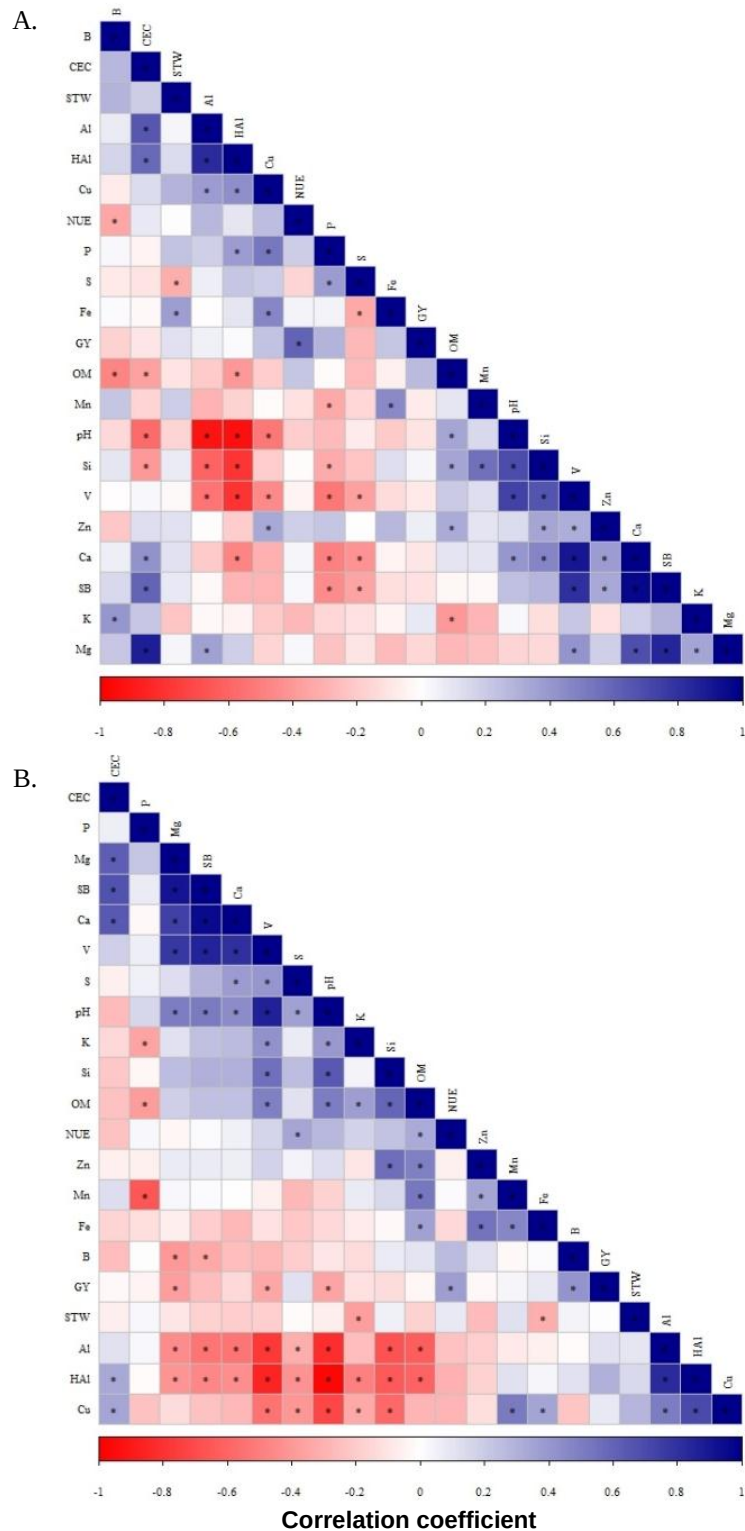
STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, NST, PST, KST, CaST, MgST, SST, BST, CuST, FeST, MnST, ZnST and SiST = Straw N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively, NGR, PGR, KGR, CaGR, MgGR, SGR, BGR, CuGR, FeGR, MnGR, ZnGR and SiGR = Grain N, P, K, Ca, Mg, S, B, Cu, Fe, Mn, Zn and Si uptake, respectively.

5. Heatmap showing the Pearson correlation among soil chemical attributes (0-0.20m depth), straw biomass, NUE and wheat grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2016 crop season.



STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, P, K, Ca, Mg, HAI, Al, S, B, Cu, Fe, Mn, Zn and Si = Soil P-resin, K, Ca, Mg, H+Al, Al, S, B, Cu, Fe, Mn, Zn and Si content, respectively, OM = Soil organic matter, pH = soil pH, SB = soil sum of bases, CEC = soil cation exchange capacity, V = soil base saturation.

6. Heatmap showing the Pearson correlation among soil chemical attributes (0-0.20m depth), straw biomass, NUE and wheat grain yield for non-inoculated treatments (A) and *A. brasilense* inoculated treatments (B) in 2017 crop season.



STW = Straw biomass, NUE = N use efficiency, GY = Grain yield, P, K, Ca, Mg, HAl, Al, S, B, Cu, Fe, Mn, Zn and Si = Soil P-resin, K, Ca, Mg, H+Al, Al, S, B, Cu, Fe, Mn, Zn and Si content, respectively, OM = Soil organic matter, pH = soil pH, SB = soil sum of bases, CEC = soil cation exchange capacity, V = soil base saturation.

APPENDIX E – Photos

1. Overview of the study area (A), liming sources application (B), area overview after liming sources application (C), Corn simple hybrid DOW2B710 (D), chemical seed treatment (E) and dry seeds after chemical treatment, in occasion of *A. brasilense* inoculation (F).

A.



B.



C.



D.



E.



F.



2. Corn planting (A), area overview after planting (B), corn development in V2/V3 stage (C), corn development in V4/V5 stage (D), nitrogen side-dressing application at V6 stage (E) corn development in V7/V8 stage (F).

A.



B.



C.



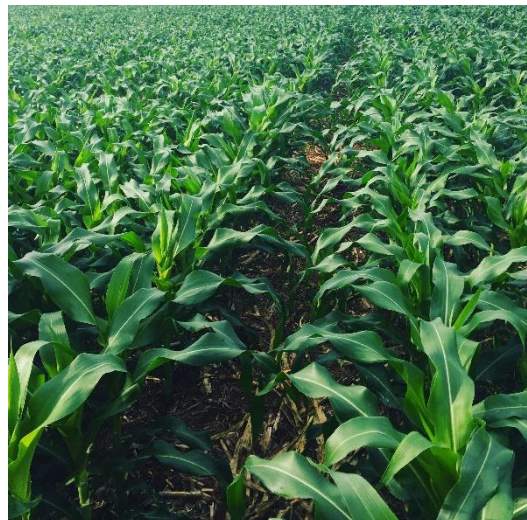
D.



E.



F.



3. Corn crop at full flowering (A), corn crop at full flowering (B), corn crop at full flowering (C), corn crop at full flowering (D), corn crop in the end of grain filling (E) and following the end of corn grain filling (F).

A.



B.



C.



D.



E.



F.



4. Corn crop at harvest time (A), corn crop at harvest time (B), corn grain harvested for final evaluations (C) and corn grain harvested for final evaluations (D).

A.



B.



C.



D.



5. Area overview after corn harvest and weed management (before wheat planting) (A and B), and wheat chemical seed treatment (C and D).

A.



B.



C.



D.



6. Wheat planting (A), area overview after planting (B), wheat development with 5 DAE (C), wheat development with 15 DAE (D), nitrogen side-dressing application at tillering (30 DAE) (E) wheat development with 45 DAE (F).

A.



B.



C.



D.



E.



F.



7. Wheat crop at beginning of flowering (A), wheat crop at full flowering (B), wheat crop at full flowering (C), wheat crop at full flowering (D), wheat crop in the end of grain filling (E) and wheat crop in the end of grain filling (F).

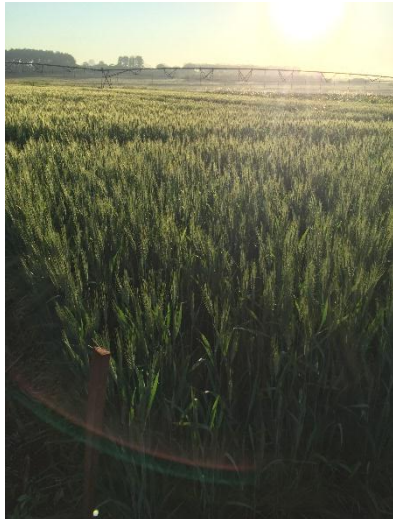
A.



B.



C.



D.



E.



F.

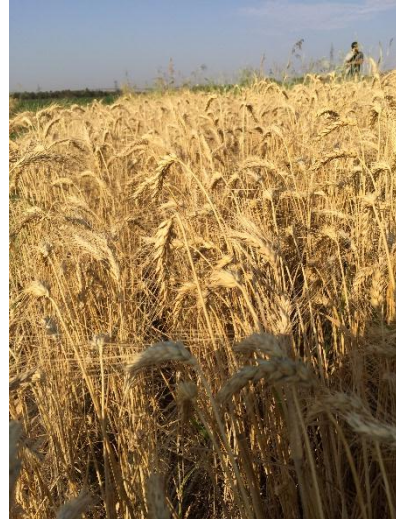


8. Wheat crop at harvest time (A), wheat crop at harvest time (B), wheat grain harvested for final evaluations (C), wheat grain harvested for final evaluations (D), lab work analysis (E) and standard curve of Si for plant tissue analysis (F).

A.



B.



C.



D.



E.



F.



9. Our research group who has always helped us in the development of the project (A) and (B), Southwest Research & Outreach center (SWROC) – College of Food, Agricultural and Natural Resource Sciences – University of Minnesota (C-E). With Prof. PhD. Paulo Humberto Pagliari at SWROC (F).

A.



B.



C.



D.



E.



F.

